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SYNTHESIS OF DIAZOKETONES DERIVED FROM α-AMINO ACIDS; PROBLEM OF SIDE REACTIONS

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Abstract - Optimum conditions of synthesis of eight diazoketones derived from optically active N-(t-butyloxycarbonyl)- and N-benzyloxycarbonylamino acids have been described. The problem of formation of by-products during Arndt-Eistert synthesis of β-homoamino acids at the stage of reaction of mixed anhydride with a weak nucleophile-diazomethane - has been discussed.

It was found already in early forties that Wolff's rearrangement of diazoketones with chiral center next to the carbonyl group proceeds with retention of configuration, without appreciable racemization.[1-3] The observation formed a basis for synthesis of homologous, optically active carboxylic acids by Arndt-Eistert method. Balenovic and co-workers obtained a series of Phth-β-homoamino acids in this way.[4] Rudinger described an elegant synthesis of L-α- and L-β-aminoadipic acids from L-glutamic acid introducing p-toluenosulfonic group as a protection of amino function.[5] However, in more recent papers concerning homologation of optically active N-(t-butyloxycarbonyl)- and N-benzyloxycarbonyl-α-amino acids, striking divergences of the physicochemical data of the compounds obtained by different research centers can be observed. This is true of both, intermediates-diazoketones and β-homoamino acids.[6-10]

Our interest in β-homoamino acids was related to the possibilities in the field of structural diagnostic created by their introduction into molecules of biologically active peptides, in place of an appropiate α-amino acid. The original project aimed at a synthesis of L-β-homophenylalanine of unquestionable chemical homogeneity. The syntheses of N-(t-butyloxycarbonyl)-L-β-homophenylalanine utilizing the sequence of Arndt-Eistert reactions described in literature seemed to lead in effect to two different compounds.[8,9]

RESULTS AND DISCUSSION

By applying general rules of methodology described by B.Penke and co-workers[10] (Scheme 1a) we obtained, with excellent yields, a few diazoketones interesting to us, derived from optically active N-(t-butyloxycarbonyl)- and N-benzyloxycarbonyl-α-amino acids 1 - 8 (Table 1).

Scheme 1

a. $Y-NH-CHR-COOH \xrightarrow[2.\,CH_2N_2,\ -5^\circ]{1.\,iBuOCOCl,\ -20^\circ} Y-NH-CHR-COCHN_2$

b. $Y-NH-CHR-COCHN_2 \xrightarrow[2.\,H^+]{1.\,Ag_2O,\ OH^-,\ 75\text{-}85^{\circ}*} Y-NH-CHR-CH_2-COOH$

Y = $C_6H_5CH_2OCO$- or $(CH_3)_3COCO$-, R - side chain of amino acid

*- the temperature of Wolff rearrangement depends on Y and R

Melting points of nearly all the diazoketones synthesized in our laboratory differ significantly from the ones listed in the paper by Penke.[10]

Table 1. Comparison of melting points of some diazoketones.

	Compound	mp, °C 10	mp, °C this work
1	Z-L-Phe-DAM	84 - 85	81,5 - 82
2	Z-D-Phe-DAM	-	81 - 82
3	Z-L-Ala-DAM	oil	89 - 90
4	Z-L-Ile-DAM	48 - 50	67 - 68
5	Boc-L-Phe-DAM	67 - 70	83,5 - 85
6	Boc-L-Tyr Bzl -DAM	-	122 - 122,5
7	Boc-L-Ile-DAM*	-	87 - 88
8	Boc-L-Pro-DAM	oil	40 - 41

DAM - diazomethane

*this compound was obtained earlier.[11]

The examination of IR, ^1H NMR and mass spectra, as well as the results of elementary analysis (experimental part, A) leads us to the belief that the diazoketones presented in this paper, i.e. intermediates in the synthesis of β-homoamino acids, are pure chemical individuals.

In order to explain the observed inconsistencies we focused our attention on specific reactivity of N-protected amino acids containing activated carboxyl group, which in case of synthesis of diazoketones 1 - 8 were mixed anhydrides. The by-products, which were not removed during extraction due to their neutral character and thus could substantially influence the degree of purity of the diazoketones, were in the first place looked for in the filtrates.

Apart from the bands characteristic of pure diazoketones ca. 2100 (CHN_2), 1700-1680 (CO uretan; only CO band of 3 has the frequency at 1720 cm^{-1}), 1640--1625 cm^{-1} (CO ketone), the IR spectra of each of the described eight crude diazoketones contained also three other bands, viz, 1825, 1750 and 1730-1720 cm^{-1}.

The paper presents the results of investigations on the oily fraction 1' (experimental part, B) left after synthesis of N-benzyloxycarbonyl-L-phenylalanyldiazomethane, since the degree of contamination of this diazoketone with by-products was the highest.

The presence of a band of high intensity at 1750 cm^{-1} proves that the post--reaction mixture contained methyl ester of N-benzyloxycarbonyl-L-phenylalanine. This was farther confirmed by mass spectrum obtained by FD technique of the oily

fraction 1', containing a peak at m/e = 313.3 related to the parent ion of methyl ester. The presence of the ester has also been identified by tlc technique; the R_F value of one of the spots present on chromatograms of 1' (experimental part,B) is identical to the R_F value of methyl ester obtained from N-benzyloxycarbonyl-L--phenylalanine and diazomethane. A gradual dissapearance of the mentioned spot accompanied by formation of a respective amide was observed on the chromatograms obtained after the reaction of 1' with dimethylamine.

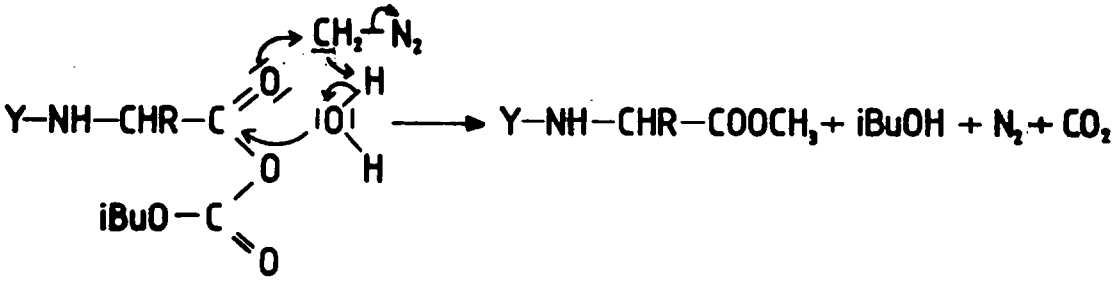
During the synthesis of N-benzyloxycarbonyl-L-prolyldiazomethane via mixed anhydride M.Cassal and co-workers isolated ca. 14% of methyl ester of N-benzyl-oxycarbonyl-L-proline from the filtrate.[7]

Large amounts of methyl ester, formed in addition to the main product - diazoketone, seem to exclude its origin from the reaction of non-reacted protected amino acid and diazomethane; formation of mixed anhydrides under standard conditions is nearly quantitative.[12] It is also confirmed by the experiments connected with the synthesis of peptide bond that mixed anhydrides are acylation reagents of relatively low activity towards water; no decrease of yield of their aminolysis is observed in solutions containing up to 30% of water.[13] For this reason we excluded the possibility of N-benzyloxycarbonyl-L-phenylalanine being formed as a result of secondary reaction.

The idea that another mechanism, different from the typical reaction of formation of methyl esters from carboxylic acids and diazomethane, can be taken into consideration arose also as a result of our observations that large and comparable concentrations of methyl ester of the protected amino acid were always found in the oily fraction 1', independently of the duration of activation of N--benzyloxycarbonyl-L-phenylalanine with isobutyl chloroformate (Scheme 1a).

We think that most of the methyl ester can be formed in the reaction of a mixed anhydride with the HOH --- CH_2N_2 adduct (Scheme 2, the way of obtaining of diazomethane from N-methyl-N-nitrosourea does not allow a complete removal of water from etheral solution) suggested here, which is competitive to the reaction with diazomethane acting as a nucleophilic reagent.

Scheme 2



Y and R see Scheme 1

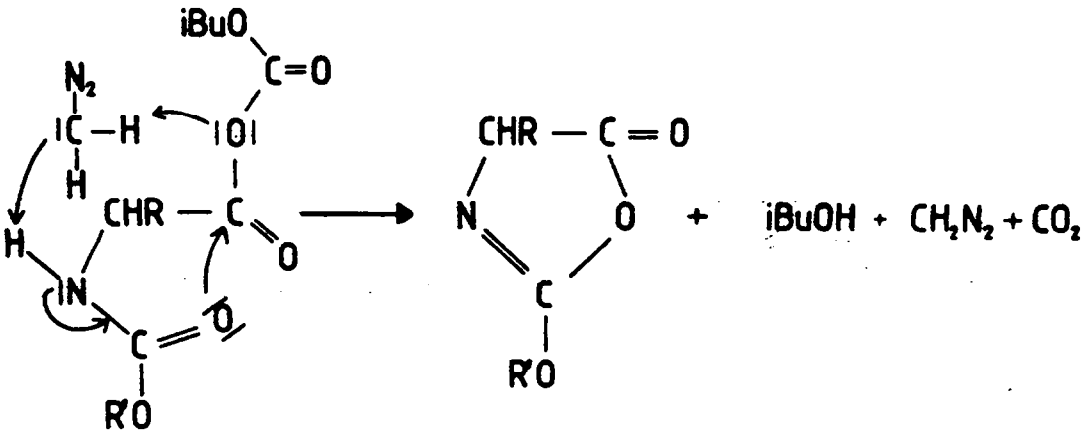
The acid-base type HOH --- CH_2N_2 adduct can not only increase the activity of water molecule as a nucleophilic reagent, but is also able to promote the formation of a tri-molecule cyclic active complex (Scheme 2) due to the specific character of diazomethane, which can at the same time act as both electro- and nucleophile. The complex can be stabilized by energetically advantageous conversions - generation of N_2 and CO_2 molecules-results in the formation of methyl ester of N-alkoxycarbonylamino acid and isobutyl alcohol.

The presence of a band at 1825 cm^{-1} in the IR spectrum of crude N-benzyl-

oxycarbonyl-L-phenylalanyldiazomethane has been attributed to the formation of a compound with activated carboxyl group. Our experiments (experimental part, B) have indicated its high reactivity towards benzyl ester of phenylalanine and dimethylamine, but much lower reactivity towards methanol. The compound did not react with diazomethane at temperatures ranging from -5 to 0°C. Such a behaviour excluded the possibility of the presence of non-reaced mixed anhydride, while suggesting that 2-benzyloxy-4-benzyl-5(4H)-oxazolone could be formed; a compound which was very controversial until Benoiton confirmed its existance in numerous papers.[14-16] In the mass spectrum of 1' we did observe a distinct peak at m/e = 281.3 related to the parent ion the discussed oxazolone. By means of tlc, carried out rapidly enough, we have also managed to trace the formation of oxazolone from N-benzyloxycarbonyl-L-phenylalanine and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide under standard conditions described by Benoiton.[14] The R_F value of 2-benzyloxy-4-benzyl-5(4H)-oxazolone corresponded to R_F = 0.63 of one of the spots present on chromatograms of oily fraction 1' (experimental part, B). We have also found out that benzyl ester of N-benzyloxycarbonylphenylalanylphenylalanine is formed as a result of reaction of 1' with benzyl ester of phenylalanine (experimental part, B).

The present results explicitly indicate the existence of 2-alkoxy-4-alkyl--5(4H)-oxazolone among the products of reaction in the synthesis of diazoketones from mixed anhydrides of N-alkoxycarbonylamino acids and diazomethane. In our opinion, in this case diazomethane can function in two ways - either as a weak nucleophilic reagent it reacts with activated carboxyl group towards formation of a diazoketone (Scheme 1a) or as a base it promotes cyclization of a mixed anhydride to 2-alkoxy-4-alkyl-5(4H)-oxazolone[17] (Scheme 3).

Scheme 3



R' = $C_6H_5CH_2$-or $(CH_3)_3C$-, R = side chain of amino acid

A case is described in literature in which only a cyclic product identified as 2-benzyl-5(4H)-oxazolone was obtained in the reaction of hippuric acid chloride and diazomethane, instead of the expected diazoketone.[18] Karrer inaccurately explains the fact claiming that diazomethane facilitated dehydrochlorination. It is very interesting that 2-benzyl-5(4H)-oxazolone, did not react with diazomethane present in excess in the reaction mixture.

The third of the „extra" absorption bands lying at 1730-1720 cm^{-1}, observed in IR spectra of crude diazoketones after extraction with $NaHCO_3$, can be attributed to the presence of N-protected amino acid formed as a consequence of the

decomposition of unstable 2-benzyloxy-4-benzyl-5(4H)-oxazolone. A peak lying at m/e = 299.3 corresponding to mass of N-benzyloxycarbonylphenylalanine can be observed in mass spectrum of the oily fraction 1'.

On the basis of the obtained experimental data we think that the inconsistencies in the description of physical properties of β-homoamino acids in literature result from the application of diazoketones which were not completely purified before the successive stage of Arndt-Eistert synthesis - Wolff's rearrangement (Scheme 1b). Since both a methyl ester and an oxazolone undergo hydrolysis under alkaline conditions then as a result, apart from an appropriate product of the rearrangement - a β-homoamino acid of unchanged configuration of the β-carbon atom, some L and D isomers of the parent amino acid are formed. All of the products are characterized by similar physical properties, hence chemically pure homologue can be difficult to obtain.

We would also like to stress the fact that low reactivity of 2-benzyloxy-4--benzyl-5(4H)-oxazolone towards diazomethane - which we have observed, justifies the omission of the question of its chiral instability[19,20,21] in the synthesis of diazoketones derived from α-amino acids protected with a urethane group.

The diazoketones 1 - 8 have been transformed into respective β-homoamino acids by Wolff's rearrangement. Their chemical homogeneity was unquestionable. In the experimental part C we describe the transformation of N-benzyloxycarbonyl- and N-(t-butyloxycarbonyl)-L-β-homophenylalanine into L-β-homophenylalanine. Both the N-protected amino acids obtained in the Arndt-Eistert reaction lead to an identical compound (experimental part, C), differing from the one described by Chaturvedi.[8]

EXPERIMENTAL

All melting points were uncorrected. IR spectra were recorded on a Specord 71 IR Spectrometer (nujol). Optical rotation were determined on a Hillger-Watts polarimeter. ^{1}H NMR spectra were recorded at 80 MHz on a Tesla BS567A spectrometer. Chemical shifts are reported as δ values in ppm downfield from tetramethylsilane as interal standard. Splitting patterns are indicated as s, singlet; d, dublet; t, triplet; q, quartet; m, multiplet; br, broad peak. FD mass spectra were recorded with MAT 711 apparatus. Analytical thin layer chromatography (tlc) was performed with Merck precoated tlc plates (silica gel 60 F_{254}). All solvents were dried and distilled by standard procedures. All diazoketones 1 - 8 were obtained in accordance with the procedure described for N-benzyloxycarbonyl-L-phenylalanyldiazomethane; average yields: 70 - 80 %.

A/ Diazoketones Synthesis

N-benzyloxycarbonyl-L-phenylalanyldiazomethane (Z-L-Phe-DAM), 1. N-benzyloxycarbonyl-L-phenylalanine (12 g, 40 mmol) was dissolved in a mixture of dry diethyl ether tetrahydrofuran (200 cm^3, 1:1) and triethylamine (5.56 cm^3, 40 mmol). Isobutyl chloroformate (5.44 cm^3, 40 mmol) was added to a cooled to -20°C and vigorously stirred solution. The stirring was continued for 15 min. while the temperature was maintained at -20°C. A very carefully dried, cooled etheral solution of diazomethane (obtained from 8 g, 80 mmol of N-methyl-N-nitrosourea) was added. The mixture was stirred for 1 h at -10°C and another 12 h was stored in a refrigerator at 5°C. The solvents were removed in vacuo to a 1/3 of starting volume and a fresh portion of diethyl ether was added. The reaction mixture was washed with a saturated, cooled $NaHCO_3$, H_2O and dried over $MgSO_4$ anh. The solvent was removed in vacuo at 5°C. Dry benzene was poured to an oily residue then evaporation was repeated and n-hexane added. The crude diazoketone 1 was

filtered off yielding 9.6 g (75 %). n-Hexane filtrate was concentrated in vacuo at 5°C and stored in refrigerator firmly closed, as 1'. After two more recrystallizations from n-hexane/benzene, a highly homogeneous crystalline, yellowish Z-L-Phe-DAM was obtained. tlc: carbon tetrachloride/tetrahydrofuran 2:0,25; n-hexane/acetone 3:2; mp 81 - 82.5°C; $[\alpha]_D^{20}$ -42° (c1, methanol); IR: 2100 (CHN_2), 1700 (CO urethane), 1625 (COCH); ^{1}H NMR δ ($CDCl_3$): 2.9 (2H,d,$CHCH_2$), 4.5 (1H,m, $CHCH_2$), 5.02 (2H,s,$CH_2C_6H_5$), 5.14 (1H,s,CHN_2), 5.37 (1H,d,NH), 7.2 (5H,m,C_6H_5), 7.27 (5H,s,C_6H_5); MS (FD) m/e: 323 (M^+), 254 (M^+-$COCHN_2$); Found: C, 67.09; H, 5.4; N, 12.5. Calc. for $C_{18}H_{17}O_3N_3$: C, 66.8; H, 5.30; N, 12.99 %.

N-benzyloxycarbonyl-D-phenylalanyldiazomethane (Z-D-Phe-DAM), 2. All analytical data as 1 except the sing of specific rotation; $[\alpha]_D^{20}$ +42 (c1, methanol).

N-benzyloxycarbonyl-L-alanyldiazomethane (Z-L-Ala-DAM), 3. The synthesis was performed in diethyl ether. The crude material was recrystallized from n-hexane/ethyl acetate and n-hexane/benzene; chrom. homogeneous, tlc: carbon tetrachloride/acetic acid/methanol 5:0.2:1; mp 89 - 90°C; $[\alpha]_D^{20}$ -60° (c2, methanol); IR: 2056 (CHN_2), 1720 (CO urethane), 1646 (COCH); ^{1}H NMR δ ($CDCl_3$): 1.3 (3H,d,$CHCH_3$), 4.32 (1H,m,$CHCH_3$), 5.1 (2H,s,$CH_2C_6H_5$), 5.42 (1H,s,CHN_2), 5.5 (1H,m,NH), 7.37 (5H, s,C_6H_5); MS (FD) m/e: 248.1 (M^+ +1), 178 (M^+ -$COCHN_2$); Found: C, 58.57; H, 5.29; N, 16.29, Calc. for $C_{12}H_{13}O_3N_3$: C, 58.2; H, 5.29; N, 16.98 %.

N-benzyloxycarbonyl-L-izoleucyldiazomethane (Z-L-Ile-DAM), 4. The synthesis was performed in diethyl ether. The crude material was recrystallized from n-hexane/ethyl acetate; chrom. homogeneous, tlc: n-hexane/acetone 2:3; mp 67 - 68°C; $[\alpha]_D^{20}$ -44° (c 0.5, methanol); IR: 2100 (CHN_2), 1688 (urethane), 1638 (COCH); ^{1}H NMR δ ($CDCl_3$): 0.83 (6H, d+t overlap., CH_2CH_3 and $CHCH_3$), 1.1 (2H,m,CH_2CH_3), 1.52 (1H, m,$CHCH_3$), 4.12 (1H,m,CHNH), 5.05 (2H,s,$CH_2C_6H_5$), 5.37 (2H,br. d+s, NH and CHN_2), 6.32 (5H,s,C_6H_5); MS (FD) m/e: 289.3 (M^+), 261.3 (M^+ -N_2), 220.2 (M^+ -$COCHN_2$); Found: C, 62.60; H, 6.65; N, 14.42, Calc. for $C_{15}H_{19}O_3N_3$: C, 62.27; H, 6.62; N, 14.52 %.

N-(t-butyloxycarbonyl)-L-phenylalanyldiazomethane (Boc-L-Phe-DAM), 5. This compound was synthesized in diethyl ether. The crude diazoketone was recrystallized from ethyl acetate/n-hexane; chrom. homogeneous, tlc: n-hexane/acetone 3:2, mp 83.5 - 85°C; $[\alpha]_D^{20}$ -40 (c1, methanol); IR: 2100 (CHN_2), 1690 (CO urethane), 1640 (COCH); ^{1}H NMR δ ($CDCl_3$): 1.07 (9H,s,$C(CH_3)_3$), 2.95 (2H,d,$CHCH_2$), 4.27 (1H,m, $CHCH_2$), 5.25 (1H,d,NH), 5.37 (1H,s,CHN_2), 7.23 (5H,s,C_6H_5); MS (FD) m/e: 289.3 (M^+), 220.1 (M^+ -$COCHN_2$); Found: C, 62.4; H, 6.40; N, 14.21, Calc. for $C_{15}H_{19}O_3N_3$: C, 62.28; H, 6.60; N, 14.51 %.

N-(t-butyloxycarbonyl)-L-(O-benzyl)tyrozyldiazomethane (Boc-L-Tyr(Bzl)-DAM), 6. This synthesis was performed in dry diethyl ether/dioxane. The crude material was recrystallized from n-pentane/benzene; chrom. homogeneous, tlc: carbon tetrachloride/acetic acid/methanol 5:0.2:1; mp 122 - 122.5°C; $[\alpha]_D^{20}$ -11 (c2, methanol); IR: 2085 (CHN_2), 1690 and 1670 (CO urethane), 1640 (COCH); ^{1}H NMR δ ($CDCl_3$): 1.4 (9H, s,$C(CH_3)_3$), 2.95 (2H,d,$CHCH_2$), 4.35 (1H,m,$CHCH_2$), 5.0 (2H,s,$CH_2C_6H_5$), 5.19 (1H,s, CHN_2), 5.1 - 5.3 (1H,d,NH), 7.00 (4H,"q",p-C_6H_4), 7.37 (5H,s,C_6H_5); MS (FD) m/e: 395.1 (M^+), 354.8 (M^+ -CHN_2); Found; C, 67.10; H, 6.41; N, 10.25, Calc. for $C_{22}H_{25}O_3N_3$: C, 66.82; H, 6.37; N, 10.62 %.

N-(t-butyloxycarbonyl)-L-izoleucyldiazomethane (Boc-L-Ile-DAM), 7. This compound has earlier been obtained and described without complete analytical data.[11] $[\alpha]_D^{20}$ -65 (c1, methanol); IR: 2100 (CHN_2), 1680 (CO urethane), 1650 (COCH); ^{1}H NMR δ

($COCl_3$): 0.87 (6H,t+d,CH_3CH_2 and CH_3CH), 1.3 (11H,s+m,$C(CH_3)_3$ and CH_2CH_3), 1.3 - - 1.73 (1H,br.m,$CHCH_3$), 4.06 (1H,m,NHCH), 5.18 (1H,d,NH), 5.42 (1H,s,CHN_2); MS (FD) m/e: 186.3 (M^+ -$COCHN_2$); Found: C, 56.20; H, 8.22; N, 16.35, Calc. for $C_{12}H_2O_3N_3$: C, 56.44; H, 8.29; N, 16.45 %.

N-(t-butyloxycarbonyl)-L-prolyldiazomethane (Boc-L-Pro-DAM), 8. The synthesis was performed in dry diethyl ether. The crude material was recrystallized from n-hexane; chrom. homogeneous, tlc: carbon tetrachloride/acetic acid/methanol 5:0.2:1; mp 40 - 41°C; $[\alpha]_{546}^{21}$ -138.7 (c1, methanol); IR: 2080 (CHN_2), 1685 (CO urethane), 1625 (COCH); ^{1}H NMR δ ($CDCl_3$): 1.42 (9H,s,$C(CH_3)_3$), 2.0 (4H, overlap. two m, CH_2-CH_2-CH), 3.45 (2H,t-like,CH_2-N), 4.25 (1H,q,CH-CH_2), 5.45 (1H,br,CHN_2); MS (FD) m/e: 170.2 (M^+ -$COCHN_2$); Found: C, 55.10; H, 7.05; N, 17.42, Calc. for $C_{11}H_{17}O_3N_3$: C, 55.20; H, 7.10; N, 17.56 %.

B/ Experiments on the Oily Residue 1'

Some analytical data of 1': IR: 1820, 1750, 1720 cm^{-1} - we described those bands as belonging to a carbonyl group of oxazolone, methyl ester and free acid, respectively (see text); MS (FD) m/e: 313.3 (M^+, methyl ester), 299.4 (M^+ free acid), 281.3 (M^+ oxazolone); tlc: dry carbon tetrachloride/tetrahydrofuran 2:0.25, R_f = 0.63 (oxazolone), R_f = 0.6 (methyl ester), R_f = 0.02 (free acid), (the chromatogram has to be developed immediately after the solution of 1' is placed on the tlc plate). To have a clear picture we did not specified those bands, peaks and spots associated with the presence of a residual diazoketone 1.

Reaction of 1' with diazomethane. 1' (0.02 g) was treated with an excess of an etheral solution of diazomethane at 5°C for 24 h. The solvent was evaporated. There was not an observable change of IR spectrum of the oily residue (the band of methyl ester was already present in IR spectrum).

Reaction of 1' with methanol. 1' (0.02 g) was dissolved in dry methanol (10 cm^3) and stored at 5°C. There was not a significant change in IR spectrum after 12 h. The mixture was left at 5°C for another 36 h. The solvent was removed in vacuo. IR: 1820 cm^{-1} - absent; tlc: R_f = 0.63 - absent; MS (FD) m/e: 281.3 - absent.

Reaction of 1' with dimethylamine. 1' (0.03 g) was dissolved in methanolic (30 %) solution of dimethylamine (10 cm^3) at 5°C. After 1 h the solvent was evaporated in vacuo. IR: 1820 - absent; tlc: R_f = 0.63 - absent, R_f = 0.15 - present (it was identified as N-benzyloxycarbonyl-L-phenylalanine dimethylamide in model reaction); MS (FD) m/e: 281.3 - absent, 326.4 - present (it was identified as M^+ of N-benzyloxycarbonyl-L-phenylalanine dimethyl-amide).

Reaction of 1' with L-phenylalanine benzyl ester. 1' (0.04 g) was dissolved in dry tetrahydrofuran, cooled to 0°C and treated with L-phenylalanine benzyl ester (obtained from L-phenylalanine benzyl ester p-toluensulfonate, (0.042 g 0.1 mmol)). The reaction mixture was kept at 0°C for 2 h and tetrahydrofuran was then removed in vacuo. Diethyl ether was added to the oily residue. The organic layer was washed with cooled 1N HCl, H_2O, dried over $MgSO_4$ anh. and evaporated in vacuo. IR: 1820 cm^{-1} - absent (this band was also not observed, if the mixture was not washed with 1N HCl); tlc: R_f = 0.63 - absent, R_f = 0.37 - present (this spot was identified as N-benzyloxycarbonylphenylalanylphenylalanine benzyl ester, in model reaction); MS (FD) m/e$^+$: 281 -absent, 536.1 - present (M^+ of N-benzyloxycarbonyl-phenylalanylphenylalanine benzyl ester).

C/ L-β-homophenylalanine (L- β Hph). 12

N-(t-butyloxycarbonyl)-L-β-homophenylalanine (Boc-L- β Hph), 9. This compound was obtained as result of Wolff's rearrangement of 5 in presence of Ag_2O and $Na_2S_2O_3$ at 81 - 85°C. A stirring mixture of Ag_2O (2 g, 8 mmol), Na_2CO_3 anh. (2 g, 9 mmol), $Na_2S_2O_3 \times 5H_2O$ (2 g, 8 mmol), and H_2O was heated until temperature of 80°C was reached. Boc-L-Phe-DAM, 7 (6.46 g, 20 mmol) was dissolved in dioxane (50 cm^3) and gradually added in ten portions, through the period of 1.5 h keeping the temperature in the range 81 - 85°C. Meantime, three portions of Ag_2O (each of 1 g) was added. After the evolution of nitrogen had ceased, the reaction mixture was cooled, diluted with H_2O, filtered and extracted with three portions of diethyl ether. The aqueous phase was cooled to 5°C, acidified with 0,1N HCl, extracted with ethyl acetate and dried over $MgSO_4$ anh. The solvent was removed in vacuo and a crude material was crystallized from n-hexane/ethyl acetate. Two more crystallizations gave homogeneous Boc-L- β Hph. Yield: 72 %; mp 100 - 102°C; $[\alpha]_D^{20}$ -15 (c2, dimethylformamide); chrom. homogeneous, tlc: benzene/acetone/acetic acid 2:1:0.2; ^{1}H NMR δ ($CDCl_3$): 1.25 (9H,s,$C(CH_3)_3$), 2.4 (2H,d,CH_2COOH), 2.77 (2H, d,$CH_2C_6H_5$), 4.03 (1H,m,NHCH), 5.0 (1H,m,NH), 7.12 (5H,s,C_6H_5), 8.3 (1H,br,s,COOH); lit.: (9) mp 98 - 100°C, $[\alpha]_D^{24}$ -13 (c2, dimethylformamide); lit.: (8) yellowish oil.

N-benzyloxycarbonyl-L-β-homophenylalanine (Z-L- β Hph), 10. This compound was obtained by procedure indicated for 9. The temperature of Wolff's rearrangement 80 - 81°C; Yield: 75 %; mp 118 - 119°C, $[\alpha]_D^{20}$ -38 (c1,AcOH); chrom homogeneous, tlc: benzene/acetone/acetic acid 2:1:0.2; ^{1}H NMR δ ($CDCl_3$): 2.3 (2H,d,CH_2COOH), 2.75 (2H,d,$CH_2C_6H_5$); 4.12 (1H,m,NHCH), 4.9 (2H,s,$OCH_2C_6H_5$), 5.25 (1H,m,NH), 7.07 (5H,s,$CHCH_2C_6H_5$), 7.15 (5H,s,$OCH_2C_6H_5$), 9.52 (1H,br.s,COOH); lit.: (8) mp 110 - - 111°C.

L-β-homophenylalanine trifluoroacetate (TFA·H-L- β Hph), 11. Boc-L- β Hph, 9, (2.02 g, 7.2 mmol) was dissolved in trifluoroacetic acid (15 cm^3). After 15 min. dry diethyl ether (20 cm^3) was added and solvents were evaporated in vacuo. This operation was repeated twice. The solidifying material was triturated with dry ether and left for 1 - 2 h in refrigerator. The solid trifluoroacetate was filtered, washed thoroughly with ether and dried in vacuo. Yield: 1.8 g (85 %); mp 131°C; chrom. homogeneous, tlc: n-butanol/acetic acid/water 4:1:5 (upper phase).

L-β-homophenylalanine (H-L- β Hph), 12. TFA H-L- β Hph, 11 (0.25 g) was dissolved in water (1 cm^3 and passed through a column with Amberlit IR 45 (acetate form). The column was washed successively with water and 10 % acetic acid. Ninhydrine positive fractions were combined and concentrated in vacuo. The residue was treated twice with dry benzene. The crude amino acid was precipitated from a solvent mixture diisopropyl ether/methanol and filtered off. Recrystallization from hot water gave 2.1 g (87 %) of H-L- β Hph; mp 210 - 211°C dec.; $[\alpha]_D^{22}$ +8.5 (c1.5,H_2O); IR: 3150 - 2700 (NH_3^+, CH_{arom}), 1650 (C-C_{arom}), 1560 (CO_2^-); ^{1}H NMR δ (trifluoroacetic acid): 2.62 - 3.12 (4H,2d overlap., $CH_2C_6H_5$ and CH_2COOH), 3.85 (1H,m,NH_3^+CH), 6.8 (3H,m,NH_3^+), 7.12 (5H,s,C_6H_5); MS (FD) m/e: 180.19 (M^+ +1); Found: C, 67.21; H, 7.32; N, 7.80, Calc. for $C_{10}H_{13}O_2N$: C, 67.02; H, 7.31;N, 7.81 %. lit.: (8) mp 225 - 226 C, $[\alpha]_D^{22}$ +22.1 (c1.5,H_2O). Hydrogenation of 10 gave also a product which was identical with 12 obtained through acidolysis of 9, but quite different from the product described in Chaturvedi's work.[8]

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