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ONE POT SYNTHESIS AND CONFORMATION OF N-t-BUTYLOXYCARBONYL, O-PHENACYL DERIVATIVES OF PROLINE AND OTHER SECONDARY AMINO ACIDS.

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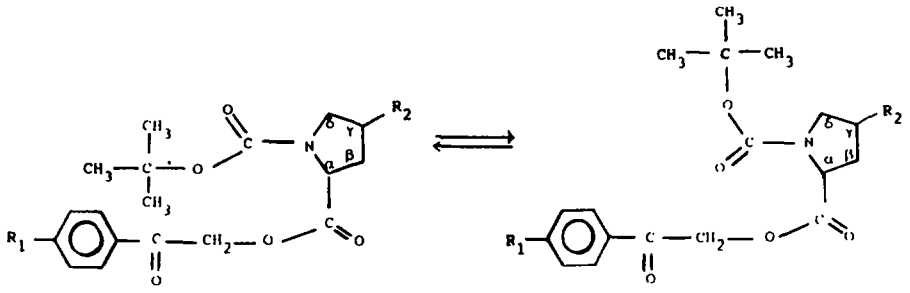
ABSTRACT

Proline, 4-hydroxyproline, azetidine-2-carboxylic acid, pipecolic acid and proline containing dipeptides were converted to N-t-butyloxycarbonyl, O-phenacyl derivatives in a one pot synthesis. ^{1}H- and ^{13}C-NMR spectroscopy of certain derivatives (Boc-Pro-PE, Boc-4Hyp-PE, Boc-Pro-4BrPE and Boc-Pip-PE) in $CDCl_3$ solution reveal the presence of cis-trans isomers in almost equal quantities. On the contrary, the ^{1}H- and ^{13}C-NMR spectra of the N-t-butyloxycarbonyl phenacyl ester of azetidine-2-carboxylic acid, show the presence of one isomer only. Similarly, only one urethane isomer was observed in solution for the protected C-terminal angiotensin II dipeptides, N-t-Boc-Pro-Phe-PE and N-t-Boc-Pro-Ile-PE. All phenacyl derivatives exhibited magnetic asymmetry attributed to steric factors.

We recently reported a method for the isolation of free amino acids from natural sources and their identification as N-t-butyloxycarbonyl (Boc) amino acid benzyl esters[1,2]. This is attained in a two step synthesis. In another report we have also described the isolation of free amino acids as N-t-Boc amino acid phenacyl esters (PE)[3].This method offers the advantage of usualy affording crystalline compounds with the potential of being studied by X-ray crystallography[4,5]. Also the ^{1}H-NMR spectra of these derivatives are characterized by an AB quartet with a unique $\Delta_\nu = \nu_2 - \nu_1$ value for each amino acid,due to the magnetic nonequivalence of the O-methylene phenacyl protons. Interestingly, N-t-Boc-proline phenacyl ester displayed two distinct AB quartets,revealing a simple alternate way of detecting, cis-trans isomerism about the urethane amide bond. Furthermore, in our work concerning structure-activity studies of angiotensin II analogues, proline has been found to play an important role in the overall conformation assumed by the angiotensin II peptide molecule[6-9].Therefore we thought

it of interest to synthesize a number of model N-t-butyloxycarbonyl, O-phenacyl derivatives of proline, proline homologs and proline containing dipeptides and study the cis-trans isomerism as revealed by ^{1}H and ^{13}C - NMR spectroscopy (Fig. 1).These derivatives were prepared in a one pot synthesis as well as by the conventional two step derivatization.



1. R_1 = H , R_2 = H
2. R_1 = H , R_2 = OH
3. R_1 = Br, R_2 = H

Figure 1. Cis-trans isomerism about the urethane bond of N-t-Boc, O-phenacyl derivatives of Pro, 4Hyp.

RESULTS AND DISCUSSION

Protection of the imino group of secondary amino acids by the Boc moiety has been achieved in good yields using t-butyl S-4, 6-dimethyl pyrimid-2-yl thiocarbonate[10]. Subsequently,protection of the carboxyl group was accomplished by esterification using benzyl bromide or phenacyl bromide[11]. In the present study,proline and other secondary amino acids such as 4-hydroxyproline, azetidine -2-carboxylic acid and pipecolic acid were derivatized in a one pot synthesis using phenacyl bromide and di-tert-butyl dicarbonate which allows the clean and rapid introduction of the acid labile Boc protecting group in amino acids, peptides and proteins[12]. One pot amino- and carboxyl-protection of amino acids may prove to have far reaching applications especially in the isolation of novel amino acids from natural sources[3].

The complexity of ^{1}H-NMR spectra often hinders detection of cis-trans isomers in proline derivatives.A reliable resonance for detecting cis-trans isomers in the N-t-Boc amino acid derivatives is the tert-butyl group,ab-

breviated Boc. On the other hand, the use of the phenacyl ester moiety , abbreviated PE, as C-terminal protecting group allows the easy crystallization of the synthesized derivatives and provides a characteristic AB quartet resonance for the phenacyl CH_2 methylene protons in a clean area (δ = 4.6 - 5.4 ppm) of the NMR spectrum for distinguishing cis-trans isomers[3-5] (Fig. 2).Thus compound 1 (Table I) in $CDCl_3$ showed an AB quartet for one isomer centred at δ = 5.399 ppm with a coupling constant of J=16 Hz and chemical shift difference Δ_ν = 0.375 ppm,while the other isomer showed an AB quartet centred at δ=5.379 ppm (J=16 Hz) but a smaller chemical shift difference (Δ_ν = 0.101 ppm).The presence of the two isomers in almost equal amounts was also obvious from the two sharp singlets at δ_1 = 4.464 and δ_2 = 1.442 ppm attributed to the Boc groups, one for each isomer. Similarly,compound 2 displayed an AB quartet centred at δ = 5.429 ppm (J=16 Hz, Δ_ν=0.323 ppm) for one isomer and another distinct AB quartet centred at δ=5.395 ppm (J=16 Hz, Δ_ν=0.099 ppm) for the second isomer. Also the Boc groups of the two isomers were identified as two sharp singlets at δ_1 = 1.465 ppm and δ_2 = 1.442 ppm (Fig. 2B). Consequently, compound 3

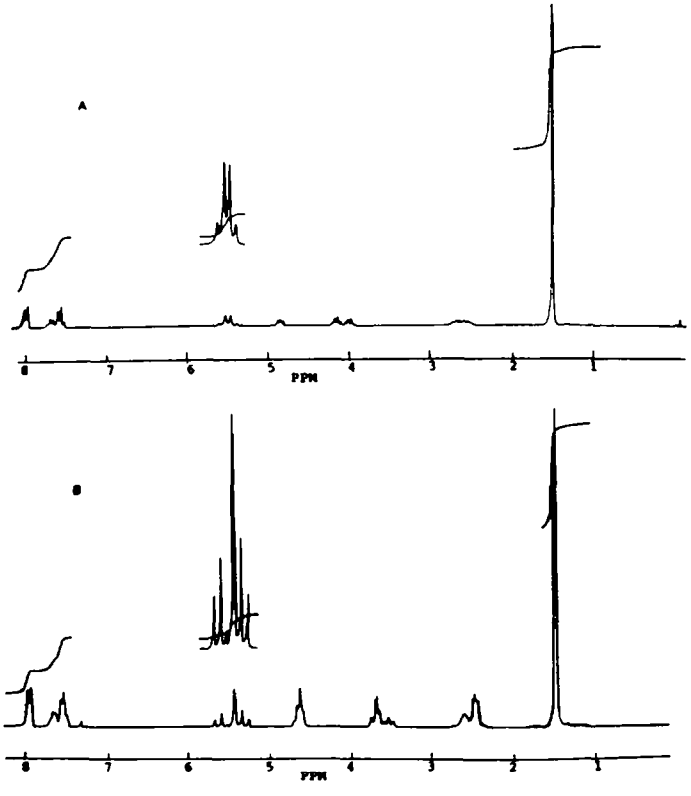


Figure 2. ^{1}H-NMR spectra of Boc-Aze-PE (A) and Boc-4Hyp-PE (B) in $CDCl_3$ + + TMS.

TABLE I.

Analytical ^{1}H NMR data for the AB quartet pattern of the phenacyl group distinguishing cis-trans isomers in N-t-Boc proline derivatives.[a]

Compound[b]	δ(ppm)	$\Delta_\nu = \nu_A - \nu_B$	ν_1	ν_2	ν_3	ν_4	J_{AB}	Boc
1. Boc-Pro-PE	5.399	0.375	5.170	5.252	5.545	5.627	16	1.464
	5.379	0.101	5.289	5.369	5.390	5.469	16	1.442
2. Boc-4HyP-PE	5.429	0.323	5.221	5.305	5.554	5.637	16	1.465
	5.395	0.099	5.304	5.388	5.403	5.487	16	1.442
3. Boc-Pro-4BrPE	5.347	0.358	5.127	5.209	5.485	5.567	16	1.464
	5.333	0.101	5.242	5.324	5.343	5.425	16	1.442
4. Boc-Pro-OBzl	5.181	0.177	5.062	5.121	5.236	5.298	16	1.464
	5.159	0.070	5.095	5.154	5.165	5.224	16	1.348
5. Trt-Pro-PE	5.345	0.269	5.170	5.251	5.438	5.520	16	-
6. Boc-Aze-PE	5.474	0.153	5.353	5.442	5.505	5.595	16	1.442
7. Boc-Pro-Phe-PE	5.423	0.189	5.288	5.371	5.477	5.559	16	1.408
8. Boc-Pro-Ile-PE	5.407	0.219	5.256	5.339	5.475	5.558	16	1.467
9. Boc-Pip-PE[c]	5.378	0.177	5.269	5.352	5.415	5.529	16	1.445
	5.411	0.065	5.350	5.420	5.415	5.485	16	1.445

[a] δ and ν value decimals are given to the third decimal point as calculated by the 200 MHz NMR's computer . Two isomers were observed for compounds 1,2,3,4,9.

[b] Numbering of compounds in text as in Table I.

[c] Two AB quartets and one A_2 singlet were observed for Boc-Pip-PE. This may be due to accidental equivalence of the two Boc resonances of the two isomers.

TABLE II

^{13}C Chemical Shifts of proline derivatives[a,b]

	Proline carbons					Protecting group carbons				
						Boc			Phenacyl	
Compound	C_α	C_β	C_γ	C_δ	-COO-	C	CH_3	O-C=O	CH_2	CO
Boc-Pro-OH[c]	58.955	30.385	24.305	46.845	178.766	80.913	28.387	155.153	-	-
	58.955	29.129	23.656	46.354	176.424	80.400	28.247	153.997	-	-
Boc-Pro-PE	59.030	31.039	24.309	46.673	172.637	79.933	28.436	154.516	66.117	192.170
	58.918	30.093	23.557	46.374	172.465	79.814	28.341	153.829	65.919	195.733
Boc-Pro-4BrPE	59.009	31.039	24.325	46.677	172.622	79.998	28.442	154.526	65.952	191.349
	58.710	30.079	23.555	46.384	172.469	79.905	28.351	153.815	65.735	190.876
Boc-4Hyp-PE	57.945	39.240	69.951	54.754	172.586	80.489	28.376	154.676	66.231	192.273
	57.536	38.557	69.256	54.555	172.764	80.313	28.282	154.056	65.936	191.764
Boc-Aze-PE[d]	60.404	20.519	48.204	-	170.937	80.132	28.304	153.046	66.151	191.668

[a] δ values in ppm are given to the third decimal point as calculated by the 200 MHz NMR's computer. Assignment was also possible by DEPT experiments.

[b] $CDCl_3$ was used as solvent with TMS as internal reference.

[c] Literature chemical shifts, in DMSO-d_6 for this compound are slightly different[21].

[d] Assignment of the ring carbon resonances was based on previously reported ^{13}C NMR data for azetidine[22].

exhibited two AB patterns centred at δ_1=5.347 ppm (J=16 Hz, Δ_ν=0.358 ppm) and δ_2=5.333 ppm (J=16 Hz, Δ_ν=0.101 ppm) and two Boc singlets at δ_1=1.464 ppm and δ_2=1.442 ppm for the two isomers.

The nonequivalence of the CH_2 benzyl methylene protons in N-t-Boc amino acid benzyl esters, and N-t-trityl dipeptide benzyl esters, has been attributed to restricted rotations[13,14]. Accordingly, the chemical shift difference observed in the AB quartet of each proline isomer, largely reflects the degree of free rotation of the phenacyl moeity in the phenacyl derivatives under scrutiny. We rationalized that the AB quartet in 1, 2 and 3 with the smaller chemical shift difference of the O-methylene phenacyl protons (Δ_ν=0.101 ppm, 0.099 ppm, 0.101 ppm respectively for 1, 2 and 3) corresponds to the trans isomer,while the AB quartet with the larger chemical shift difference (Δ_ν=0.375 ppm, 0.323 ppm, 0.358 ppm) corresponds to the cis isomer. Rotations of the Boc moeity affect markedly the phenacyl group in the isomer assuming a cis urethane conformation. Examination of molecular models indicate that the phenacyl moeity in the trans isomer is less hindered by the bulky Boc group than the phenacyl moeity in the cis isomer[14]. A trans urethane bond conformation would allow a ro-

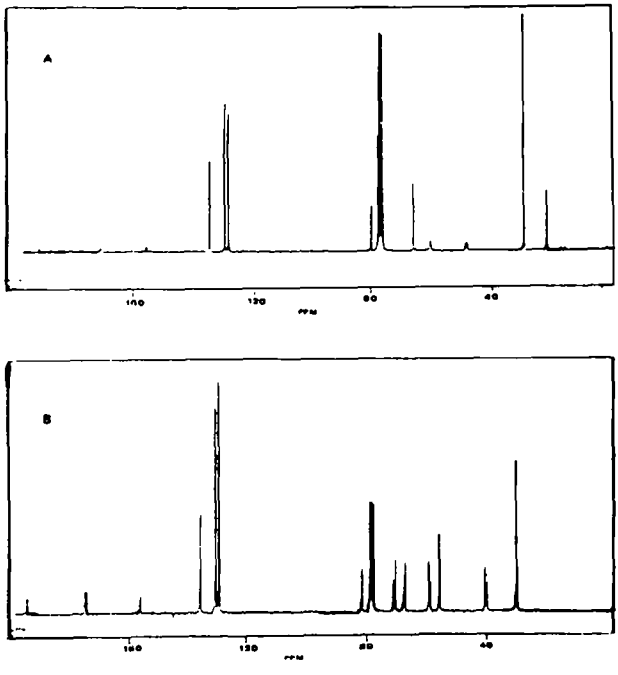


Figure 3. ^{13}C-NMR spectra of Boc-Aze-PE (A) and Boc-4Hyp-PE (B) in $CDCl_3$+ +TMS.

tational freedom for the two CH_2 phenacyl protons thus locating them in similar or perhaps identical environments.This would result in reduced or diminished chemical shift difference.

Similar considerations can be applied for N-t-Boc-Pro-OBzl (4). Two AB quartets were also observed for the cis and trans benzyl methylene protons (Table I). However,their dispersion was considerably smaller for the cis isomer (Δ_ν=0.177 ppm) and almost eliminated for the trans isomer (Δ_ν= =0.070 ppm), when compared with the dispersion of the phenacyl methylene protons (Table I).

Temperature studies in DMSO-d_6+D_2O indicated that restricted rotation was a major factor contributing to the nonequivalence of the CH_2 phenacyl protons and the spliting of the Boc resonance in phenacyl derivatives. At room temperature, both isomers of 1 showed slightly different chemical shift differences than observed in $CDCl_3$. Thus,one isomer showed Δ_ν=0.325 ppm, while the other showed Δ_ν=0.091 ppm, which could suggest a change in the population of several different conformers or a change in solvent effect on chemical shifts of the conformers. At 80° the chemical shift difference appeared to change substantially for the one isomer (Δ_ν=0.121 ppm from 0.325 ppm) (cis) but not for the other one (Δ_ν=0.089 ppm from 0.091 ppm) (trans). At 135° only one AB quartet for the phenacyl methylene protons (Δ_ν=0.070 ppm) and only one A_2 singlet for the Boc group were observed indicating a rapid cis-trans isomerization. At 160° the AB quartet was

TABLE III

^{13}C Chemical shifts[a]

Boc-Pro-Ile-PE

Carbon	Pro	Ile	Boc, PE
α	61.106	59.615	-
β	30.919	37.718	-
γ	24.690	24.344	-
	-	15.445	-
δ	46.991	11.617	-
Boc(CH_3)	-	-	28.291
Boc(-C-)	-	-	80.428
PE(CH_2)	-	-	66.311

[a] Assignment of carbon resonances was based on previous ^{13}C-NMR data for Pro,Ile[16,17] and distortionless enhancement by polarization transfer (DEPT) experiments.

converted to an A_2 singlet and thus it seems reasonable to postulate that at high temperature the geminal protons become equivalent through free rotation in the molecule. These studies could suggest that the Boltzmann population of the two isomers become more equal at higher temperatures. Alternatively,the observed greater closing of the AB pattern in one of the two isomers could also suggest a steric effect of the bulky Boc group on the phenacyl CH_2 protons for this isomer,a fact which is only compatible with a cis urethane bond conformation.

Replacement of the Boc group in compound 1 by trityl, affords N-Trt-Pro-PE (5) which as expected exhibited only one AB quartet at lower field, δ=5.345 ppm,suggesting a strong anisotropic effect of the trityl group on the phenacyl CH_2 methylene protons.The shielding effect suggests furthermore a close proximity of the phenacyl moeity with the N-trityl group in 5 indicating a stacking itneraction between the aromatic rings of the two groups[13].

The ^{1}H-NMR data of 1, 2 and 3 which confirm the presence of the two isomers in $CHCl_3$ solution are in agreement with the ^{13}C-NMR data. Indeed the ^{13}C-NMR of these derivatives show two resonances for each carbon (Table II). Previous studies have shown that the γ proline carbon resonances of the trans and cis conformation have distinct and non overlapping ranges of chemical shifts[15,16]. Thus the γ carbon resonance of the cis conformer in 1 was found at δ=23.557 ppm and for the trans conformer at δ= =24.309 ppm. Similarly, the γ carbon resonance of the cis conformer in 3 was found at δ=23.555 ppm and for the trans conformer at δ=24.325 ppm . Replacement of the γ-methylene proton with hydroxyl, results in a downfield shift of the β, γ, δ proline ring carbons. The resonances of these carbons in compound 2 are in agreement with those of free 4-hydroxyproline[17] (Fig. 3B) (Table II). The cis and trans isomers of 1, 2 and 3 are present in almost equal amounts indicated by the same intensity resonances for each respective carbon (Fig. 1).

Interestingly,it was observed that both the ^{1}H- and ^{13}C-NMR spectra of the Boc phenacyl derivative of L-azetidine-2-carboxylic acid (6), a four membered ring proline homolog,reveal the presence of only one isomer.Thus the ^{1}H-NMR spectrum of 6 displays one distinct AB quartet for the O-methylene phenacyl protons centred at δ=5.474 ppm with a chemical shift difference Δ_ν=0.153 ppm. Only one singlet was also observed at δ=1.445 ppm for the Boc methyl protons (Fig. 2A). Similarly, the ^{13}C-NMR spectrum of 6 in $CHCl_3$, show only one resonance for each carbon in agreement with the presence of one isomer as suggested by the ^{1}H-NMR data (Fig. 3A).It is known that biologically active peptide analogues of angiotensin II (ANGII) , containing proline or sarcosine at position seven, exist in solution as a

mixture of conformers due to cis-trans isomerism about the X-Pro or X-Sar amide bond[8]. However, it is not yet known which conformer is the active one. Therefore, replacement of proline with azetidine in angiotensin II , and other active peptides could be of interest in terms of structure-activity relationship and could provide important information concerning the required configuration for potency. Preliminary experiments carried out by our group have shown that replacement of L-proline with L-azetidine-2--carboxylic acid in ANGII,ANGIII and [Sar^1]ANGII produced extremely potent angiotensin II and III analogues.The biological activities of these analogues in relation to the His-Aze configuration will be published elsewhere.

Turning to the N-t-Boc, phenacyl derivatives 7 and 8 of Pro-Phe and Pro-Ile which constitute the C-terminal dipeptides for the angiotensin II super-agonist [Sar^1]ANGII and antagonist [Sar^1, Ile^8] ANGII, ^{1}H- and ^{13}C-NMR spectroscopy in $CDCl_3$ indicate the presence of only one urethane bond isomer.Thus the ^{1}H-NMR of 7 shows one AB quartet at δ=5.423 ppm and one Boc singlet at δ=1.408 ppm. Similarly,the spectrum of compound 8 displays one AB quartet at δ=5.407 ppm and one Boc singlet at δ=1.467 ppm. In agreement,the ^{13}C-NMR shows a single resonance for each carbon (Table III).

EXPERIMENTAL

Capillary melting points were taken on a Buchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl Zeiss precision polarimeter (0.005°).A Varian XL 200 instrument was used to record ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$,using tetramethylsilane as internal standard. Infrared spectra were recorded with a Perkin Elmer 457 instrument. Analytical thin-layer chromatography (t.l.c.) was performed on Merck silica gel 60 F_{254} films (0.25 mm layer thickness) precoated on glass plates with the solvent systems; A, chloroform - methanol (9:1); B, hexane-ethyl acetate (6:4) and visualized by UV,iodine and ninhydrin (containing 3% trifluoroacetic acid in butanol).Elemental analyses were done by the Microanalytical Laboratory of the National Hellenic Research Foundation,and data fall within $\pm$0.3% of the theory.Flash column chromatography was carried out on $CHCl_3$ packed silica gel column[16]. Free aminoacids (Pro, 4Hyp, Aze, Pip) and free dipeptides (Pro-Phe, Pro-Ile) were purchased from the Protein Research Foundation. Boc-Pro-OBzl and Trt-Pro-PE were prepared as previously described[1,11]. Boc-amino acid phenacyl esters 1, 2, 3, 6 and 9 and Boc-dipeptide phenacyl esters 7 and 8 were prepared from the corresponding free aminoacids or dipeptides according to the general proce -

dures for the N-t-Boc protection of amino group[10] and the phenacylation of the carboxyl group[11]. Protected dipeptides 7 and 8 were also synthesized by the mixed anhydride method,using Boc-Phe-PE and Boc-Ile-PE as starting materials. Treatment of these derivatives (0.1 mmol) with 50% solution of CF_3COOH in CH_2Cl_2 (6 ml) for 20 min at room temperature afforded the trifluoroacetate salts which precipitated with addition of ether (20 ml) and were collected by filtration. Boc-Pro-OH was condensed with phenylalanine phenacyl ester trifluoroacetate and isoleucine phenacyl ester trifluoroacetate by the mixed anhydride method[19,20]. All derivatives were characterized by ^1H-, ^{13}C-NMR spectroscopy and elemental analyses. Distortionless enhancement by polarization transfer (DEPT) experiments were carried out to assign the ^{13}C chemical shifts of the proline derivatives.

TABLE IV.

Physical properties of N-t-Boc-amino acid and N-t-Boc dipeptide phenacyl esters.

Compound	Yield[a] %	Mp[b]	Crystallization solvent[b]	R_f[c] Rf_A	Rf_B	$[a]_D^{20}$[d]	Empirical formula[e]
Boc-Pro-PE	91	80-2°C	EtOAc/Petr.Ether	0.67	0.33	-93.73	$C_{18}H_{23}O_5N$
Boc-4Hyp-PE	87	121-2°C	EtOAc/Petr.Ether	0.43	0.07	-78.68	$C_{18}H_{23}O_6N$
Boc-Pro-4BrPE	55	80° C	Ether/Petr.Ether	0.60	0.33	-70.95	$C_{18}H_{22}O_5NBr$
Boc-Aze-PE	65	56-7°C	$CHCl_3$/Petr.Ether	0.65	0.3	-130.97	$C_{17}H_{21}O_5N$
Boc-Pro-Phe-PE	85	63-4°C	Ether/Petr.Ether	0.66	0.15	-72.66	$C_{27}H_{32}O_6N_2$
Boc-Pro-Ile-PE	82	100-1°C	EtOAC/Petr.Ether	0.68	0.19	-70.61	$C_{24}H_{34}O_6N_2$

[a]Yields for the phenacylation of N-t-Boc-aminoacids and N-t-Boc dipepti - des. Yields for the one pot synthesis varied between 35-45%. Yields of the two protected dipeptides (Pro-Phe, Pro-Ile) synthesized by the mixed anhydride method were 65% and 62% respectively.

[b]Boc-Pip-PE was crystallized from ethyl acetate/petroleum ether,m.p.=78°.

[c]Only single spots were detected for loads of at least 50 mg. Letters indicate solvent systems given in Experimental Section.

[d]Optical rotations were measured for 1% concentrations in $CHCl_3$.

[e]Elemental analyses:
(Found: C, 64.79; H, 6.93; N, 4.19. Calc. for $C_{18}H_{23}O_5N$: C, 64.87; H, 6.91; N, 4.20%).
(Found: C, 61.96; H, 6.57; N, 3.99. Calc. for $C_{18}H_{23}O_6N$: C, 61.89; H, 6.59; N, 4.01%).
(Found: C, 52.34; H, 5.32; N, 3.39. Calc. for $C_{18}H_{22}O_5NBr$: C,52.43; H, 5.34; N, 3.40%).
(Found: C, 63.87; H, 6.56; N, 4.40. Calc. for $C_{17}H_{21}O_5N$: C, 63.95; H, 6.58; N, 4.39%).
(Found: C, 67.35; H, 6.68; N, 5.81. Calc. for $C_{27}H_{32}O_6N_2$: C, 67.50; H, 6.67; N, 5.83%).
(Found: C, 64.43; H, 7.60; N, 6.26. Calc. for $C_{24}H_{34}O_6N_2$: C, 64.57; H, 7.62; N, 6.28%).

General procedure for a two step synthesis of N-t-Boc, O-phenacyl esters of proline and secondary aminoacids. N-t-Boc proline phenacyl ester: N-t-Boc-proline was firstly synthesized as previously reported using t-butyl S-4,6-dimethyl pyrimid-2-yl thiocarbonate[10]. Subsequently, N-t-Boc-proline (0.215 g, 1 mmol) was added to a solution of triethylamine (0.101 g, 1 mmol) in ethyl acetate (10 ml). Phenacyl bromide (0.199 g, 1 mmol) was then added and the mixture was stirred at room temperature for 24 h. The reaction mixture was then treated with a solution of 5% sodium bicarbonate (10 ml) followed by extraction with ethyl acetate (2X20 ml). The ethyl acetate was washed with water (3X10 ml)and dried over anhydrous sodium sulfate; the solvent was removed in vacuo. The crude N-t-Boc-proline phenacyl derivative was obtained in semi-crystallized form. Recrystallization from ethyl acetate/pet.ether (8:2) afforded 0.305 g (91%); m.p. = 80 -82°C; $[a]_D^{25}$ = -93.73 (C1, $CHCl_3$).

General procedure for a one pot synthesis of N-t-Boc, O-phenacyl esters of proline and secondary amino acids. N-t-Boc-proline phenacyl ester. To a stirred solution of proline (1.15 g, 10 mmol) and triethylamine (1.44 ml, 10 mmol) in water (5 ml), di-tert butyl dicarbonate (2.18 g, 10 mmol) in dioxane (5 ml) was added. After three hours at room temperature, triethylamine (1.44 ml, 10 mmol) was added and then a solution of phenacyl bromide (2.00 g, 10 mmol) in dioxane (2 ml).The reaction mixture was kept stirring at room temperature for 5 hours. Water (80 ml) and ethyl acetate (80 ml) were then added. The ethyl acetate was then washed with 5% sodium bicarbonate (80 ml), 5% hydrochloric acid (80 ml) and water (80 ml). The solvent was then evaporated in vacuo.The remaining oily residue (2.030 g) was found to be homogenious on t.l.c. and was crystallized easily from ethyl acetate/pentane, affording 0.960 g. A second crop of crystals (0.408 g) was obtained from the mother liquid (yield 42%).

N-t-Boc-proline-phenylalanine phenacyl ester. Mixed anhydride method[19,20]. To a chilled solution of N-t-Boc proline (1.6 mmol) in dry THF (3 ml), N-methyl morpholine (1.6 mmol) and isobutyl-chlorocarbonate (1.6 mmol) were added. The mixture was kept for 15 min at 0° and another 15 min at room temperature and then mixed with 1 mmol phenylalanine phenacyl ester trifluoroacetate and N-methyl morpholine (1 mmol) in THF (2 ml). After 3 h at room temperature the solvent was evaporated in vacuo.The remaining residue was taken up with ethyl acetate (10 ml), washed with 5% $NaHCO_3$ (10 ml X 2), 10% citric acid (10 ml) and water (10 ml) and dried over Na_2SO_4. The solvent was evaporated under vacuum and the oily residue (0.330g) was crystallized in the refrigerator.It was recrystallized from ethyl acetate -hexane. Yields and physical properties are given in Table IV.

N-t-Boc-proline-isoleucine phenacyl ester. This compound was prepared from N-t-Boc proline (1.6 mmol) and isoleucine phenacyl ester trifluoroacetate (1 mmol) in a similar manner to that described for N-t-Boc-proline-phenylalanine phenacyl ester. The product was crystallized from ethyl acetate / hexane. Yields and physical properties are given in Table IV.

N-t-Boc-proline 4-bromophenacyl ester. This compound was prepared from N-t-Boc-proline (1 mmol) and 4-bromophenacyl bromide in a similar manner to that described for N-t-Boc-proline phenacyl ester. Yields and physical properties are given in Table IV.

Abbreviations: Standard abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature as found in Biochemistry 14, 449 (1975)., Biochem. J. 126, 773 (1972). All optically active amino acids used were of the L configuration. Other abbreviations used are: Pro, proline; 4Hyp, 4-hydroxyproline; 4BrPE, 4 bromo phenacyl; Aze, azetidine-2-carboxylic acid; Pip, pipecolic acid; Boc, t-butyloxycarbonyl; PE, phenacyl; PEBr, phenacyl bromide; Ile, isoleucine; Phe, phenylalanine; Trityl and Trt, triphenylmethyl; Bzl, benzyl; EtOAc, ethyl acetate; DCC , dicyclohexyl carbodiimide; HOBt, 1-hydroxybenzotriazole; t.l.c.; thin-layer chromatography; $CHCl_3$; chloroform; CF_3COOH, trifluoroacetic acid; Et_2O, diethyl ether; ANGII, angiotensin II.

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