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High-performance liquid chromatographic enantiomer separation and determination of absolute configurations of phosphinic acid analogues of dipeptides and their α-aminophosphinic acid precursors

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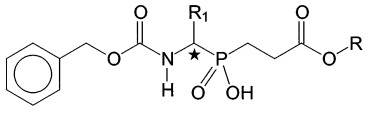
Abstract—The enantiomers of *N*-benzyloxycarbonyl-phosphinic pseudodipeptides and their *N*-benzyloxycarbonyl-α-aminophosphinic acid precursors as well as various other structural analogues were separated on a set of cinchona alkaloid-derived chiral anion-exchangers by HPLC in the reversed-phase mode. Semi-preparative scale chromatography provided single enantiomers in 100 mg quantities. The configurations of the enantiomers were assigned indirectly by enantioselective chromatography on the basis of the elution order and was confirmed by enantiomeric reference compounds.

1. Introduction

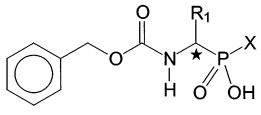
Phosphinic acid peptides, also called phosphinic pseudopeptides, are a class of compounds, which have a peptide bond substituted by a non-hydrolysable phosphinic acid moiety. Many of the members in this compound class are effective enzyme inhibitors, e.g. of Zn–metalloproteases, aspartic acid proteinases, serine proteinases and many others.[1] It is to be expected that the activity is not equal for the individual stereoiso-

(a)



R_1	R = CH_3	R = H
i-butyl	**1**	**5**
phenyl	**2**	**6**
benzyl	**3**	**7**
2-phenylethyl	**4**	**8**

(b)



R_1	X	
i-butyl	H	**9**
phenyl	H	**10**
benzyl	H	**11**
2-phenylethyl	H	**12**
phenyl	CH_3	**13**
phenyl	C_2H_5	**14**
phenyl	OH	**15**

Figure 1. Structures of (a) *N*-benzyloxycarbonyl-protected phosphinic acid pseudodipeptides **1**–**8** and (b) their precursors **9**–**12** as well as some structurally related reference compounds **13**–**15**.

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mers, but resides preferentially in one of them. The study of the stereospecificity of action of those compounds depends on methods that allow the preparative production of the individual stereoisomers in sufficient amount and stereochemical purity. In addition, stereoselective analytical assays are required for the determination of their enantiomeric purity as well as for stereoselective bioanalytical investigations.

Herein we report a stereoselective liquid chromatographic method for phosphinic pseudodipeptides employing cinchona-alkaloid derived chiral stationary phases. Since the target compounds leave the synthesis protocol as *N*-benzyloxycarbonyl-derivatives (Fig. 1a), these derivatives can be used directly without deprotection of either the N-terminal or C-terminal protecting group, although other *N*-derivatives, e.g. *N*-9-fluorenylmethoxycarbonyl (FMOC), *N*-3,5-dinitrobenzyloxycarbonyl or 2,4-dinitrophenyl derivatives are supposed to provide much better enantioselectivities than the Z-protecting group. The developed enantioselective chromatographic methods allowed us to separate the enantiomers of the target *N*-benzyloxycarbonyl-derivatives of phosphinic pseudopeptide esters **1**–**4** as well as the corresponding hydrolyzed compounds with free C-terminal carboxylic groups **5**–**8**, along with their aminophosphinic acid precursors **9**–**12** (Fig. 1). Furthermore, some other structurally related analogues **13**–**15** have been investigated as reference compounds (Fig. 1). For biological activity tests, the enantiomers of Z-Leuψ(PO_2HCH_2)GlyOMe **1** and Z-Phgψ(PO_2HCH_2)-GlyOMe **2** were prepared in 100 mg quantities by semi-preparative HPLC in the batch mode. The same chromatographic separation system was used for determining the enantiomeric purity of the prepared Z-phosphinic pseudodipeptide ester enantiomers. This also allowed us to indirectly assign the absolute configurations of the individual enantiomers, which was then verified with a sample obtained by a stereospecific synthesis. Thus, the proposed methods provide also a means for the control of the quality and effectiveness of stereoselective synthesis concepts of such chiral entities.

The presented methodologies should be of interest, since reports about enantiomeric separation of chiral aminophosphinic acids with free acidic functionality are scarce. Only a few separations of phosphinic acids and their derivatives have been published, e.g. CE with cyclodextrins as chiral additives to the background electrolyte.[2–4] Diastereomers of phosphinic pseudotetrapeptides N- and C-terminal amino acids with an (*S*)-configuration and two other configurations either (*R*) or (*S*)-have been separated by conventional CE without any chiral selector, for the purpose of determining the dissociation constants.[5,6]

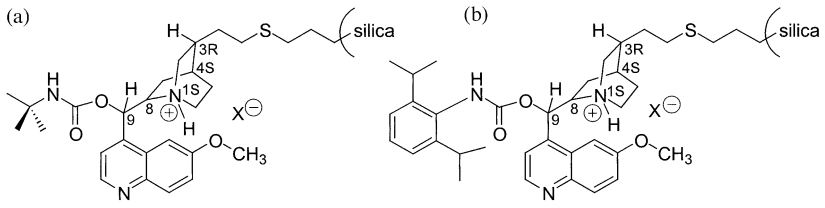
2. Results and discussion

2.1. Separation of phosphinic pseudodipeptide ester derivatives (monovalent acids)

The phosphinic acid group of the pseudo-dipeptides has a pK_a of $\sim$1.8.[5,6] It is dissociated at the mobile phase pH 5.6 and is thus negatively charged, whilst the quinuclidine of the cinchonan carbamate selectors (Fig. 2) is protonated and positively charged. Accordingly, an ion-exchange process can be established in which the quinuclidinium ion of the chiral selectors represents the fixed positive charge of the anion-exchange type CSP that interacts with the oppositely ionized solute by strong ionic interactions. This primary electrostatic interaction, which is active over a long distance and attracts the solute towards the ion-exchange binding site, is located in the center of a cleft-like hydrophobic binding pocket that is pre-formed by a number of lipophilic and bulky moieties, viz. planar quinoline ring, bulky *tert*-butyl residue at the carbamate group, and the rigid quinuclidine ring itself (Fig. 3a).[7] The polar interaction sites in the center of the binding site are thus embedded in a hydrophobic surrounding which can strengthen the electrostatics of the selector-analyte interactions.

N-Derived amino acids, e.g. 3,5-dinitrobenzoylated leucine, have been shown to fit favorably into this cleft of the binding pocket featuring besides H-bond-mediated ionic interaction, hydrogen bonding between the solute's amide and the selector's carbamate, as well as face-to-face π-π-interaction (Fig. 3a). The herein investigated pseudodipeptide derivatives may bind to the cinchonan carbamate by essentially the same binding mechanism (Fig. 3b) and can therefore also be separated into the enantiomers on the investigated cinchonan carbamate CSPs (see Table 1).

The additional P-alkyl group on the phosphorus atom of the phosphinic acid, as compared to carboxylic, phosphonic, or sulfonic acid analogues, however,



CSP 1: AX QD-1(8*R*,9*S*)
CSP 2: AX QN-1(8*S*,9*R*)
CSP 3: AX QD-2 (8*R*,9*S*)
CSP 4: AX QN-2 (8*S*,9*R*)

Figure 2. Structures of the investigated chiral anion-exchange type cinchonan-based chiral stationary phases.

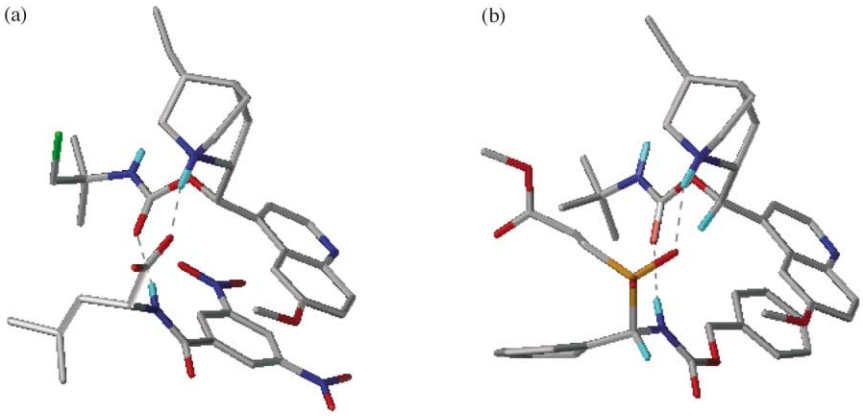


Figure 3. X-Ray crystal structure (a) and tentative binding model (b) of the selector-analyte ion-pair with the favorable stereochemistry for tight binding. (a) *O*-9-(β-Chloro-*tert*-butylcarbamoyl)quinidine in complex with (*R*)-3,5-dinitrobenzoyl leucine,[7] and (b) *O*-9-(*tert*-butylcarbamoyl)quinidine in complex with (*S*)-Z-Phgψ(PO_2HCH_2)GlyOMe, (*S*)-**2**.

Table 1. Chromatographic data of phosphinic pseudopeptides obtained with different anion exchange type cinchonan-based chiral stationary phases[a]

Compound	k_1	α	R_S	e.o.[b]	k_1	α	R_S	e.o.[b]
	CSP 1, *O*-9-(*tert*-butylcarbamoyl) quinidine				**CSP 2**, *O*-9-(*tert*-butylcarbamoyl) quinine			
1	3.22	1.15	1.76	(*R*)-(−)	1.91	1.08	1.14	(*S*)-(+)
2	7.07	1.17	2.18	(*R*)-(+)	5.62	1.18	2.53	(*S*)-(−)
3	4.11	1.00	–	–	3.21	1.00	–	–
4	4.79	1.12	1.48	(*R*)-(−)	4.76	1.03	0.41	(*S*)-(+)
5	4.07	1.00	–	–	8.70	1.00	–	–
6	17.02	1.08	0.95	(*R*)-(+)	18.53	1.13	1.63	(*S*)-(−)
7	7.69	1.11	1.14	(*S*)-(+)	11.32	1.11	1.22	(*R*)-(−)
8	11.74	1.00	–	–	13.57	1.00	–	–
	CSP 3, *O*-9-(2,6-diisopropylphenylcarbamoyl) quinidine				**CSP 4**, *O*-9-(2,6-diisopropylphenylcarbamoyl) quinine			
1	1.59	1.08	<0.5	(*R*)-(−)	3.42	1.06	0.62	(*S*)-(+)
2	3.34	1.05	<0.5	(*R*)-(+)	6.47	1.14	1.49	(*S*)-(−)
3	1.98	1.32	2.40	(*R*)-(−)	4.15	1.20	1.89	(*S*)-(+)
4	2.60	1.06	<0.5	(*R*)-(−)	5.56	1.00	–	–
5	4.82	1.09	0.84	(*R*)-(−)	5.39	1.00	–	–
6	11.70	1.09	<0.5	(*R*)-(+)	11.81	1.15	1.95	(*S*)-(−)
7	5.81	1.36	2.69	(*R*)-(−)	5.79	1.47	5.62	(*S*)-(+)
8	8.56	1.05	<0.5	(*R*)-(−)	9.10	1.00	–	–

[a] Column dimensions, 150×4 mm I.D.; mobile phase, methanol–50 mM sodium phosphate buffer (80:20; v/v) (pH_a 5.6); temperature, 40°C; flow rate, 1 mL/min; UV detection, 250 nm.
[b] e.o., elution order, i.e. assigned configuration of the first eluted enantiomer.

appears to put larger steric demands on the size of the binding pocket which is to a great extent defined in the relatively rigid selector molecule. The additional voluminous residue fills the empty space in the binding pocket of the complex meaning it can more easily collide with other bulky groups with the result being detrimental for effective molecular recognition and chiral separation. Indeed, both retention factors of the second eluted enantiomer, i.e. the high-affinity enantiomer, as well as the enantioselectivity constantly decreased in the order H>CH_3>C_2H_5 as the X-substituent within the series of comparable Z-PhgPO_2H-X homologues (see Fig. 1b; with R_1 being a phenyl group). For example, on the *tert*-butylcarbamoyl-quinidine CSP (Chiral AX QD-1, **CSP 1**) the retention factors k_2 diminished from 8.51 for **10** over 7.34 for **13** to 7.10 for **14**, while enantioselectivity values α declined in the same order from 1.28 over 1.22 to 1.11 (Table 2). The corresponding target phosphinic pseudodipeptide analogue **2** with X=2-(methoxycarbonyl)ethyl was a little better separated with $\alpha=1.17$ ($k_1=7.07$, $k_2=8.29$, $R_S=2.18$) than the ethyl group-bearing analogue **14**. On the other hand, the replacement of H by OH as the X-substituent (Fig. 1b) led to the bis-protic phosphonic acid congener **15**, which in accordance with the ion-exchange mechanism showed a stronger retention ($k_1=12.43$) than the phosphinic acid **10** ($k_1=6.65$) with minor effect on enantioselectivity

$\alpha = 1.30$ ($R_S = 2.63$) when compared to **10** ($\alpha = 1.28$, $R_S = 3.23$) (Table 2). Similar trends were also observed on the other investigated CSPs.

Although in the tentative binding models (Fig. 3b) it appears that the alkyl, arylalkyl and phenyl groups, respectively, at the stereogenic center of the Z-protected phosphinic pseudodipeptide esters point towards free space, they make a significant contribution to the binding and enantioselectivity. Besides different solvation effects, the aminophosphinic acid residue can come into contact with the bulky carbamate residues and interact with this moiety by steric and/or Van der Waals type interactions. Alternatively, in the hydro-organic environment this binding contribution can also be interpreted as a hydrophobic interaction with significant effect on observed enantioselectivities. For example, for both the *tert*-butylcarbamoylated CSPs, the α values

Table 2. Chromatographic data of phosphinic acid precursors and structurally related compounds obtained with different anion-exchange type cinchonan-based chiral stationary phases[a]

Compound	k_1	α	R_S	e.o.[b]	k_1	α	R_S	e.o.[b]
	CSP 1, *O*-9-(*tert*-butylcarbamoyl) quinidine				**CSP 2**, *O*-9-(*tert*-butylcarbamoyl) quinine			
9	3.64	1.19	2.15	(*R*)-(−)	1.86	1.14	1.36	(*S*)-(+)
10	6.65	1.28	3.23	(*R*)-(+)	6.26	1.23	3.40	(*S*)-(−)
11	4.96	1.04	0.5	(*R*)-(−)	4.57	1.00	1.00	–
12	5.56	1.18	2.21	(*R*)-(−)	5.37	1.10	1.33	(*S*)-(+)
13	6.02	1.22	2.10	(*R*)-(+)	5.63	1.21	2.81	(*S*)-(−)
14	6.40	1.11	1.15	(*R*)-(+)	6.17	1.14	1.90	(*S*)-(−)
15	12.43	1.30	2.63	(*R*)-(+)	12.37	1.23	2.77	(*S*)-(−)
16[c]	3.16	1.25	2.62	(*S*)-(−)	4.28	1.19	2.86	(*R*)-(+)
17[c]	7.00	1.14	1.46	(*S*)-(+)	7.92	1.13	1.91	(*R*)-(−)
18[c]	5.82	1.24	2.83	(*S*)-(+)	7.17	1.15	2.41	(*R*)-(−)
	CSP 3, *O*-9-(2,6-diisopropylphenylcarbamoyl) quinidine				**CSP 4**, *O*-9-(2,6-diisopropylphenylcarbamoyl) quinine			
9	2.60	1.21	2.51	(*R*)-(−)	3.53	1.18	2.19	(*S*)-(+)
10	4.69	1.25	3.22	(*R*)-(+)	6.31	1.27	3.04	(*S*)-(−)
11	3.51	1.46	4.75	(*R*)-(−)	5.00	1.38	4.76	(*S*)-(+)
12	4.30	1.23	2.55	(*R*)-(−)	6.43	1.16	2.02	(*S*)-(+)
13	4.40	1.13	1.76	(*R*)-(+)	6.37	1.18	2.06	(*S*)-(−)
14	4.90	1.04	<0.5	(*R*)-(+)	7.21	1.14	1.58	(*S*)-(−)
15	7.03	1.27	3.27	(*R*)-(+)	10.35	1.26	3.12	(*S*)-(−)
16[c]	3.63	1.26	3.43	(*S*)-(−)	3.58	1.22	2.63	(*R*)-(+)
17[c]	7.43	1.03	<0.5	(*S*)-(+)	8.71	1.08	0.97	(*R*)-(−)
18[c]	6.28	1.45	4.62	(*S*)-(+)	6.81	1.39	4.69	(*R*)-(−)

[a] Column dimensions, 150×4 mm I.D.; mobile phase, methanol–50 mM sodium phosphate buffer (80:20; v/v) (pH_a 5.6); temperature, 40°C; flow rate, 1 mL/min; UV detection, 250 nm.

[b] e.o., elution order; assigned configuration of the first eluted enantiomer.

[c] **16**, *N*-benzyloxycarbonyl-leucine; **17**, *N*-benzyloxycarbonyl-phenylglycine; **18**, *N*-benzyloxycarbonyl-phenylalanine.

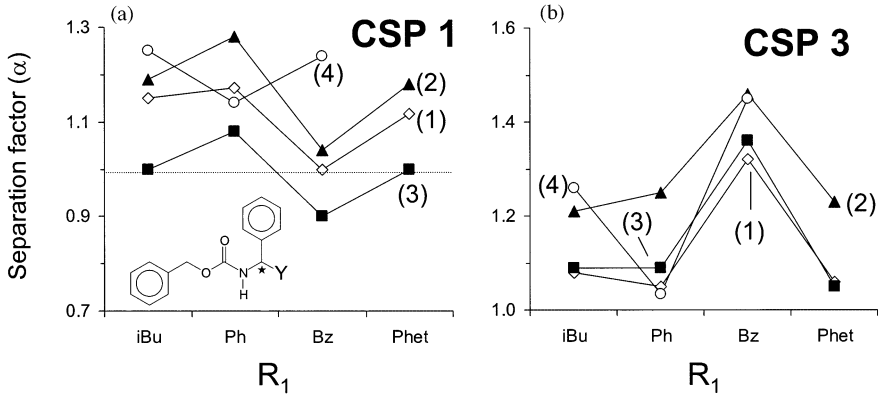


Figure 4. Effect of the side chain residue R_1 on enantioselectivity α of various phosphinic acid derivatives and carboxylic acid analogues on **CSP 1** (a) and **CSP 3** (b). (1) Y = -$PO_2H(CH_2)_2COOCH_3$, (2) Y = -PO_2H_2, (3) Y = -$PO_2H(CH_2)_2COOH$, and (4) Y = -COOH. R_1: iBu = isobutyl, Ph = phenyl, Bz = benzyl, Phet = 2-phenethyl. The separation factor α is here defined as $k_{(S)}/k_{(R)}$ yielding a value below 1 for compound **7** with Bz residue and Y = -$PO_2H(CH_2)_2COOH$ on **CSP 1**, which indicates reversed elution order compared to the other compounds of the series (vide supra). For the experimental conditions see Tables 1 and 2.

decrease in the order phenyl>isobutyl>2-phenylethyl> benzyl (see also Fig. 4a, curve 1). Most remarkably the latter, i.e. the benzyl congener, which had not been separated under the given conditions on either of the both *tert*-butyl carbamate phases, is the solute yielding the highest enantioselectivity value on both the diisopropylphenyl carbamate CSPs (Fig. 4b, curve 1). This is a typical observation for bis-aromatic compounds such as *N*-benzyloxycarbonyl and *N*-benzoyl α-amino acids with a benzyl side chain on the diisopropylphenyl cinchonan carbamate CSPs also featuring two aromatic binding sites. While the α values of the amino acids with aliphatic residues are substantially reduced on the diisopropylphenyl phase when compared to the *tert*-butyl CSP analogue, enantioselectivity, e.g. bis-aromatic Z-Phe or Bz-Phe, is enhanced. This can be explained by a spatial fit of the aromatic moieties for a simultaneous π-π-interaction between the quinoline ring and the Z-group, as well as the diisopropylphenyl residue and benzyl side chain. Improper spatial requirements for simultaneous bi-valent π-π-interaction of the aromatic moieties in the corresponding phenyl and 2-phenethyl-congeners in contrast led to a substantial loss in the chiral recognition ability of the diisopropylphenyl cinchonan carbamate selectors for these two solutes. Here it is noteworthy that on both the *tert*-butyl and 2,6-diisopropylphenyl carbamate CSPs, the entire series of the phosphinic acid precursors (Fig. 4a and b, curve 2) yielded higher enantioselectivities than the corresponding phosphinic pseudodipeptide ester congeners (curve 1).

Compounds **1** and **4** showed the highest degree of resolution on the *tert*-butyl carbamoyl quinidine CSP (**1**, $\alpha = 1.15$; $R_S = 1.8$ and **4**, $\alpha = 1.12$; $R_S = 1.5$), compound **2** the best enantiomer separation on the *tert*-butyl carbamoyl quinine CSP (**2**, $\alpha = 1.18$; $R_S = 2.5$ but also sufficient resolution on the corresponding pseudo-enantiomeric quinidine analogue CSP 1; **2**, $\alpha = 1.17$; $R_S = 2.2$), while compound **3** on the other hand was considerably better resolved on the 2,6-diisopropylphenyl carbamoyl quinidine CSP (**3**, $\alpha = 1.32$; $R_S = 2.4$) (Table 1). Remarkably, only the 2,6-diisopropylphenyl carbamoyl quinidine CSP revealed an enantiomer separation capability for all 4 ester derivatives **1**–**4**, although with exception of compound **3** only at low levels (R_S typically <0.5). This clearly agrees with our earlier findings that the cinchonan carbamate CSPs with the 2,6-diisopropylphenyl residue show broader enantioselectivity than the corresponding *tert*-butyl analogues which however afford higher enantioselectivity for many classes of compounds. Representative chromatograms of the phosphinic pseudodipeptide ester derivatives are depicted in Figure 5 and of the corresponding aminophosphinic acid precursors in Figure 6.

The chromatographic results for the phosphonic pseudopeptides in Table 1 as well as those for the precursors and analogues in Table 2 moreover largely reflect the expected pseudo-enantiomeric behavior of quinidine and quinine carbamate selectors as indicated by the reversal of the elution order on the corresponding Chiral AX QD (CSP **1** and **3**) and QN CSPs (CSP **2** and **4**). However, it became quite evident that the corresponding quinidine and quinine selectors are not true enantiomers, but actually diastereomers (opposite configurations at C_8 and C_9, but identical configurations at the stereogenic centers at N_1, C_3, and C_4). The α values of the quinidine and quinine CSP counterparts, though exhibiting mostly similar levels of enantioselectivity, are not equal as would be expected for real enantiomers on the CSPs with similar selector loadings. The selectivity differences between pseudo-enantiomeric counterparts once more underlines the stringent demands for the steric and functional fit of the binding geometries of the selectors.

On the basis of our binding model for *N*-carbonylated amino acids shown in Figure 3, which is not only backed by X-ray crystal structures of selector-analyte

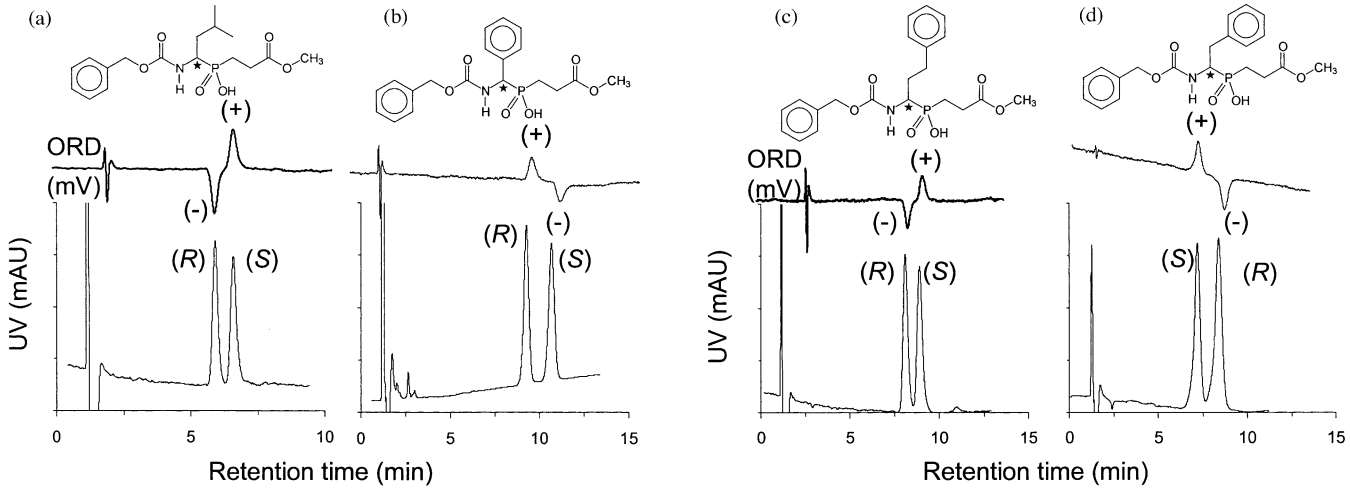


Figure 5. Chromatograms of the direct HPLC enantiomer separation of compounds **1** (a), **2** (b), and **4** (c) on *O*-9-(*tert*-butylcarbamoyl) quinidine based **CSP 1**, and (d) compound **3** on *O*-9-(2,6-diisopropylphenylcarbamoyl) quinine based **CSP 4**. For the experimental conditions, see Table 1.

co-crystallisates.[7] but also by solution-phase NMR as well as molecular modeling experiments (molecular dynamic simulations) on selector–analyte complexes,[8] the absolute configuration of the first eluted enantiomer of the Z-aminophosphinic acid derivatives was chromatographically assigned to be (*R*) on the quinidine phase, taking into account the change in CIP priorities caused by the phosphor atom. As a tacit assumption, a homology principle between the *N*-carbonylated amino acids like dinitrobenzoyl or benzyloxycarbonyl amino acids and herein investigated *N*-benzyloxycarbonylated aminophosphinic acids was thereby assumed. Moreover, it was supposed that the overall primary binding and chiral recognition mechanism does not change significantly when compared to the *N*-carbonylated amino acids derivatives so that the tentative binding model shown in Figure 3b still holds. Thus, it can be seen that the (*S*)-enantiomer of **2** fits very well with the favorable binding requirements and is therefore able to exhibit a higher affinity towards the quinidine carbamate selector. It should hence be eluted second. To verify this the (*R*)-enantiomer of Z-Phgψ(PO_2HCH_2)GlyOMe **2** was prepared by stereoselective synthesis. As predicted the (*R*)-enantiomer eluted first on the quinidine CSP, whilst the (*S*)-enantiomer showed a higher affinity to the selector confirming the validity of the chromatographic assignment of the absolute configurations of aminophosphinic acids, as previously demonstrated for amino phosphonic acids.[9] The change of the specific rotation within the series for the Z-phosphinic pseudodipeptide ester with a phenyl group as the R_1 residue (compound **2**) originated from structure (chromophore) induced reversal of the sign of rotation and does not reflect a reversal in the elution order, as could have been argued before verification with the enantiomerically pure **2**. Moreover, the results with the enantiomeric precursors, viz. (*R*)-(−)-**9**, (*R*)-(+)-**10**, and (*R*)-(−)-**11** are fully in line with the above assignment (see Table 2) and also confirm the correctness of the chromatographic determination of the absolute configurations for the investigated aminophosphinic acid derivatives.

2.2. Separation of phosphinic pseudodipeptides with free carboxylic acid at the C-terminus (bis-acidic compounds)

By hydrolysis of the esters **1**–**4** the bis-acidic phosphinic pseudodipeptides **5**–**8** with free C-terminal carboxylic group are obtained. A second acidic group in the analyte molecules has implications for the separation mechanism, as it can compete with the phosphinic acid group for binding at the fixed ionic site of the CSPs, and thus scramble the above discussed chiral recognition mechanism. However, the phosphinic acid group is more acidic and so is preferred as the negatively ionized binding site. Also the existence of topologically favorable additional interactions such as the H-bond between analytes' carbamate N–H and the selector's carbamate carbonyl can be used as strong arguments in favor of adherence to the discussed binding mechanism even in the hydrolyzed analytes. Accordingly, the phosphinic acid group is supposed to dominate the chiral recognition mechanism, while the additional carboxylic group merely adds a significant retention increment.

The chromatographic data presented in Table 1 underpins this hypothesis. There is only one exception to this general rule within the series: Compound **7**, which features a benzyl side chain residue, shows a reversed elution order on both of the *tert*-butyl carbamoylated CSPs (**CSP 1** and **CSP 2**), but not on the 2,6-diisopropylphenyl carbamoylated analogues (**CSP 3** and **CSP 4**). This can be explained by a more favorable arrangement of the solute upon primary ionic interaction with the carboxylic group, supported by additional other interactions such as π-π-interaction with the aromatic group of the side chain instead of the phenyl ring of the protection group. The fact that both pairs of interaction sites, i.e. the phosphinic acid group and the phenyl of the Z-protection group as well as the C-terminal carboxylic group and phenyl of the side chain, are separated by five atoms and thus topologically very similar, can be invoked to indicate a competitive binding mode. Since single enantiomers of **7** or **3** were not

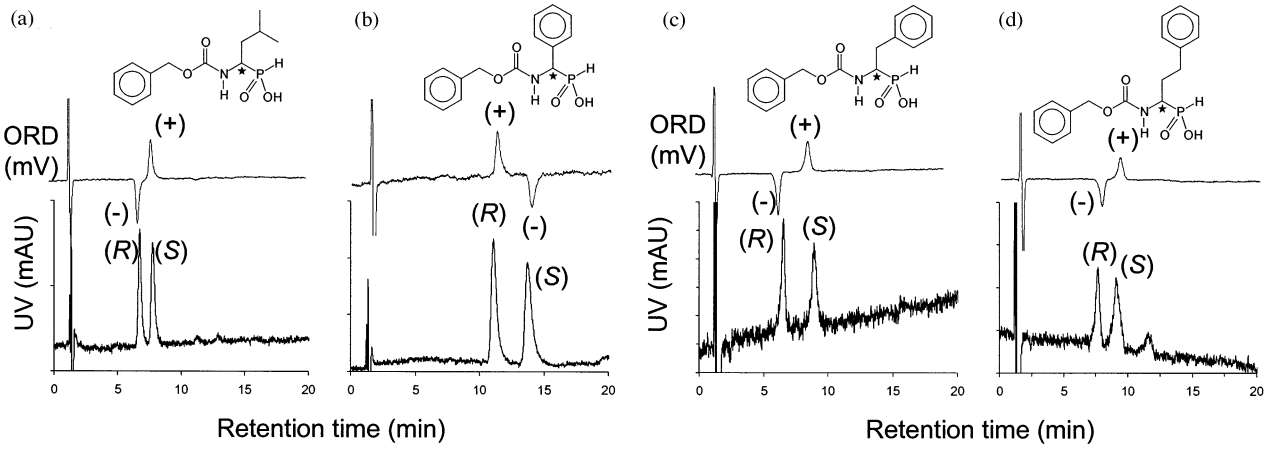


Figure 6. Chromatograms of the direct HPLC enantiomer separation of compounds **9** (a) and **10** (b) on *O*-9-(*tert*-butylcarbamoyl) quinidine based **CSP 1**, and of compounds **11** (c) and **12** on *O*-9-(2,6-diisopropylphenylcarbamoyl) quinidine based **CSP 3**. For the experimental conditions, see Table 1.

available, the confirmation of the reversed elution order was carried out chromatographically. Compound **3** was separated chromatographically on a semi-preparative scale on **CSP 4** providing single enantiomers of **3**. The first eluted enantiomer was assigned to be the (*S*)-(+)-enantiomer and was after hydrolysis to (*S*)-(+)-**7** with 1 M LiOH (ca. 3 h at 60°C) re-injected on **CSP 2**, with a racemic sample as a reference. Thus it was clearly seen that the (*S*)-(+)-enantiomer eluted after the (*R*)-(−)-enantiomer of **7** on **CSP 2**, which means that the elution order was reversed on both of the *tert*-butyl carbamate phases when compared to all the other resolved phosphinic pseudodipeptide esters and di-acid solutes of the series due to different binding and chiral recognition mechanisms.

In any case, the retention factors of the bis-acidic compounds **5**–**8** were increased on all CSPs compared to the corresponding ester analogues (Table 1), which is very much in accordance with the primary anion-exchange retention mechanism. With the exception of **7,** all the compounds of the series with the hydrolyzed ester group (Fig. 4, curve 3) are separated with lower α values than the corresponding esters (Fig. 4, curve 1) on **CSP 1** and **CSP 2**. In contrast, on **CSP 3** the difference in enantioselectivities for the ester and carboxylic acid pseudodipeptides are more or less negligible (Fig. 4b, curves 1 and 3). Noticeably, on all four CSPs, compound **7**, with the benzyl side chain at the stereogenic center, was resolved with the highest selectivity, e.g. 1.47 ($R_S = 5.62$) on the 2,6-diisopropylphenylcarbamoyl quinine-based **CSP 4**. Compound **6** with the phenyl ring as the side chain also gave the highest resolution on **CSP 4**, but was separated with a significantly lower separation factor ($\alpha = 1.15$) and resolution ($R_S = 1.95$), while compounds **5** and **8** with the isobutyl and 2-phenylethyl residue, respectively, were only poorly separated under the given conditions and solely on **CSP 3** (e.g. **5**, $\alpha = 1.09$ and $R_S = 0.84$; **8**, $\alpha = 1.05$ and $R_S < 0.5$). In this context it must be noted, however, that the conditions have not been specifically optimized for the present compounds. On the contrary, standard conditions as established previously for aminophosphonic acid derivatives have been utilized throughout.[9] Hence, better separations could be obtained, in particular for the bis-acidic compounds after a systematic optimization of the major influential experimental variables such as pH, eluent modifier and type, temperature, and flow rate. However this would have been beyond the focus of the present investigation.

2.3. Semi-preparative enantiomer separation and application for enantiomeric excess determination

Compounds **1** and **2** were separated on a semi-preparative scale on the *tert*-butylcarbamoylquinidine **CSP 1**. Despite high loading (ca. 40 mg) the resolution was still satisfactory (see Fig. 7). No advanced fraction collection and peak shaving technology was utilized, but only two fractions corresponding to the first and second eluted enantiomer were collected with a cut in the peak valley. The direct chromatographic enantiomeric purity control afforded an enantiomeric excess ee of 98% for the first eluted (*R*)-enantiomer and 82% for the second

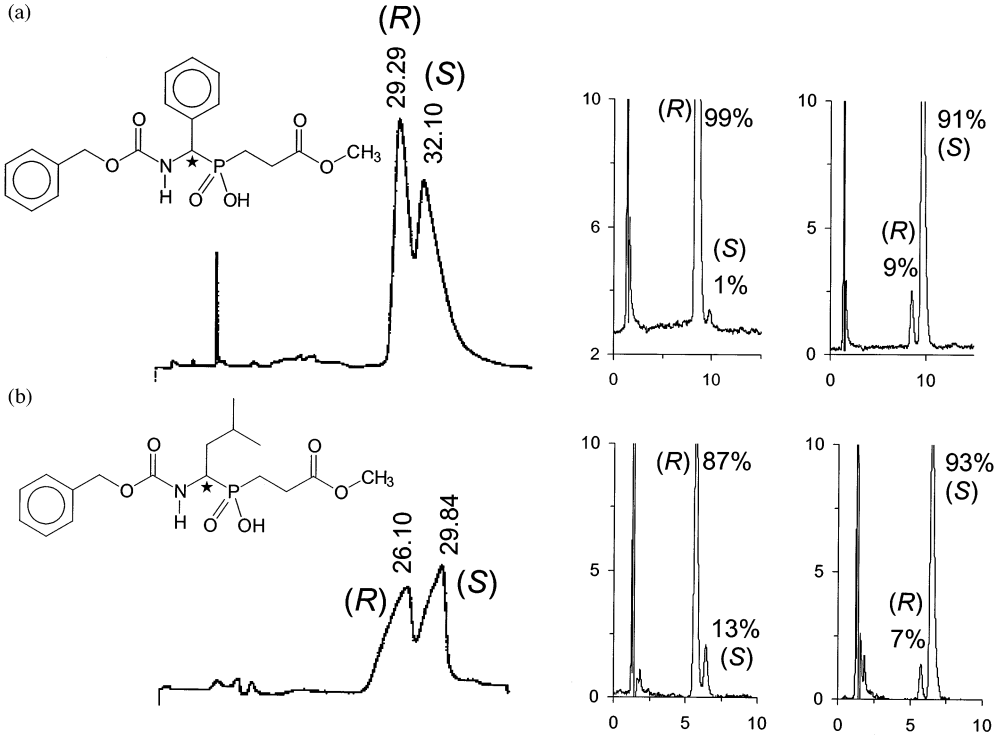


Figure 7. Semi-preparative separation of compound **2** (a) and compound **1** (b) on *O*-9-(*tert*-butylcarbamoyl) quinidine based **CSP 1** (column dimension, 250×16 mm I.D.) and analytical control of the enantiomeric purity of the individual fractions. For other conditions see Section 4.

eluted (*S*)-enantiomer of Z-Phgψ(PO_2HCH_2)Gly-OMe **2** (see Fig. 7a) as well as an ee of 74% for the first eluted (*R*)-enantiomer and 86% for the second eluted (*S*)-enantiomer of Z-Leuψ(PO_2HCH_2)Gly-OMe **1** (Fig. 7b). The modest ee values for the second eluted enantiomer of **2** and the first eluted enantiomer of **1** has to be sought in the tailing and fronting, respectively, of the peaks during semi-preparative chromatography. If a higher enantiomeric purity is required, recycling of a middle fraction is strongly recommended and certainly allows the production of enantiomers in ee exceeding 98%.

Figure 8 on the other hand shows the chromatograms monitored in the course of the enantiomeric purity determination of the enantiomeric reference compound (*R*)-(+)-**2** that was prepared by stereoselective synthesis. It can be seen that the compound has a high ee (ca. 98%). The fact that the impurity is eluted in front of the main component still allows its correct quantitation. Also the *N*-benzyloxycarbonyl-α-aminophosphinic acids that were used as enantiomeric reference compounds and precursors showed high enantiomeric excess, namely 98%, >98%, and >99% ee for (*R*)-(−)-**9**, (*R*)-(−)-**11**, and (*R*)-(+)-**10**, respectively.

3. Conclusion

The concept of enantioselective anion-exchange employing quinidine and quinine carbamate CSPs turned out to be highly suitable for the enantiomer separation of the target phosphinic pseudopeptides as well as their α-aminophosphinic acid precursors. Obviously, these test compounds largely adhered to a selector-analyte binding and chiral recognition mechanism which was previously proposed for *N*-carbonylated amino acid derivatives and was thoroughly validated spectroscopically. Hence, the separation method could be exploited for the chromatographic determination of the absolute configurations of the *N*-derivatized aminophosphinic acids and phosphinic pseudodipeptides on the basis of the tentative binding model previously proposed for *N*-carbonylated amino carboxylic and phosphonic acids. These results have been confirmed with enantiomeric samples that were obtained by stereoselective synthesis. The presented enantioselective chromatographic method thus proved its usefulness in analytical and preparative enantiomer separation of phosphinic acid derivatives and their stereochemical description as well as enantiomeric purity control.

4. Experimental

4.1. Materials

The *N*-benzyloxycarbonyl-α-aminophosphinic acid pseudodipeptide ester derivatives **1**–**4** depicted in Figure 1 were synthesized by a Michael addition reaction of methyl acrylate to the corresponding Z-protected 1-aminoalkylphosphinic acid preactivated into its trivalent silyl ester according to the procedure described.[10] This synthesis yielded compounds **1**–**4** as racemates. Compound **2** was also synthesized in its enantiomeric form by the same reaction protocol but starting from

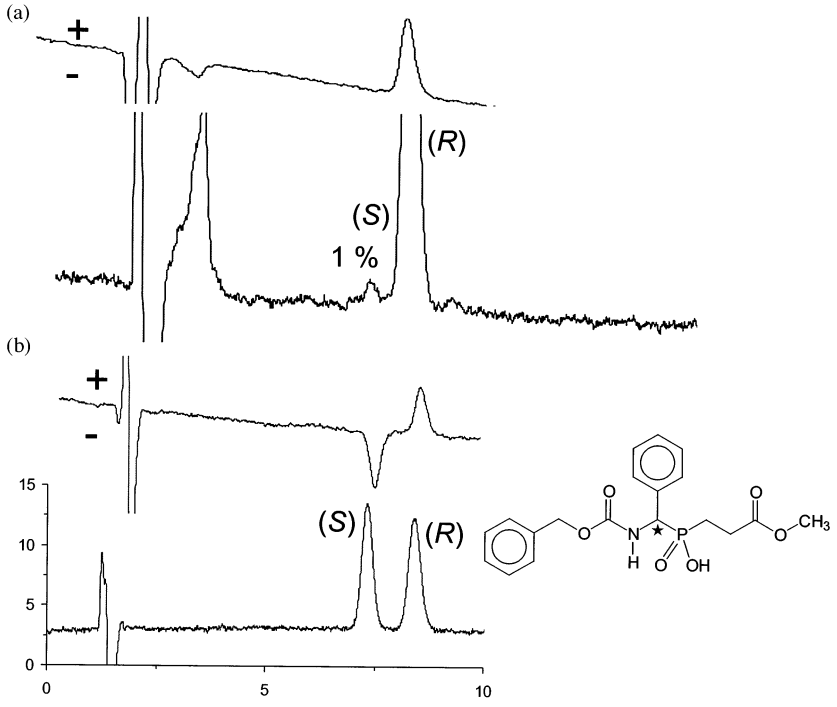


Figure 8. Enantiomeric purity determination of (*R*)-(+)-**2** synthesized by a stereoselective method on *O*-9-(*tert*-butylcarbamoyl) quinine based **CSP 2**, on which the enantiomeric impurity is eluted in front of the main enantiomer peak. For the experimental conditions, see Table 1.

the (*R*)-(+)-enantiomer of *N*-benzyloxycarbonyl-1-amino-1-phenylmethylphosphinic acid **10**, which was obtained by fractional crystallization of racemic **10** as diastereomeric salts with (*R*)-(+)-1-phenylethylamine as previously published.[11,12] Similarly, the (*R*)-(−)-enantiomers of **9** and **11** were obtained from the corresponding racemates and (*R*)-(+)-1-phenylethylamine, and the racemic *N*-benzyloxycarbonyl-1-aminoalkylphosphinic acids **9**–**12** by a procedure described previously.[11] Compounds **13**–**15** were from a previous study.[2] The bisacidic phosphinic pseudodipeptide compounds **5**–**8** were obtained by hydrolysis of the corresponding Z-phosphinic acid pseudodipeptide esters **1**–**4** with lithium hydroxide. Thus, 10 mg of the respective compound **1** to **4** were dissolved in 1 mL 1 M lithium hydroxide and kept at 60°C for 3 h. The HPLC control of the hydrolysis showed the reaction had gone to completion after 2.5 h.

The chiral stationary phases, Chiral AX QD-1, QN-1, QD-2, and QN-2 (Fig. 2) were from Bischoff Chromatography (Leonberg, Germany) and were also commercially available from Iris Technologies (Lawrence, KS, USA). Methanol was of HPLC grade and supplied by Merck (Darmstadt, Germany). Sodium dihydrogen phosphate of analytical grade was employed to prepare the buffer and was also from Merck. The pH of the mobile phase was adjusted with phosphoric acid thus representing the apparent pH (pH_a) of the hydro-organic mixture. The mobile phase was filtered through a 0.2 μm Nylon membrane filter and degassed by sonication prior to use.

4.2. Instrumentation and HPLC method

HPLC experiments were performed with a Hitachi–Merck HPLC system which consisted of a L-7100 intelligent pump, L-7400 UV–vis detector, L-7200 autosampler, D-7000 interface, HSM 7000 chromatography data station software from Merck (Darmstadt, Germany) and a Jasco OR-990 optical rotation detector (Jasco, Groß-Umstadt, Germany). The HPLC column was thermostated at 40°C with a column thermostat from W.O. Electronics (Langenzersdorf, Austria). Column dimensions were 150×4 mm I.D. The mobile phase consisted of methanol–50 mM sodium dihydrogen phosphate buffer (80:20; v/v) (pH_a 5.6). The flow rate for the analytical runs was 1 mL/min. The phosphinic acid derivatives were dissolved in a mobile phase at a concentration of ca. 1 mg/mL where 20 μL of the sample solution were injected. The detection wavelength was set at 250 nm.

4.3. Semi-preparative chromatography

Compounds **1** and **2** (see Fig. 1) were separated into the enantiomers on a semi-preparative scale employing a Chiral AX QD-1 column (Bischoff Chromatography, Leonberg Germany) containing **CSP 1**, 15 μm particles (column dimension 250×16 mm I.D.) using the same mobile phase as for analytical scale separations at a flow rate of 4.5 mL/min and ambient temperature. The amount injected onto the column per run was 35 mg of **1** and 45 mg of **2**. Both the enantiomers were collected separately and the pooled product fractions evaporated to dryness. The residue was dispersed in 1 M hydrochloric acid had been dried with the phosphinic dipeptide ester extracted with ethyl acetate. After the organic phase had been dried with sodium sulfate and the solvent evaporated, a white crystalline product was obtained after stirring with petroleum ether.

Acknowledgements

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