Amino Acids (1993) 4: 157–167

Amino
Acids


# High-performance liquid chromatographic resolution of (*R*, *S*)-α-alkyl-α-amino acids as diastereomeric derivatives

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Accepted August 10, 1992

**Summary.** A number (27) of racemic α-alkyl-α-amino acids (AAA) were derivatized either with *o*-phthaldialdehyde (OPA) in combination with *N*-*t*-butoxycarbonyl-L-cysteine (Boc-Cys) or *N*-acetyl-L-cysteine (Ac-Cys), or with $N^2$-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide (Marfey's reagent). The resolution of the diastereoisomers formed was investigated by reversed-phase ($C_{18}$) high-performance liquid chromatography (HPLC) using gradient elution conditions employing sodium phosphate buffers of pH 7.2 together with acetonitrile, and fluorescence detection at 344 nm (excitation) and 443 nm (emission) for the OPA/Boc-Cys or OPA/Ac-Cys derivatives. For the diastereomers formed by derivatization with Marfey's reagent triethylammonium phosphate buffers of pH 3.0 (pH 7.2 for acidic AAA) together with acetonitrile, and u.v. detection at 340 nm were used. Whereas with Marfey's reagent all diastereomers of AAA showed complete, or almost complete, resolution, only 8, or 11, respectively of the diastereomers formed by derivatization with OPA/Boc-Cys or OPA/Ac-Cys were resolved under the chromatographic conditions used.

**Keywords:** Amino acids – Nonprotein amino acids – α-Alkyl-α-amino acids – High-performance liquid chromatography – Enantiomer separation – *o*-Phthaldialdehyde – *N*-Acetyl-L-cysteine – *N*-*t*-Butoxycarbonyl-L-cysteine – Marfey's reagent

## Introduction

Nonprotein α-alkyl-α-amino acids (AAA) of the structural type $H_2NC(R^1R^2)COOH$ ($R^1$, $R^2$ = alkyl, alkaryl; $R^1$, $R^2 \neq H$) are of interest since they have been found to be constituents of polypeptide antibiotics of the peptaibiotic family (Brückner et al., 1991). Some AAA exert reversible inhibitory action on amino acid decarboxylases, and α-methyldopa is used therapeutically as an enzyme

inhibitor (Smirk, 1963)). Very recently, a lowering of serum triglycerides in mice by oral administration of (*R*, *S*)-α-butyl-α-amino-*n*-butyric acid (α-But-Abu) and (*R*, *S*)-α-pentyl-α-amino-*n*-butyric acid (α-Pent-Abu) was reported (Lubec et al., 1991). The most simple AAA, namely α-aminoisobutyric acid (Aib), has been employed as an unmetabolizable amino acid in resorption studies (Riggs and Walker, 1960). Furthermore, owing to their ability to withstand proteolytic cleavage of peptide-bonded AAA (Payne et al., 1970) and to stabilize α-helical (Schmitt and Jung, 1985) or $3_{10}$-helical (Toniolo and Benedetti, 1991) secondary structures, AAA have also attracted attention as building blocks for peptide drug design (Cordopatis and Theodoropoulos, 1983; Cann et al., 1987). As a result of its natural microheterogeneity and helical structure, the Aib-peptide paracelsin (Przybylski et al., 1984) has served as a probe for the retention behaviour of reversed-phase stationary phases in HPLC (Lork et al., 1989). As a consequence of the bulky $C^{\alpha}$-side chain groups of AAA, a drastically reduced reactivity was observed in peptide bond formation (Schmitt and Jung, 1985), and peptides containing AAA have served as models for testing novel peptide bond forming reagents (Altherr and Heimgartner, 1991; Coste et al., 1990). Steric constraints are also responsible for the much lower derivatization yields of AAA in comparison to α-amino acids (AA) in derivatization procedures (Cronin et al., 1979; Brückner et al., 1987; Duchateau et al., 1989), and the $C^{\alpha}$-hydrogen substitution by bulky groups is the cause of the relatively lower resolution factors observed in the analytical separation of AAA enantiomers. The use of chiral AAA for peptide syntheses in place of the most frequently employed nonchiral Aib, however, necessitates reliable methods for either their stereoselective synthesis (Seebach et al., 1983; Schöllkopf, 1985) or the preparative resolution of racemic mixtures (Kruizinga et al., 1988), as well as highly effective analytical methods for finally proving the optical purity of the AAA obtained by these procedures. In continuation of the gas chromatographic studies described recently for the resolution of (*R*, *S*)-AAA (Brückner and Langer, 1991), we now report on their liquid chromatographic separation as diastereomers obtained by derivatization with *o*-phthaldialdehyde (OPA) together with *N*-acetyl-L-cysteine or *N*-*t*-butoxycarbonyl-L-cysteine (Nimura and Kinoshita, 1986; Buck and Krummen, 1987) or with $N^2$-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide (FDNP-Ala-$NH_2$, Marfey's reagent; Marfey, 1984).

## Materials and methods

### *Instruments*

Gradient elution was performed with two dual-head pumps Model 2200 (Bischoff Analysentechnik, Leonberg, FRG) controlled by a Shimadzu C-R3A integrator (Shimadzu, Kyoto, Japan) that was also used for the printout of the chromatograms. Samples were injected manually into the HPLC column by means of a Model 7125 injection valve with a 20-μl injection loop (Rheodyne Inc., Cotati, CA, USA). As the detector either a fluorescence detector Model 8400 (Shimadzu) or a variable wavelength detecor Model 788 (Micromeritics) was used. The HPLC column (125 mm × 4.6 mm i.d., pre-column 20 mm × 4.6 mm i.d.) was packed with 3 μm Spherisorb ODS 2 (Shandon, Runscorn, Cheshire, UK). The column was kept at 25°C. Eluents were degassed by sonication (Bransonic 32 sonicator; SGI Laborbedarf, Darmstadt, FRG).

*Abbreviations and sources of α-alkyl-α-amino acids*

α-Alkyl-α-amino acids (AAA) are considered as being formally derived from their corresponding α-amino acids (AA) via $C^{\alpha}$-hydrogen substitution by alkyl or aralkyl groups and can also be regarded as $C^{\alpha,\alpha}$-disubstituted glycines or dialkylated glycines. Thus, $C^{\alpha}$-hydrogen substitution in protein α-AA by a methyl group leads to the series of α-methyl-α-amino acids and $C^{\alpha}$-hydrogen substitution in alanine (Ala) or α-amino-*n*-butyric acid (Abu) by an alkyl group leads to the series of α-alkylalanines (α-alkyl-Ala; alkyl = ethyl (Et), *n*-propyl (Prop), *n*-butyl (But), *n*-pentyl (Pent), *n*-hexyl (Hex), *n*-heptyl (Hept), *n*-octyl (Oct), and α-alkyl-α-Tamino-*n*-butyric acids (α-alkyl-Abu; alkyl = *n*-propyl to *n*-hexyl).

The following (*R*, *S*)-AAA were purchased from Sigma Chemical Company, St. Louis, MO, USA: α-methylleucine (α-Me-Leu), α-methylserine (α-Me-Ser), α-methylmethionine (α-Me-Met), α-methylaspartic acid (α-Me-Asp), α-methylglutamic acid (α-Me-Glu), α-methylornithine (α-Me-Orn), α-methylhistidine (α-Me-His), α-methylphenylalanine (α-Me-Phe), α-methyl-*meta*-methoxyphenylalanine (α-Me-*m*-PheOMe), α-methyl-*para*-tyrosine (α-Me-*p*-Tyr), α-methyldopa (α-Me-Dopa), α-methyltryptophane (α-Me-Trp). α-Methyl-*meta*-tyrosine (α-Me-*m*-Tyr) was from Serva, Heidelberg, FRG, and α-ethylphenylglycine (α-Et-Phg) was from *EMKA* Chemie, Markgröningen-Talhausen, FRG. α-Alkylalanines (alkyl = Et to Oct) and α-alkyl-α-amino-*n*-butyric acids (alkyl = Prop to Pent) were synthesized in our laboratory according to the Strecker procedure from the respective ketones (from Fluka, Buchs, Switzerland) and potassium cyanate. (*S*)-α-Me-*p*-Tyr and (*S*)-α-Me-Dopa were from Sigma, (*S*)-α-Me-Met, (*S*)-α-Me-Ser, and (*R*)-α-Me-Phe were kindly donated by Prof. D. Seebach, *ETH* Zürich, Switzerland. The series (*S*)-α-Et-Ala to (*S*)-α-But-Ala and (*S*)-α-Prop-Abu to (*S*)-α-But-Abu were obtained by digestion of racemic AAA with acylase I or carboxyxpeptides I (Brückner and Currle, 1987).

*Reagents used for derivatization procedures*

Marfey's reagent was from Sigma and can also be synthesized asccording to an improved procedure (Brückner and Keller-Hoehl, 1990). *N*-Acetyl-L-cysteine (Ac-Cys) was from Sigma, *N-tert.*-butoxycarbonyl-L-cysteine (Boc-Cys) was from Novabiochem, Läufelfingen, Switzerland, and *o*-phthaldialdehyde (OPA) was from Fluka. The follwing chemicals were from Merck, Darmstadt, FRG and were of analytical (*p.a.*) or chromatographic grade: boric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate dodecahydrate, ethanol (100%) (EtOH), methanol (MeOH), chloroform, acetone, hydrochloric acid (37%), acetonitrile (ACN), tetrahydrofuran (THF), triethylamine (TEA), ortho-phosphoric acid (85%), dimethyl sulfoxide (DMSO).

*Preparation of reagents used for derivatization of AAA and derivatization conditions*

Derivatization of AAA with OPA/*N*-acyl-cysteines: OPA (10 mg) was dissolved in EtOH (300 μl), 0.4 M sodium borate buffer of pH 10 (22 ml), and Boc-Cys (or Ac-Cys) (30 mg) were added; the reagents were stored at 4°C. For derivatization 5 mM AAA (10 μl) in pH 10 sodium borate buffer was diluted with borate buffer (490 μl), the respective OPA/Ac-Cys (or Boc-Cys) reagent (2 ml) was added and, after 10 min reaction time at 25°C, 5-μl aliquots were injected onto the HPLC column.

Preparation of stock solutions of AAA. 100 mM solutions of AAA in bidistilled water were used with the exceptions of the series α-Hex-Ala to α-Non-Ala which had to be dissolved in MeOH-$CHCl_3$ 4 : 1 (v/v), and α-Me-Dopa, α-Me-*p*-Tyr, α-Et-Phg, and α-Me-*m*-PheOMe which were dissolved in 1 M sodium borate buffer (pH 6.85) under heating and sonification.

*Derivatization of AAA with Marfey's reagent*

FDNP-Ala-$NH_2$ (1 mg) in acetone (100 μl), 100 mM AAA (50 μl) and 1 M sodium hydrogen carbonate (20 μl) were heated in a closed test tube for 60 min at 40°C. 2 M HCl (10 μl) was added at room temperature, the mixture was evaporated to dryness over NaOH pellets *in*

*vacuo* (at ca. 0.02 mm Hg), the residue was dissolved in DMSO (10 ml) and 10-$\mu$l aliquots were subjected to HPLC.

*Gradient elution of AAA derivatized with OPA/Acyl-Cys reagents*

Elution conditions I

Buffer A: 12.5 mM sodium phosphate buffer, pH 7.2 (990 ml) and THF (10 ml); buffer B: 12.5 mM sodium phosphate buffer, pH 7.2 (500 ml) and ACN (500 ml); linear gradient 0% B to 40% B in 40 min; flow rate 1. 5 ml/min in cases I–IV.

Elution conditions II

Buffer A: 12.5 mM NaOAc, pH 7.2 (850 ml); buffer B: 12.5 mM NaOAc, pH 7.2 (500 ml) and ACN (500 ml); linear gradient 0% B to 25% in 40 min.

Elution conditions III

Buffer A: 12.5 mM NaOAc, pH 7.2 (850 ml) and ACN (150 ml); buffer B: 12.5 mM NaOAc, pH 7.2 (500 ml) and ACN (500 ml); linear gradient 0% B to 60% B in 30 min.

Elution conditions IV

Buffer A: 12.5 mM NaOAc, pH 7.2 (995 ml) and THF (5 ml); isocratic elution.

*Gradient elution of AAA derivatized with Marfeys reagent*

Elution conditions V

Eluent A: 50 mM Triethylammonium phospate (TEAP) buffer, pH 3 (900 ml) and ACN (100 ml); eluent B: 50 mM TEAP buffer, pH 3 (500 ml) and ACN (500 ml); linear gradient from 0% B to 100% B in 60 min; flow rate of 2 ml/min in cases V and VI.

Elution conditions VI

Eluent A: 50 mM TEAP buffer, pH 7.2 (900 ml) and ACN (100 ml); eluent B: 50 mM TEAP buffer, pH 7.2 (500 ml) and ACN (500 ml); gradient elution from 0% B to 100% B in 60 min.

For preparation of TEAP buffer, TEA (6.93 ml) was dissolved in bidistilled water (950 ml) and pH 3.0 or pH 7.2, respectively, were adjusted by addition of ortho-phosporic acid; then the volume was made up to 1 liter.

## Results and discussion

The structures of the diastereomeric derivatives obtained either by reaction of OPA together with Ac-Cys or Boc-Cys and (*R*)-α-ethylalanine as the most simple, chiral α-alkyl-α-amino acid, or by reaction of this amino acid with Marfey's reagent, are shown in Fig. 1a and Fig. 1b, respectively.

Net retention times $t_{R_1}$ and $t_{R_2}$ of the diastereomers of (*R*, *S*)-AAA formed by reaction of OPA together with the chiral thiols Ac-Cys and Boc-Cys, or with Marfey's reagent, and differences in net retention times $\Delta t_R$ and gradient elution conditions are given in Table 1. Sections of chromatograms are shown in Figs. 2 and 3. Owing to the large differences in hydrophobicities of the diastereomeric derivatives, suitable gradient elution conditions had to be selected in order to

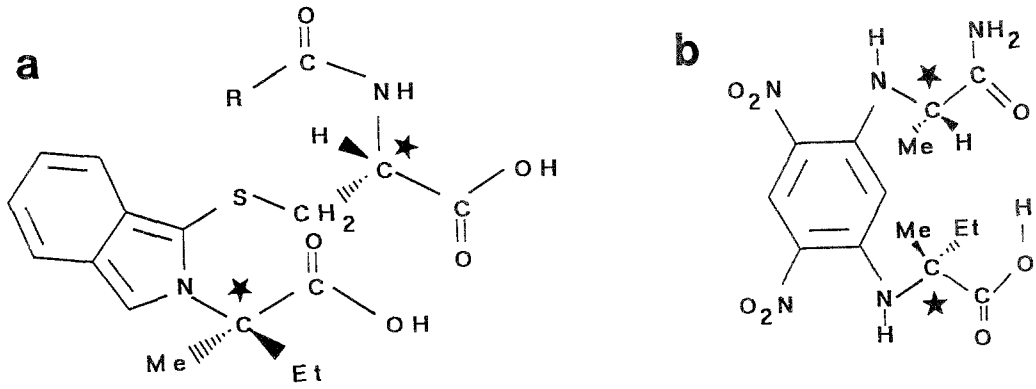


**Fig. 1. a** Structure of the diastereomeric isoindole derivative formed by reaction of *o*-phthaldialdehyde (OPA) with *N*-acetyl-L(= *R*)-cysteine (Ac-Cys) or *N*-*t*-butoxycarbonyl-L-cysteine (Boc-Cys) and (*R*)-α-ethylalanine [(*R*)-isovaline], R = methyl (in Ac-Cys) or *t*-butoxy (in Boc-Cys); and **b** structure of the diastereomer formed by reaction of Marfey's reagent [$N^2$-(5-fluoro-2,4-dinitrophenyl)-L(= *S*)-alanine amide] with (*R*)-α-ethylalanine; *Me* methyl; *Et* ethyl; asterisks indicate chiral centres

achieve reasonable elution times. Under these conditions, 8 of the 27 AAA investigated were resolved by derivatization with OPA/Boc-Cys (Fig. 2a) and 11 were almost baseline separated by derivatization with OPA/Ac-Cys (Fig. 2b). Resolution decreased in the homologous series α-Et-Ala to α-But-Ala, and no separation was achieved for the series α-Pent-Ala to α-Non-Ala or α-Prop-Abu to α-Hex-Abu by derivatization with either OPA/Ac-Cys or OPA/Boc-Cys.

Since it had been recognized that AAA are much less reactive in derivatization reactions as compared to AA (Cronin et al, 1979; Weinstein and Grinberg, 1985; Brückner et al., 1987; Duchateau et al., 1989) a reaction time of 10 min was used for AAA instead of the ca. 2 min usually used for derivatization of AA with OPA/thiols. From the detector response, however, it was obvious that in particular α-Et-Phg, α-Me-Val and α-alkyl-Abu gave low derivatization yields. Addition of enantiomerically pure AAA to the racemates showed that under the conditions used, without exception, after derivatization with OPA/Ac-Cys or Boc-Cys, the diastereomer containing the (*R*)-AAA elutes before that containing the (*S*)-AAA (tested for α-Et-Ala, α-Me-Metz and α-Me-Phe). It should be noted, however, that in the case of DL-Asp and derivatization with OPA/Ac-Cys, a pH-dependence of the elution order of diastereomers is observed.

For diastereomers of (*R*, *S*)-AAA obtained by derivatization with Marfey's reagent, the net retention times $t_R$ and $\Delta t_R$ values are given in Table 1 and sections of the chromatograms are shown in Fig. 3. It can be seen that baseline resolution, or almost baseline resolution, was achieved for all AAA. The (*S*)-AAA elute before (*R*)-AAA (tested for α-Et-Ala, α-Me-Ser, α-Me-Met, α-Me-p-Tyr, α-Me-Phe, and α-Me-Dopa). This chromatographic behaviour of diastereomers of AAA is in agreement with that of AA where large differences in $\Delta t_R$ values after derivatization with Marfey's reagent or, more generally, chiral variants of Sanger's reagent (Brückner and Gah, 1991) were recognized. The $\Delta t_R$ values of AAA, however, are smaller than those of the respective AA from which the AAA is formally derived from (e.g. (*R*, *S*)-Leu shows higher resolution in comparison

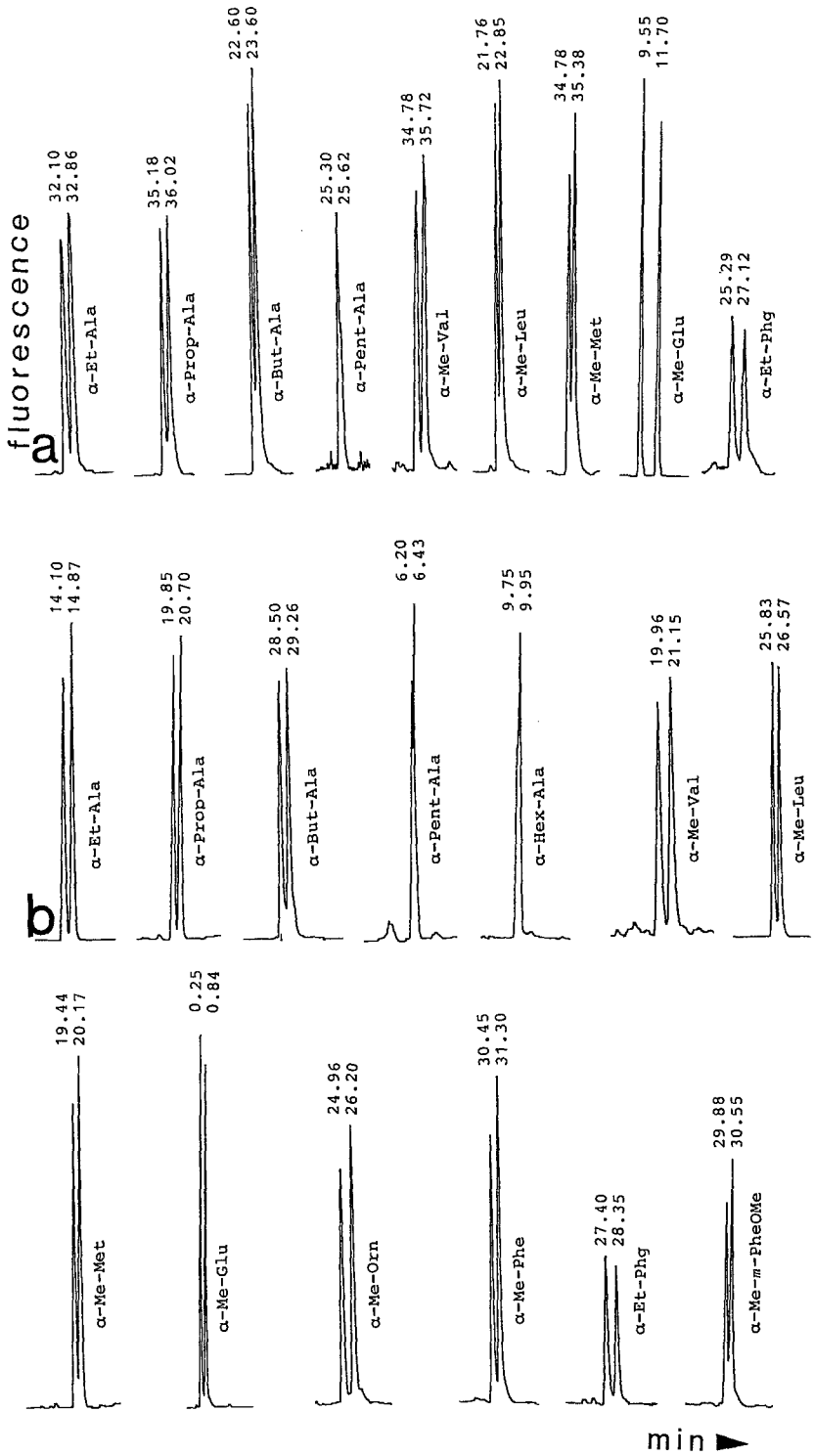


**Fig. 2.** Sections of chromatograms and net retention times of (*R, S*)- α-alkyl-α-amino acids (AAA obtained after derivatization with **a** OPA/*N*-*t*-butyloxycarbonyl-L-cysteine (Boc-Cys), and **b** with OPA and *N*-acetyl-L-cysteine (Ac-Cys). (For abbreviations of AAA in Figures 2–4 see Materials and methods and for gradient elution conditions see Table 1.). Fluorescence detection at 344 nm (excitation) and 443 nm (emission)

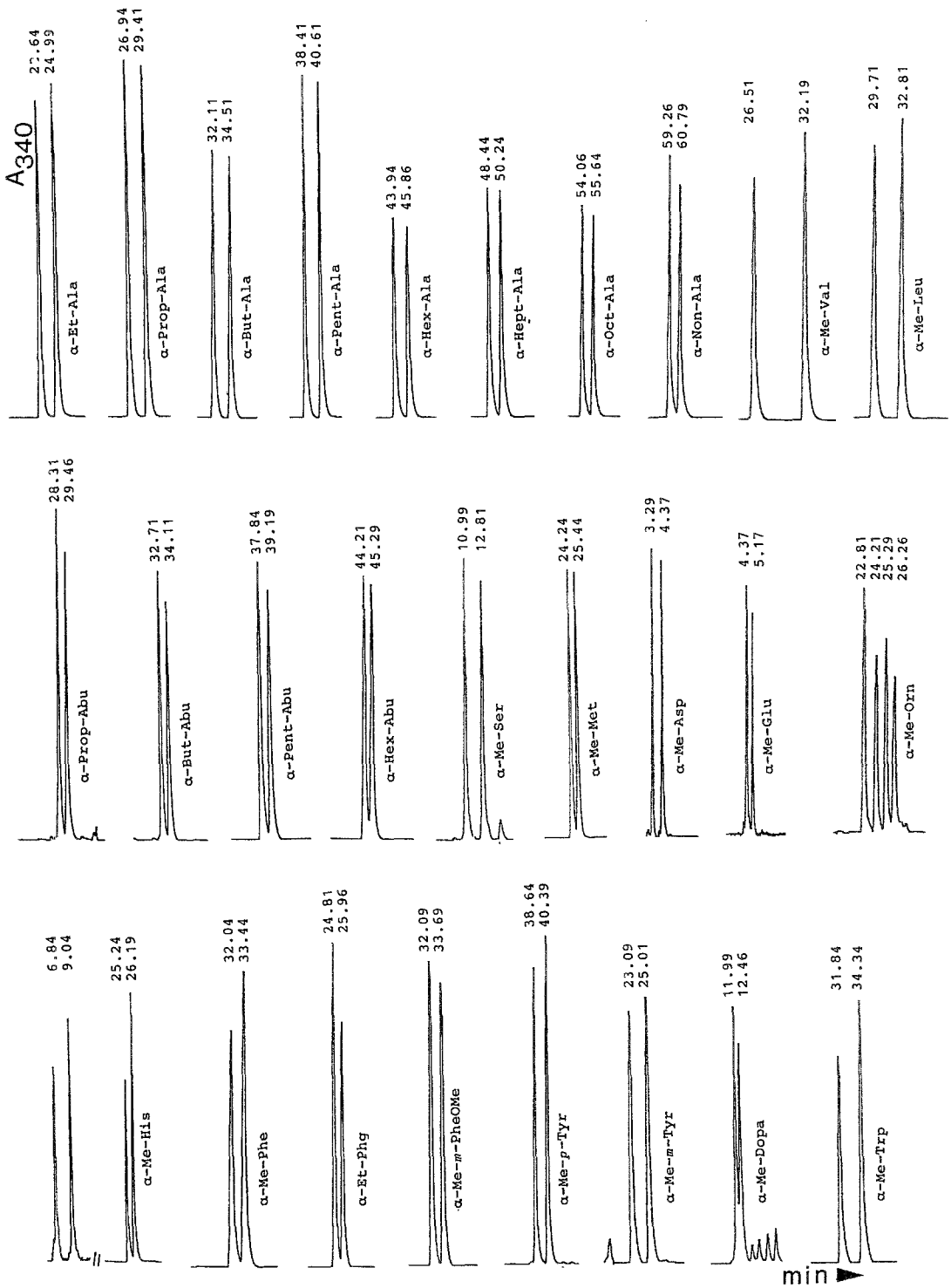


**Fig. 3.** Sections of chromatograms and net retention times of AAA obtained after derivatization with $N^2$-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide (Marfey's reagent). Absorbance at 340 nm ($A_{340}$)

**Table 1.** Net retention times ($t_{R_{1,2}}$) of the first ($t_{R_1}$) and second ($t_{R_2}$) eluted diastereomer, and differences of net retention times ($\Delta t_R$), of (*R*, *S*)-α-alkyl-α-amino acids (AAA) after derivatization with *o*-phthaldialdehyde (OPA) together with *N*-*t*-butoxycarbonyl-L-cysteine (Boc-Cys) or *N*-acetyl-L-cysteine (Ac-Cys), or with $N^2$-(5-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (Marfey's reagent)

| (*R*, *S*)-AAA[a] | Reagent OPA/Boc-Cys | | | OPA/Ac-Cys | | | Marfey's reagent | | |
|---|---|---|---|---|---|---|---|---|---|
| | EC[b] | $t_{R_{1,2}}$ | $\Delta t_R$ | EC | $t_{R_{1,2}}$ | $\Delta t_R$ | EC | $t_{R_{1,2}}$ | $\Delta t_R$ |
| α-Et-Ala | I | 32.10<br>32.86 | 0.76 | I | 14.10<br>14.87 | 0.77 | V | 22.64<br>24.99 | 2.35 |
| α-Prop-Ala | I | 35.18<br>36.02 | 0.84 | I | 19.85<br>20.70 | 0.85 | V | 26.94<br>29.41 | 2.47 |
| α-But-Ala | II | 22.60<br>23.60 | 1.00 | I | 28.50<br>29.26 | 0.76 | V | 32.11<br>34.51 | 2.40 |
| α-Pent-Ala | II | 25.30<br>25.62 | 0.32 | III | 6.20<br>6.43 | 0.23 | V | 38.41<br>40.61 | 2.20 |
| α-Hex-Ala | III | 16.72 | n.r. | III | 9.75<br>9.95 | 0.20 | V | 43.94<br>45.86 | 1.92 |
| α-Hept-Ala | III | 19.39 | n.r. | III | 12.79 | n.r. | V | 48.44<br>50.24 | 1.80 |
| α-Oct-Ala | III | 22.15 | n.r. | III | 15.80 | n.r. | V | 54.06<br>55.64 | 1.58 |
| α-Non-Ala | III | 24.85 | n.r. | III | 18.67 | n.r. | V | 59.26<br>60.79 | 1.53 |
| α-Me-Val | I | 34.78<br>35.72 | 0.94 | I | 19.96<br>21.15 | 1.19 | V | 26.51<br>32.19 | 5.68 |
| α-Me-Leu | II | 21.76<br>22.85 | 1.09 | I | 25.83<br>26.57 | 0.74 | V | 29.71<br>32.81 | 3.10 |
| α-Prop-Abu | I | 28.67 | n.r. | I | 22.85 | n.r. | V | 28.31<br>29.46 | 1.15 |
| α-But-Abu | III | 10.95 | n.r. | I | 25.37 | n.r. | V | 32.71<br>34.11 | 1.40 |
| α-Pent-Abu | III | 13.92 | n.r. | I | 33.87 | n.r. | V | 37.84<br>39.19 | 1.35 |
| α-Hex-Abu | III | 17.47 | n.r. | III | 11.06 | n.r. | V | 44.21<br>45.29 | 1.08 |
| α-Me-Ser | I | 18.62 | n.r. | I | 2.62 | n.r. | V | 10.99<br>12.81 | 1.82 |
| α-Me-Met | I | 34.78<br>35.38 | 0.60 | I | 19.44<br>20.17 | 0.73 | V | 24.24<br>25.44 | 1.20 |
| α-Me-Asp | I | 7.59 | n.r. | IV | 0 | n.r. | VI | 3.29<br>4.37 | 1.08 |
| α-Me-Glu | I | 9.55<br>11.70 | 2.15 | IV | 0.25<br>0.84 | 0.59 | VI | 4.37<br>5.17 | 0.80 |
| α-Me-Orn | I | 16.04<br>16.92 | 0.88 | I | 24.96<br>26.20 | 1.24 | V | 22.81<br>24.21 | 1.40[c] |
| | | | | | | | | 25.29<br>26.26 | 0.97[d] |
| α-Me-His | I | 24.62 | n.r. | I | 1.60 | n.r. | V | 6.84<br>9.04 | 2.20[c] |
| | | | | | | | | 25.24<br>26.19 | 0.95[d] |

**Table 1** (*continued*)

| (*R*, *S*)-AAA[a] | Reagent OPA/Boc-Cys | | | OPA/Ac-Cys | | | Marfey's reagent | | |
|---|---|---|---|---|---|---|---|---|---|
| | EC[b] | $t_{R_{1,2}}$ | $\Delta t_R$ | EC | $t_{R_{1,2}}$ | $\Delta t_R$ | EC | $t_{R_{1,2}}$ | $\Delta t_R$ |
| α-Me-Phe | II | 26.77 | n.r. | I | 30.45<br>31.30 | 0.85 | V | 32.04<br>33.44 | 1.40 |
| α-Et-Phg | II | 25.29<br>27.12 | 1.83 | I | 27.40<br>28.35 | 0.95 | V | 24.81<br>25.96 | 1.15 |
| α-Me-*m*-PheOMe | II | 27.77 | n.r. | I | 29.88<br>30.55 | 0.67 | V | 32.09<br>33.69 | 1.60 |
| α-Me-*p*-Tyr | I | 22.68 | n.r. | I | 12.62 | n.r. | V | 38.64<br>40.39 | 1.75 |
| α-Me-*m*-Tyr | | n.d. | | | n.d. | | V | 23.09<br>25.01 | 1.92 |
| α-Me-Dopa | | n.d. | | | n.d. | | V | 11.99<br>12.46 | 0.47 |
| α-Me-Trp | I | 38.62 | n.r. | I | 26.58 | n.r. | V | 31.84<br>34.34 | 2.50 |

[a] For abbreviations see Materials and methods; [b] *EC* gradient elution conditions I–V, see Materials and methods; [c] monosubstituted diastereomer; [d] disubstituted diastereomer; *n.r.* not resolved; *n.d.* not determined.

(*R*, *S*)-α-Me-Leu although both are baseline resolved). In particular the series α-Pent-Ala to α-Non-Ala and α-Prop-Abu to α-Hex-Abu, which are not resolved by derivatization with OPA/Ac-Cys or Boc-Cys, are satisfactorily separated by derivatization with Marfey's reagent. The basic AAA α-Me-Orn and α-Me-His gave rise to mono- and disubstituted diastereomers resulting in four stereoisomers under the conditions used (*cf.* Fig. 3). The relatively large differences in $\Delta t_R$ values of the diastereomers of AAA are, in analogy to those of AA, explained by the formation of an intramolecular hydrogen bond between the carboxy group of the respective AAA and the carboxamide group of the reagent in the *S-S* diastereomer but not in the *R-S* diastereomer; first letter corresponds to the configuration of the AAA, second to that of Ala in the reagent (Brückner and Gah, 1991). As in the cases of OPA/Ac-Cys or OPA/Boc-Cys, it was seen that AAA are much less reactive than AA upon derivatization with Marfey's reagent.

In conclusion, derivatization of AAA with Marfey's reagent in particular is considered to be a highly suitable method for the liquid chromatographic resolution of AAA using the diastereomeric approach.

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Received February 3, 1992