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Enantiomeric resolution of derivatives of α-aminophosphonic and α-aminophosphinic acids by high-performance liquid chromatography and capillary electrophoresis

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Abstract

Resolution of chiral α-aminophosphonic and α-aminophosphinic acid esters analogous to phenylalanine or phenylglycine is achieved by HPLC with chiral cellulose or amylose phases. Resolution of the corresponding racemic acids is successfully performed by capillary electrophoresis. For derivatives analogous to phenylalanine, enantioselectivity of both analytical methods is depending on functional groups at the ring, the groups at the phosphorus atom as well as the structure of protecting groups. Suitable resolution conditions were found for all aminophosphonic and aminophosphinic acid derivatives either at HPLC by variation of chiral stationary phases and the eluent or at CE by using different β-cyclodextrin derivatives at specific conditions.

Keywords: Enantiomer separation; Aminophosphinic acids; Aminophosphonic acids; Phosphinic acids; Phosphonic acids

1. Introduction

α-Aminophosphonic and α-aminophosphinic acids are compounds analogous to amino acids and therefore, they have potentially biological activity. Their synthesis has been described by asymmetric hydrogenation of prochiral precursors [1–4]. As physiological properties of these substances are dependent on stereoisomeric configuration, estimation of optical purity of reaction products is of great importance. This requires elaboration of suitable analytical methods. Enantiomeric resolution of some other classes of organophosphorus compounds succeeded by high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) [5–9]. A great number of chiral stationary phases (CSPs) for HPLC are commercially available allowing resolution of a great diversity of racemates. However, for each substance the suitable chromatographic system has to be found. Cellulose and amylose phases, developed by Okamoto and co-workers show especially high chiral recognition for many chiral compounds [10,11]. In this work, α-aminophosphonic and α-aminophosphinic acid derivatives were separated into the stereoisomers by HPLC on different CSPs or by CE using cyclodextrin derivatives.

2. Experimental

HPLC was performed with a liquid chromatograph 1090 series II equipped with diode array detection (DAD) (Hewlett-Packard) and Chiralyser (IBZ Messtechnik, Hannover, Germany). Separations were carried out on Chiralcel OD-H or Chiralpak AD analytical columns 250×4.6 mm I.D. (Daicel).

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CE was performed with a BioFocus 3000 (Bio-Rad). β-Cyclodextrin was purchased from Merck, methyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin and poly(vinyl alcohol) (PVA) were purchased from Aldrich. The background electrolyte was 100 m*M* borate buffer, 0.05% PVA, pH between 7 and 9.8, cyclodextrin concentration between 10 and 30 m*M*. The capillary was an uncoated fused-silica column, 17 cm×50 μm. The capillary temperature was 22°C.

3. Results and discussion

Enantiomeric resolution of α-aminophosphonic acid methyl esters 1 succeeded by HPLC on the cellulose based CSP Chiralcel OD-H. Table 1 shows, that in almost all cases excellent resolutions are obtained with hexane–2-propanol eluents. The compound carrying a 4-F substituent exhibits the highest R_S value. However compound 1f with a *m*-F substituent demonstrates the smallest. Baseline resolution was not achieved for this derivative on Chiralcel OD-H. These results agree with investigations of Caccamese et al. [12], who observed for the HPLC analysis of *N*-arylamino-1arylmethylphosphonate esters, that the resolution of the compound with a 3-F substitution of the phenyl ring is worse than the separation for the corresponding 4-F-substituted compound.

The baseline resolution of 1f was obtained on Chiralpak AD with hexane–ethanol (95:5) as eluent (R_S=1.61).

The *S* enantiomer of 1a was less retained than the corresponding *R* derivative on the CSP Chiralcel OD-H. Using a CSP containing *tert*.-butylvalinamide as a chiral centre [13] a reversed peak order was obtained. Here the different retention times of the enantiomers are owing to the most part on the enantioselective hydrogen bonds between racemates and the amide groups of the CSP. Both polysaccharide CSPs possess a conformation of a left-handed helix and besides the possibility of hydrogen bonding the enantiomers can enter the grooves of the helix, to achieve chiral resolution of racemates [10,11]. While the methyl ester of 1a is better resolved on Chiralcel OD-H (α=1.3) than on the valinamide CSP (α=1.1), the valinamide CSP is particularly suitable for the resolution of the corresponding sterically more pretentious isopropyl ester (Chiralcel OD-H: α=1.2, valinamide CSP: α=1.4). The resolution of the corresponding ethyl ester is good in both chromatographic systems.

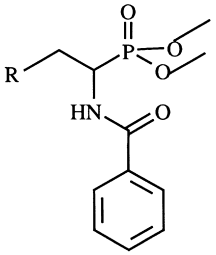
Analysis of *N-tert*.-butoxycarbonyl (Boc) derivatives in comparison to *N*-benzoyl-protected derivatives requires eluents with smaller polarity. Resolution of Boc-protected derivative analogous to 1a succeeds for instance with hexane–2-propanol (99:1) or with hexane-*tert*.-butyl methyl ether eluents.

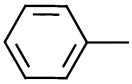
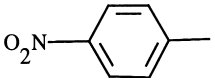
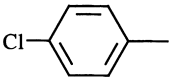
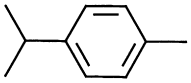
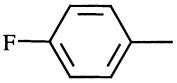
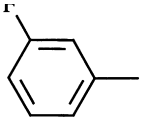
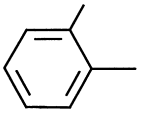
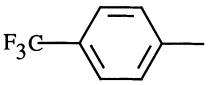
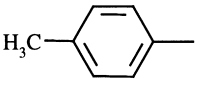
Also aminophosphinic acid esters give evidence for enantioselective interactions on CSP Chiralcel OD. The unsaturated precursors, carrying only one asymmetric centre at the phosphorus atom, are resolvable on both Chiralcel OD-H and Chiralpak AD. For resolution of hydrogenation products a chromatographic system is necessary, allowing resolution of the two enantiomeric precursors as well as the four stereoisomeric reaction products. For this, CSP Chiralpak AD was more suitable than Chiralcel OD-H. Fig. 1 shows one example of a resolution.

Assortment of peaks to both the one enantiomeric pair as well as the diastereomers was performed by comparison of automatically recorded UV spectra. Fig. 2 demonstrates resolution of two enantiomeric pairs of an other aminophosphinic acid ester with their corresponding UV spectra. All investigated diastereomeric aminophosphinic acid derivatives exhibited clear differences in their UV spectra.

In contrast to the aminophosphonic acid derivatives, in which an assignment of distinct peaks to the corresponding enantiomers was possible by X-ray studies of the enantiomers, there is at present in the case of aminophosphinic acid derivatives no possibility for an unambiguous correlation of the individual peaks to their absolute configuration. For this aim an X-ray analysis should also be performed for these compounds. Table 2 demonstrates in addition to k' values of the four stereoisomers the α values of enantiomeric pairs of the *N*-benzoylaminophosphinic acid ethyl esters at Chiralpak AD together with an optimised eluent. Resolution succeeds in all investigated cases with the same column. The differences between eluents, necessary for an optimal resolution, are substantially higher than at the analysis of the aminophosphonic acid esters.

Table 1
Enantiomeric resolution of 1-(*N*-benzoyl)aminophosphonophenylalanine methyl esters on Chiralcel OD-H[a]



Compound	R	Eluent	k_1'	k_2'	α	R_S
1a		Hexane–2-propanol (90:10)	2.48	3.25	1.31	2.81
1b		Hexane–2-propanol (90:10)	6.48	8.32	1.28	1.83
1c		Hexane–2-propanol (90:10)	5.11	6.96	1.36	2.71
1d		Hexane–2-propanol (90:10)	7.88	9.84	1.25	2.32
1e		Hexane–2-propanol (90:10)	2.03	2.66	1.31	4.04
1f		Hexane–ethanol (95:5)	4.29	4.65	1.08	1.44
1g		Hexane–2-propanol (95:5)	1.93	2.48	1.29	2.55
1h		Hexane–2-propanol (90:10)	1.81	3.03	1.65	3.27
1i		Hexane–2-propanol (90:10)	1.78	2.22	1.25	2.48

[a] Relative retention $\alpha = k_2'/k_1'$, resolution factor $R_S = 1.18[t_2 - t_1/w_{1/2(1)} + w_{1/2(2)}]$.

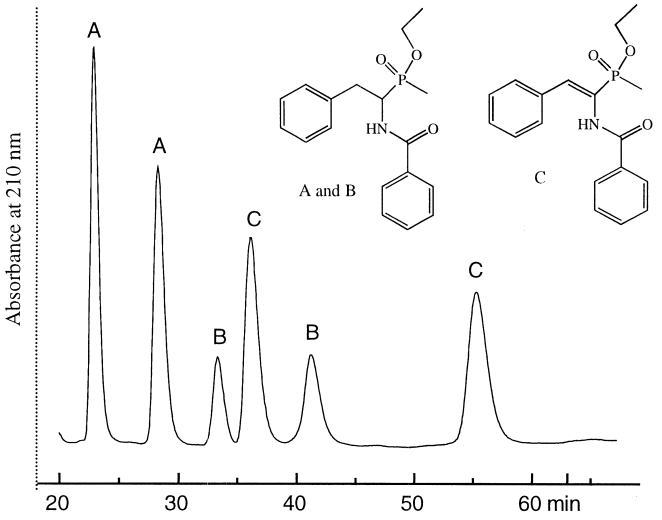


Fig. 1. Resolution of the prochiral precursors (C) and the four products of asymmetric hydrogenation (two enantiomeric pairs A and B). CSP: Chiralpak AD, eluent: hexane–ethanol (95:5).

To optimise the enantioselectivity, the concentration of the alcohol modifier in hexane was varied for 2-propanol and ethanol. Resolution of different aminophosphinic acid esters at Chiralpak AD exhibits a strong dependence of enantioselectivity on the composition of eluent. Small alterations of the alcohol proportion in hexane or the change from ethanol to isopropanol effect large differences of retention times, also often an alteration of peak order. Fig. 3 demonstrates this with an example. The

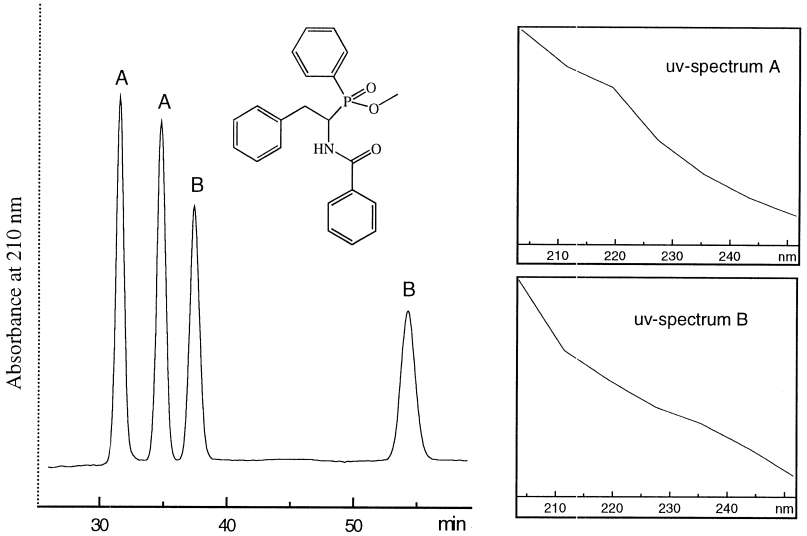
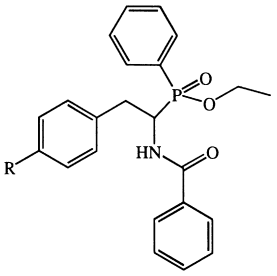


Fig. 2. Resolution of four hydrogenation products (pairs A and B) and the corresponding UV spectra of diastereomers. CSP: Chiralpak AD, eluent: hexane–2-propanol (9:1).

Table 2
Enantiomeric resolution of 1-(*N*-benzoyl)aminophosphinophenylalanine ethyl esters on the CSP Chiralpak AD



R	Eluent	k'_1	k'_2	k'_3	k'_4	α (1)	α (2)
H	Hexane–ethanol (9:1)	1.66	2.68	2.87	3.81	1.61 (k'_2/k'_1)	1.33 (k'_3/k'_4)
CH_3	Hexane–ethanol (98:2)	11.6	14.84	17.62	21.04	1.28 (k'_2/k'_1)	1.19 (k'_3/k'_4)
F	Hexane–ethanol (95:5)	4.6	7.0	7.8	8.8	1.9 (k'_4/k'_1)	1.13 (k'_3/k'_2)
$CH(CH_3)_2$	Hexane–ethanol–2-propanol (95:5:6)	1.47	1.78	2.79	3.12	2.12 (k'_4/k'_1)	1.57 (k'_3/k'_2)
NO_2	Hexane–ethanol–2-propanol (90:8:2)	5.57	7.22	13.16	24.14	4.33 (k'_4/k'_1)	1.82 (k'_3/k'_2)

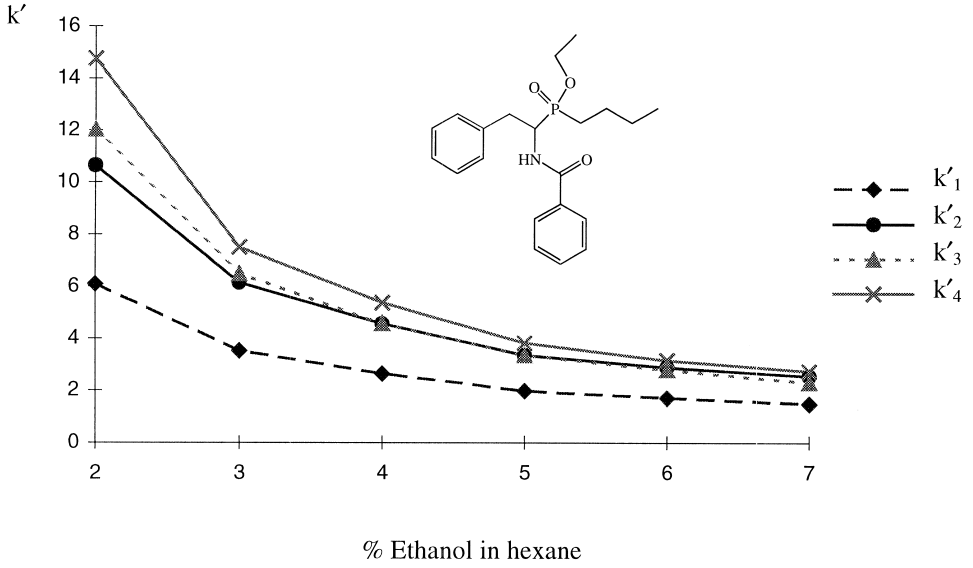


Fig. 3. Dependence of k' values of stereoisomers on the eluent composition.

hexane eluent containing 1% to 2% ethanol results in an elution profile of peaks 1 and 2 as the first enantiomeric pair as well as peaks 3 and 4 as the second one, but the increase to 7% ethanol alters the elution profile, representing peaks 1 and 3 as the first and peaks 2 and 4 as the second enantiomeric pair, respectively.

In addition to *N*-benzoyl-protected aminophosphinic acid esters, derivatives with carbobenzoxy (CBZ)-protecting groups can be resolved with this system into the stereoisomers, as is shown in Fig. 4a. Resolution of a derivative analogous to phenylglycine was also successful (Fig. 4b).

While enantiomeric resolution of aminophosphonic and aminophosphinic acid esters was successful for HPLC, resolution of the corresponding acids was performed by CE. Aminophosphinic acids contain only one asymmetric carbon atom and because of the fast racemisation no chiral phosphorus. Therefore for both aminophosphonic as well as aminophosphinic acids the resolution of enantiomers but not of diastereomers is required.

In CE, the enantiomer separations can be accomplished by use of different chiral buffer additives, such as crown ethers, bile salts, chiral surfactants and proteins. Native and modified cyclodextrins are the most widely used chiral selectors. The mechanism of these resolutions was investigated for many examples [14–18]. Inclusion-complexation between cyclodextrin and racemate enables chiral recognition. The relatively hydrophobic cavities of cyclodextrin are able to host parts of molecules. β-Cyclodextrin is suitable for inclusion-complexes with molecules composed of one or two condensed aromatic groups. Interactions between the analytes and the relatively hydrophilic outside of the cyclodextrin are important for the resolution also.

β-Cyclodextrin was also successfully used for the enantiomeric resolution of some aminophosphonic acid derivatives [19]. Our investigated aminophosphonic acids were excellently separated in their enantiomers by a β-cyclodextrin-containing buffer system (Table 3).

pH dependence of enantioselectivity is similar for different derivatives. All compounds are good separable in their enantiomers at pH 9.0 to 9.8. Resolution decreases at smaller pH as is shown in Fig. 5.

Structural alteration of compounds to be investi-

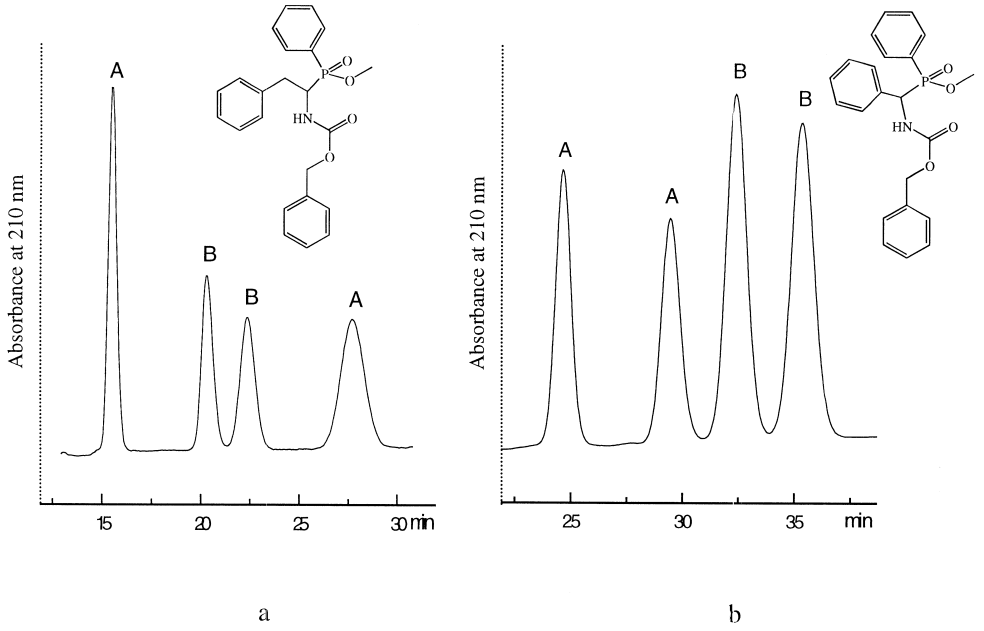
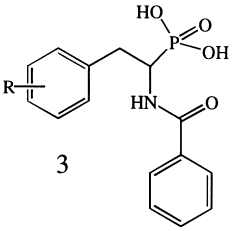


Fig. 4. Enantiomeric resolution of *N*-CBZ-aminophosphinic acid esters. CSP: Chiralpak AD, eluent: hexane–2-propanol.

Table 3
Chiral resolution of aminophosphonic acids[a]



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R	$t_{mig\ 1}$	$t_{mig\ 2}$	R_S
H	15.44	17.29	8.49
2-F	17.06	19.98	6.59
3-F	17.11	19.18	3.86
4-F	14.80	17.30	8.33
4-Cl	16.04	16.96	3.34
4-CH_3	16.89	18.32	5.27

[a] $R_S=1.177t_1-t_2/W_{1/2\ (1)}+W_{1/2\ (2)}$. Run buffer: borate, 0.05% PVA, 10 m*M* β-cyclodextrin, run voltage: 10 kV, pH: 9.6.

gated results in differences of enantioselectivity and requires often application of modified β-cyclodextrins to get a good resolution of enantiomers.

All investigated aminophosphonic acids were better resolved in their enantiomers with added β-cyclodextrin in the boric acid buffer than the corresponding aminophosphinic acids or aminophosphonic acid half esters, as is shown in Fig. 6a.

A separation mechanism based on inclusion-complexation of compounds to be separated with the cyclodextrin was employed. It should be similar for all derivatives, where hydrophobic interactions of the phenyl residue with the β-cyclodextrin are especially important. Aminophosphonic acids deliver one OH group more for the hydrophilic interactions with the OH groups of cyclodextrin. This effects an increased stability of complexes and longer migration times, resulting in a positive effect on enantioselectivity.

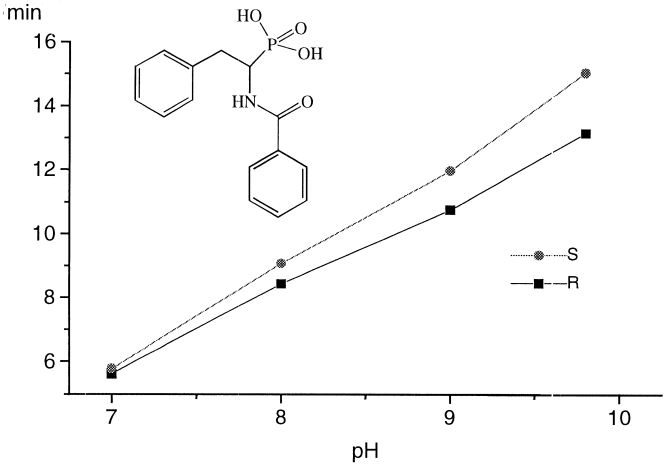

Fig. 5. Dependence of enantiomeric resolution of one aminophosphonic acid on pH. Run buffer: borate, 0.05% PVA, 10 m*M* β-cyclodextrin, run voltage: 12 kV.

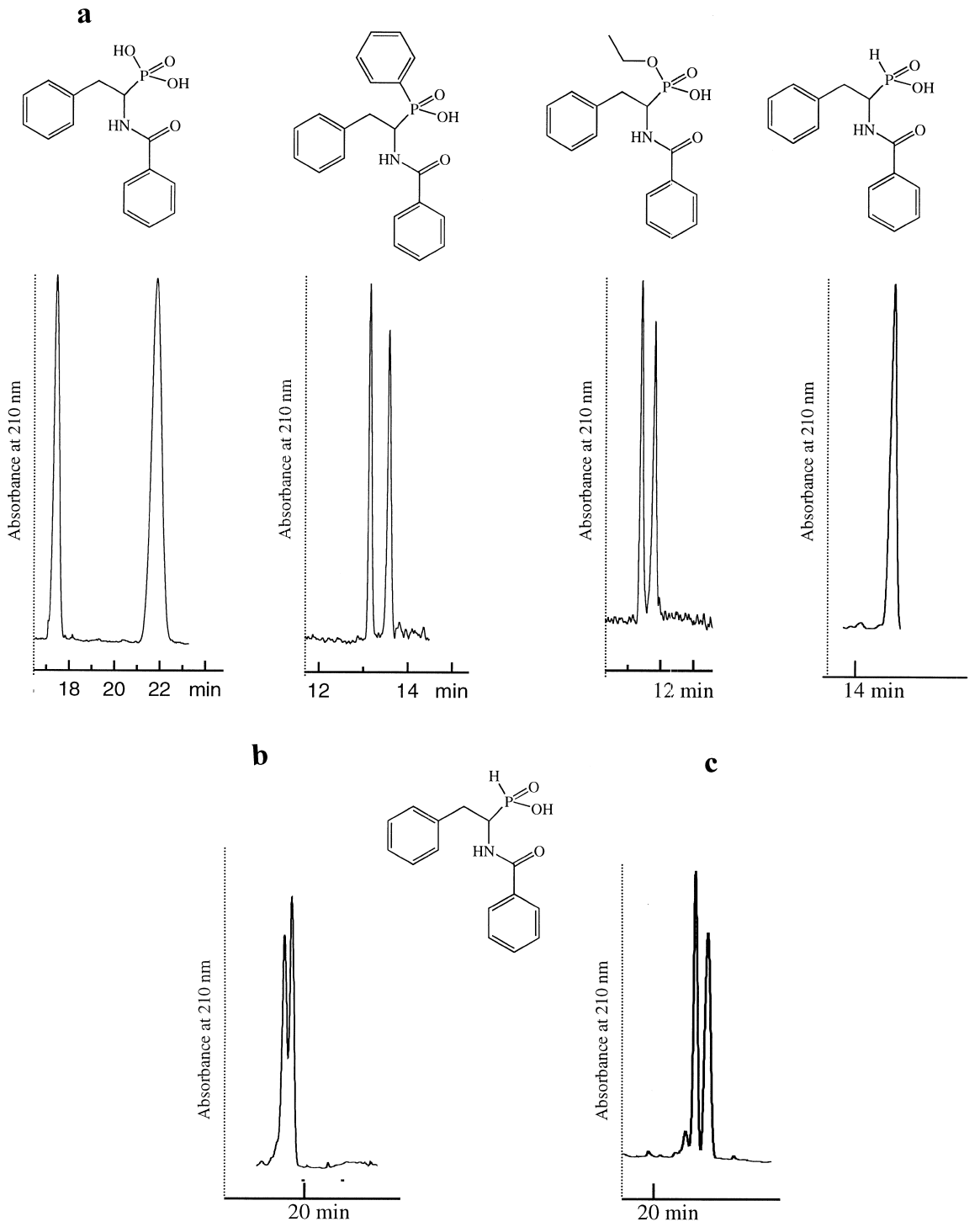

Fig. 6. (a) Dependence of enantiomeric resolution on substituents at the phosphorus atom. Run buffer: borate, 0.05% PVA, 15 m*M* β-cyclodextrin, (b) and (c) Dependence of resolution on the methyl-β-cyclodextrin concentration of the buffer. Run buffer: borate, 0.05% PVA, (b) 20 m*M* methyl-β-cyclodextrin, (c) 30 m*M* methyl-β-cyclodextrin.

This system exhibits no enantioselectivity of aminophosphonous acid. Higher working concentrations of methyl-β-cyclodextrin enable a complete resolution (Fig. 6b and c). Methyl-β-cyclodextrin possesses a better solubility in buffer and less possibilities for hydrophilic interactions in comparison to β-cyclodextrin.

The enantiomeric resolution is further dependent on substituents at the phenyl residue (Fig. 7). While derivatives without substituent or with a *p*-F substituent are separated well, no baseline resolution was achieved for the *p*-methyl substituted derivative.

Further, the structure of protecting group influences the enantioselectivity of resolution. With a 10 m*M* β-cyclodextrin containing buffer system the *N*-benzoylaminophosphinic acid analogous to phenylalanine is separated in their enantiomers well, but the corresponding CBZ-protected compound exhibits no resolution. However, the CBZ-protected derivative analogous to phenylglycine is separated at this system well. Resolution of the *N*-CBZ-compound analogous to phenylalanine was performed with methyl-β-cyclodextrin (Fig. 8).

4. Conclusions

All investigated α-aminophosphonic and α-aminophosphinic acid esters were resolved into the stereoisomers by HPLC by variation of CSPs and the eluent. Optimisation of the resolution of the corresponding acids by CE was achieved by changing type and concentration of β-cyclodextrin derivatives and the pH of the run buffer.

Acknowledgements

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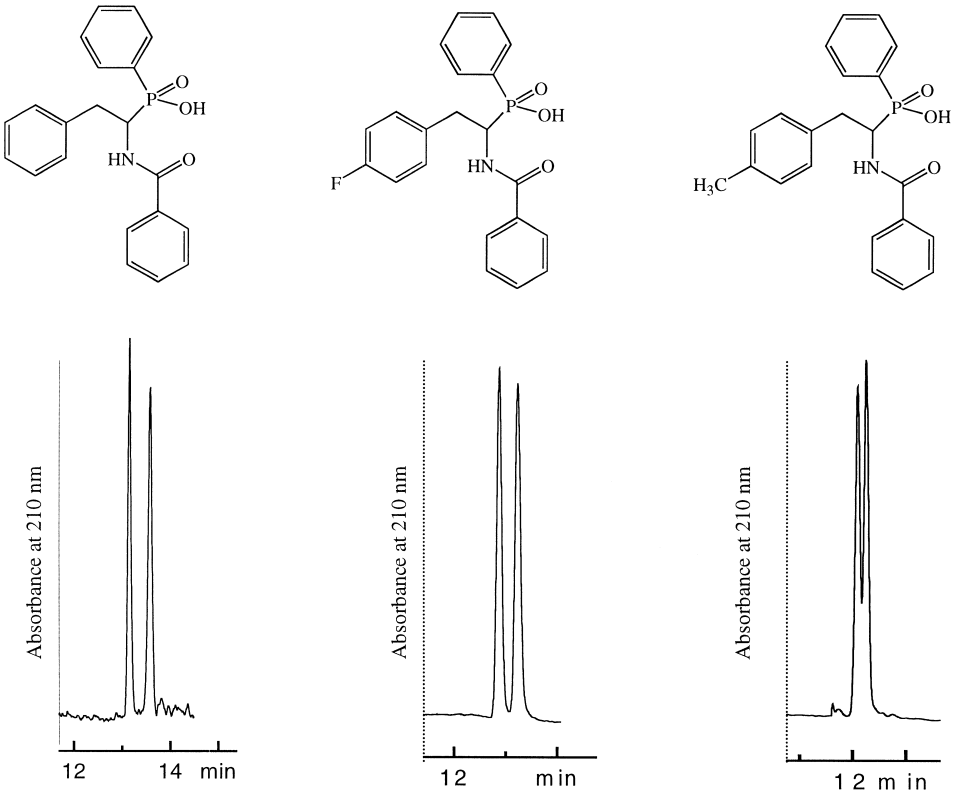

Fig. 7. Dependence of enantiomeric resolution on substituents at the phenyl residue. Run buffer: borate, 0.05% PVA, 15 m*M* β-cyclodextrin, run voltage: 10 kV, pH: 9.3.

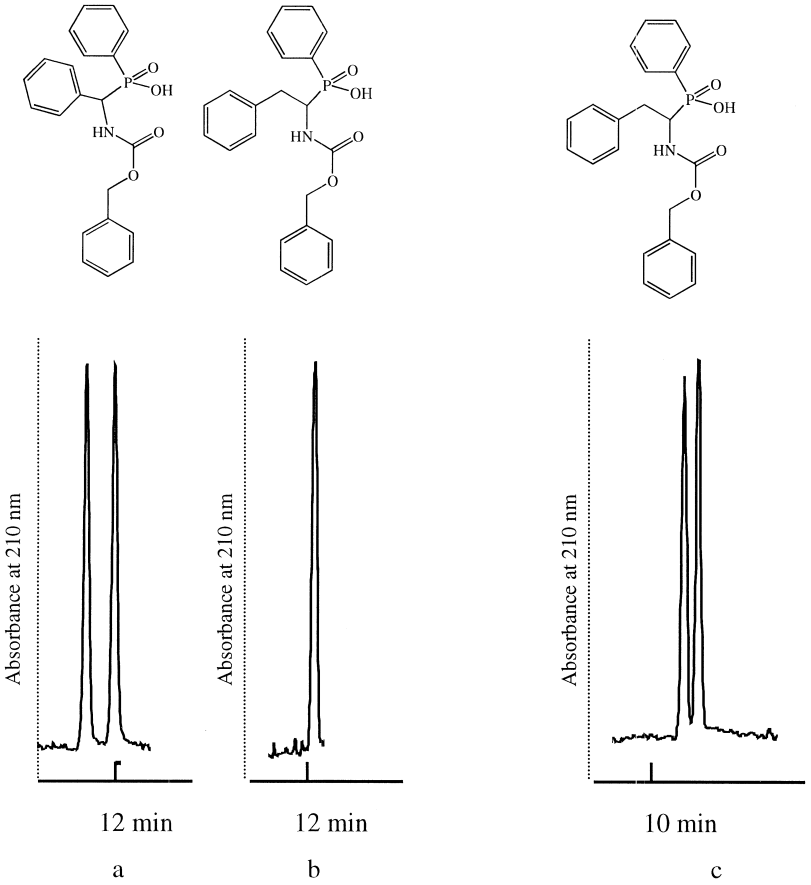

Fig. 8. Enantiomeric resolution of *N*-CBZ-aminophosphinic acids. Run buffer: borate, 0.05% PVA (a), (b) 10 m*M* β-cyclodextrin, (c) 10 m*M* methyl-β-cyclodextrin.

A. Simao for the racemic aminophosphonous acid and Mrs. A. Modler for technical assistance.

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