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Optically Active Aromatic Amino Acids. Part V: Some *N-t*-Butyloxycarbonyl-*O*-methyl-L-tyrosine Analogues with Ring Substitution at Position 3

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Abstract: Six new derivatives of Boc-L-Tyr(Me)-OH have been prepared, with the following substituents at ring position 3: $-CO_2Me$, $-CO_2Et$, –CHO, $-CH_2OH$, $-CH_2OBzl$ and –(E)–CH=NOH. © 1997 European Peptide Society and John Wiley & Sons, Ltd.

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New unusual amino acids, whether obtained from natural sources or synthesized, are of interest for diverse reasons. One application is in the synthesis of peptide analogues with improved chemical and biological properties [1,2]. The tyrosine hydroxyl group is sometimes a key feature in peptide–receptor interaction. Elimination or blocking of this group often causes a dramatic decrease in biological activity [3–6]. Replacement of this group with others, such as $-NH_2$, $-NO_2$, –F, –Me, $-N_3$ can also result in a reduction of biological activity [5,6].

In the present work new substituents were introduced into the aromatic ring of tyrosine, and the phenol group itself was methylated (Figure 1). The initial compound in each synthesis was the aldehyde (**1**) as described before [7]. This compound, when oxidized by potassium permanganate in neutral aqueous medium, gives the corresponding blocked carboxytyrosine [7]. In order to replace Ac with Boc blocking groups, this was hydrolysed with 4 M HCl, as described before [8] for the D isomer; the crude hydrochloride (**2**) was esterified with methanol (or ethanol) in the presence of thionyl chloride of the diester obtained (**3Me** and **3Et**). The amino group was blocked by di-*tert*-butyldicarbonate. The blocked diethyl diester (**4Et**) is a low-melting crystalline substance. The dimethyl diester (**4Me**) was obtained analogously, not by crystallization. Brief alkaline hydrolysis (KOH in alcohol) of either diester gave the corresponding monoester (**5Me** or **5Et**).

The structures of the monoesters obtained were confirmed by UV absorption measurements in neutral and alkaline media. The UV spectrum of *o*-methoxybenzoic acid in transition from –COOH to $-COO^-$ form shows a hypsochromic effect at minimum and maximum absorption values, accompanied by a lowered molar extinction value (ε); see Table 1 and Figure 2 [7]. The same hypsochromic shift is shown by UV spectra of (**11**) and its anion.

In order to obtain a blocked 3′-hydroxymethyl-L-tyrosine (**7**), the following scheme was applied.

A short reaction time was used for the **N**-protection of aldehyde (**6**), but the product was accom-

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N-Ac-*O*-Me-3′-CHO-L-Tyr-OEt ($\xrightarrow{3\text{ M HCl}}$)
(**1**)

O-Me-3′-CHO-L-Tyr-OH × HCl ($\xrightarrow[2.\ \text{DCHA}]{1.\ \text{Boc}_2\text{O}}$)
(**6**)

N-Boc-*O*-Me-3′-CHO-L-Tyr-OH × DCHA ($\xrightarrow[\text{or } H_2/PtO_2]{(i\text{-PrO})_3\text{Al}}$)
(**7DCHA**)

N-Boc-*O*-Me-3′-CH_2OH-L-Tyr-OH
(**8**)

panied by tarry contaminants. Chromatography was avoided by preparation of the dicyclohexylammonium salt which was purified by precipitation from chloroform by addition of hexane.

The reduction of aldehyde (**7**) can be performed using aluminium isopropoxide, by the Meerwein–Ponndorf–Verley method [10]; however, the product yield is low (38%). An easier and more efficient method is catalytic reduction with hydrogen in the presence of platinum dioxide.

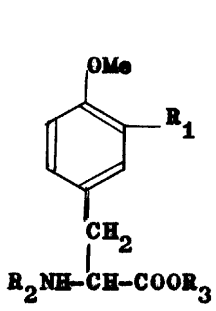
It is known that protection of the hydroxyl group in amino acid side chains (e.g. Ser, Thr) is not absolutely necessary. However, in some peptide syntheses, especially in the solid phase, side reactions may take place [11]. To prevent this when using amino acid (**8**), its *O*-benzyl derivative (**9**) was obtained according to Turan and Manning's method for *N-tert*-butyloxycarbonyl-L-homoserine [12].

The last tyrosine analogue described here, (**10**), was obtained from the corresponding aldehyde (**1**) in a three-stage synthesis. The first stage was a conversion of –CHO group into –CH=NOH; in the second stage the amino and carboxyl groups were unblocked as described earlier [13]. In the *O*-Me-3′-(*E*)-CHNOH-L-Tyr-OH obtained in this way, the amino group was blocked again but with a *tert*-butyloxycarbonyl group, as in the case of other amino acids described here.

The tyrosine analogues described in this work have been incorporated into leucine enkephalin analogues. Some analogues exhibit agonist potency in the guinea pig ileum assay. The results will be presented in a forthcoming paper.

EXPERIMENTAL PART

Melting points (uncorrected) were determined on a Boätius Microscope. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.47 MHz) spectra were recorded on a



R_1	R_2	R_3	
-CHO	Ac	Et	1
-COOH	Ac	Et	11/Lit. [7]
-COOMe/Et/	Boc	H	5Me,5Et
-CHO	Boc	H	7
$-CH_2OH$	Boc	H	8
$-CH_2OBzl$	Boc	H	9
-/E/-CH=NOH	Boc	H	10

Figure 1 Chemical structure of Tyr analogues.

Table 1 Wavelength (λ) and Molar Extinction (ε) Values at UV Absorption Extremes of Various Carboxylic Acids, Measured in Different Solvents

Acids	Solvents			
	EtOH		EtOH + H_2O + NaOH	
	λ_{max} (nm)	λ_{min} (nm)	λ_{max} (nm)	λ_{min} (nm)
o-Methoxybenzoic acid	292(ε3180)	258(ε1100)	280(ε2100)	254(ε600)
N-Ac-*O*-Me-3′-COOH-L-Tyr-OEt	292(ε2330)	260(ε650)	285(ε2330)	258(ε500)
N-Boc-*O*-Me-3′-COOMe-L-Tyr-OH	302(ε3210)	264(ε450)	302(ε3170)	263(ε280)

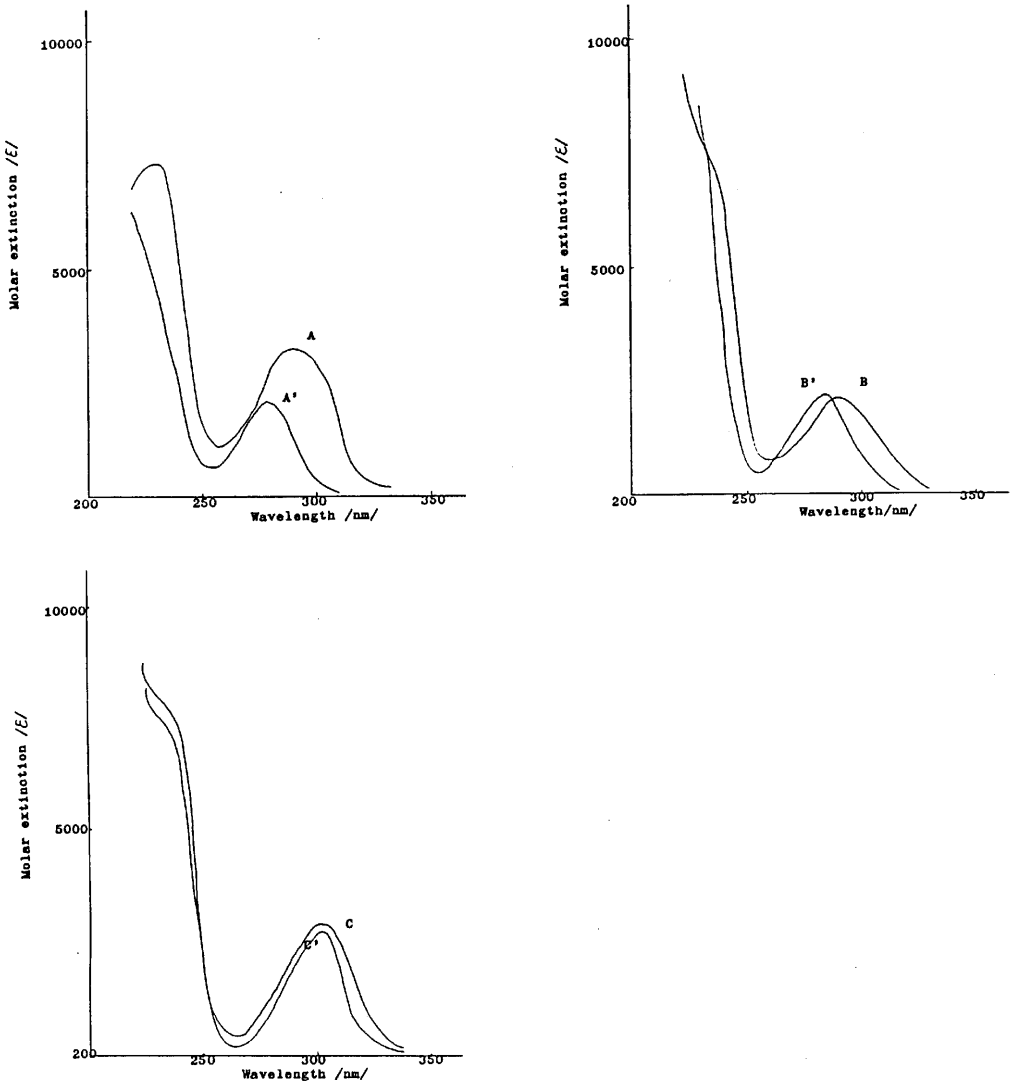


Figure 2 UV spectra of ethanol solutions: *o*-methoxybenzoic acid (A), *N*-Ac-*O*-Me-3′-COOH-L-Tyr-OEt (B), and *N*-Boc-*O*-Me-3′-COOMe-L-Tyr-OH (C) and their spectra in the mixture of EtOH + H_2O + NaOH (A′, B′ and C′ respectively).

Bruker-HX-72 in deuterochloroform in p.p.m. (referred to TMS). MS were recorded on an LKB-9000 instrument at 70 and 15 eV, and FID-MS on an Finnigan MAT 95 instrument. UV spectra were obtained on an Unicam SP 500 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241MC spectropolarimeter with a 1 dm cell. Kieselgel 60F_{254} plates (from Merck) were used for TLC, in the following solvent systems: (A) butanol-acetic acid-water (4:1:1); (B) propanol-ammonia (67:33); (C) ethyl acetate-ethanol (1:1). Compounds were visualized with UV light and chlorine gas according to procedure in [14] and formyl group with 2,4-dinitrophenyl-hydrazine.

O-Methyl-3′-carboxy-L-tyrosine hydrochloride (2)

The compound was obtained according to isomer D [8], m.p. 211–222 °C (decomp.) $[\alpha]_D^{20} = -3.0$ ($c = 1.5$, H_2O), $[\alpha]_D^{20} = -27.8°$ ($c = 1.4$, phosphate buffer pH 7, 0.2 M). Lit. value (for isomer D [8]: m.p. 212–222 °C(decomp.), $[\alpha]_D^{22} = +6.0°$ ($c = 0.7$, H_2O); $[\alpha]_D^{22} = +25.6°$ ($c = 0.6$, phosphate buffer pH 7, 0.2 M).

O-Methyl-3′-carboxyethyl-L-tyrosine ethyl ester hydrochloride (3Et)

To a solution of amino acid hydrochloride (**2**) (5.51 g, 20.0 mmol) in MeOH (200 ml) thionyl chloride (1 ml)

was added dropwise. The mixture was refluxed for 4.5 h and excess methanol was removed by evaporation. The crude ester hydrochloride was dissolved in saturated $NaHCO_3$ (60 ml) and extracted with CH_2CL_2 (360 ml). The combined organic phases were washed with water and dried over $MgSO_4$. The solution of free amine was concentrated to 50 ml, cooled and 15% HCl/EtOH (10 ml) was added. The white crystals of (**3Et**) were collected. Yield 5.29 g (15.9 mmol, 80%), m.p. 197–198 °C, $[\alpha]_D^{20} = +25.6°$ ($c = 1.7$, EtOH). TLC: R_F (B) 0.68. Found: C, 54.34; H, 6.80; N, 4.30; Cl, 11.00%. $C_{15}H_{22}NO_5Cl$ (331.80), requires C, 54.30; H, 6.68; N, 4.22; Cl, 10.69%. ^{1}H-NMR: 1.06–1.39 (6H,2t,CH_2CH_3) 3.09–3.39 (2H,m,CH_2), 3.82 (3H, s,OCH_3), 4.07–4.39 (4H,2q,CH_2CH_3,m), 6.86–7.98), (aromatic protons), 8.79 (3H,s, NH_3^+). MS, m/z (% rel. int.): 295 (1.6,M), 250 (8.9,M-OC_2H_5), 222 (9.6,M-$COOC_2H_5$), 193 (37.9,M-H_2NCHCOO C_2H_5) 179 (11.38, M-CH_2NH_2-$CHCOOC_2H_5$).

O-Methyl-3′-carboxymethyl-L-tyrosine Methyl Ester Hydrochloride (3Me)

The dimethyl ester was obtained like diethyl ester but from a half amount of substrates. Yield 2.23 g (7.4 mmol, 74%), m.p. 194–195 °C, $[\alpha]_D^{20} = +31.0°$ ($c = 1.0$, EtOH). Found: C, 51.52; H, 6.29; N, 4.99; Cl, 11.68%. $C_{13}H_{18}NO_5Cl$ (303.74) requires C, 51.41; H, 5.97; N, 4.61; Cl, 11.67%.

N-tert-Butyloxycarbonyl-*O*-methyl-3′-carboxyethyl-L-tyrosine Ethyl Ester (4Et)

To a solution of diester hydrochloride (**3Et**) (1.49 g, 6.0 mmol) in DMF (14 ml) triethylamine (0.84 ml, 6.0 mmol) was added dropwise under cooling and stirring. To the above suspension di-*tert*-butyldicarbonate (4.31 g, 6.0 mmol) was added. After 20 h of stirring, the mixture was evaporated to dryness and dissolved in chloroform. The solution was washed with 1 M HCl, water, $NaHCO_3$ sat., and water, dried over $MgSO_4$ and evaporated to the oily mass. After a night under light petroleum white crystals of (**3Et**) were collected. Yield 1.80 g (4.55 mmol, 76%), m.p. 39–45 °C, $[\alpha]_D^{20} = +8.8°$ ($c = 1.0$, EtOH). TLC: R_F (B) 0.79. Found: C, 59.96; H, 7.38; N, 3.81%. $C_{20}H_{29}NO_7$ (395.45) required C, 60.75; H, 7.39; N, 3.54%.

N-tert-Butyloxycarbonyl-*O*-methyl-3′-carboxymethyl-L-tyrosine Methyl Ester (4Me)

N-protected dimethyl ester (**4Me**) was obtained as an oil from diester (**3Me**) like N-protected diethyl ester (**4Et**). TLC: R_F (B) 0.79. ^{1}H-NMR: 1.40 (9H,s,$(CH_3)_3C$), 2.99–3.11 (2H,m,CH_2), 3.70 (3H,s,$COOCH_3$), 3.86 (3H,s,OCH_3), 3.87 (3H,s,$COOCH_3$), 4.22–4.58 (1H,m,CH), 4.98–5.02 (1H,d,NH), 7.18–7.59 (3H,m,aromatic protons), 7.65 (1H,broad, COOH). ^{13}C-NMR: 28.29 (t-Boc,CH_3), 37.36 (C_B), 51.95 (Phe-$COOCH_3$), 52.23 (C-$COOCH_3$), 54.53 (C_A), 56.09 (Phe-OCH_3), 79.98 (t-Boc,$C(CH_3)_3$), 155.02 (t-Boc,C=O), 166.41 (Phe-$COOCH_3$), 112.33, 120.01, 127.81, 132.56, 134.25, 158.33 (aromatic carbons), 172.16 (C-$COOCH_3$). The crystalline product (**4Me**) was obtained as a by-product in the synthesis of (**5Me**).

N-tert-Butyloxycarbonyl-*O*-methyl-3′-carboxyethyl-L-tyrosine (5Et)

To a solution of diester (**4Et**) (0.99 g, 2.5 mmol) in EtOH (5 ml) 2.2 M KOH/EtOH (1.14 ml, 2.5 mmol) was added. After 2 h at room temperature the solution was evaporated to the glassy mass, dissolved in $NaHCO_3$ sat. (17 ml) and extracted with diethyl ether (3×). The water phase was cooled, acidified with 4 M HCl and extracted with diethyl ether. The combined organic phases were washed with water and dried over $CaCl_2$ (or $MgSO_4$) and evaporated to the glassy mass. The crude (**5Et**) (0.59 g, 1.6 mmol, 64%) was obtained. Unfortunately the product was contaminated with a small amount of *N*-Boc-*O*-Me-3′-COOH-L-Tyr-OH (probably), TLC: R_F (B) 0.40. Purification was accomplished by repeated dissolving in $NaHCO_3$ sat., cooling, acidifying and extracting like above. After drying and evaporating of extract 0.45 g (1.22 mmol, 49%) of pure product (**5Et**) was obtained as a glassy mass. ^{1}H-NMR: 1.21-1.41 (12H,m,$C(CH_3)_3$, CH_2CH_3), 3.00–3.19 (2H,m,CH_2), 3.67 (3H,s,OCH_3), 4.31–4.38 (2H,q,CH_2CH_3), 4.56, 4.58 (1H,d,CH), 5.03 (1H,s,NH), 6.89–7.59 (3H,m,aromatic protons).

Dicyclohexylammonium Salt of (5Et)

To a solution of (**5Et**) (0.45 g, 1.22 mmol) in chloroform (2 ml) equimolar amount of dicyclohexylamine was added. The product precipitated after adding of light petroleum. Yield 0.42 g (0.76 mmol, 63%), m.p. 137–139 °C, $[\alpha]_D^{20} = +28.1°$ ($c = 1.3$, EtOH). Found: C,

65.10; H, 8.75; N, 5.34%. $C_{30}H_{48}N_2O_7$ (548.72) requires C, 65.67; H, 8.82; N, 5.10%.

N-tert-Butyloxycarbonyl-*O*-methyl-3′-carboxymethyl-L-tyrosine (5Me)

To a solution of diester (**4Me**) (2.80g, 7.62mmol) in MeOH (8.5 ml) 2.19 M KOH/MeOH (3.48 ml, 7.62 mmol) was added. After 2 h at room temperature the solution was evaporated to the glassy mass, dissolved in mixture of $NaHCO_3$ sat. + H_2O (1:1) and extracted (3×) with chloroform. The combined organic phases were washed with $NaHCO_3$ sat. and water, dried over $MgSO_4$, and evaporated. Unreacted diester (**4Me**) (0.2 g) was obtained, m.p. 81–97 °C. ^{1}H-NMR spectrum confirmed the structure and was the same as for amorphous diester (**4Me**) obtained above. The aqueous phase (after extraction with chloroform) was cooled and acidified with 3 M HCl and extracted with chloroform. After separation the organic phase was washed with water, dried over $MgSO_4$ and evaporated to the glassy mass. Yield 2.3 g (6.5 mmol, 85%). TLC: R_F (A) 0.84, R_F (B) 0.65. ^{1}H-NMR: 1.41 (9H,s,$(CH_3)_3$C), 3.02–3.16 (2H,m,CH_2), 3.67 (6H,s,OCH_3,$COOCH_3$), 4.55, 4.57 (1H,d,CH), 5.05, 5.08 (1H,d,NH), 6.90–7.61 (3H,m,aromatic protons), 7.67 (1H,broad,COOH). ^{13}C-NMR: 28.29 (t-Boc,CH_3), 38.29 (C_B), 52.01 (Phe-$COOCH_3$), 55.29 (C_A), 56.06 (Phe-OCH_3), 80.08 (t-Boc,$C(CH_3)_3$), 155.73 (t-Boc, C=O), 166.95 (Phe-$COOCH_3$), 112.38, 119.79, 128.49, 132.73, 134.65, 158.15 (aromatic carbons), 176.73 (C-COOH).

Dicyclohexylammonium Salt of (5Me)

The product was obtained like the compound (**5Et**), m.p. 158–162° C, $[\alpha]_D^{20} = +28.8°$ (C = 1.5, EtOH). Found: C, 64.60; H, 8.75; N, 5.05%. $C_{29}H_{46}N_2O_7$ (534.68) requires C, 65.14; H, 8.67; N, 5.24%.

O-Methyl-3′-formyl-L-tyrosine hydrochloride (6)

A solution of *N*-acetyl-*O*-methyl-3′-formyl-L-tyrosine ethyl ester (**1**) (5.87 g, 20.0 mmol) (prepared according to [7] in 3 M HCl (100 ml) was refluxed under argon for 2 h. Evaporation gave yellowish powder with equimolar yield. The product has no melting point up to 300 °C. $[\alpha]_D^{20} = -4.3°$ (c = 1.8, H_2O). TLC: R_F (A) 0.13, R_F (B) 0.46. Lit. value: m.p. as above, $[\alpha]_D^{25} = -6.8°$ (c = 1.0, H_2O) [15, 16].

N-tert-Butyloxycarbonyl-*O*-methyl-3′-formyl-L-tyrosine dicyclohexylammonium salt (7DCHA)

To a solution of hydrochloride (**6**) (10.4 g, 40 mmol) in DMF (100 ml) di-*tert*-butyldicarbonate (9.9 g, 44.0 mmol) and triethylamine (18.0 ml, 128.0 mmol) were added. The mixture was stirred for 4 h at room temperature and evaporated. The semi-solid mass was dissolved in 1 M KOH (40 ml) and extracted with hexane (3×) and ethyl acetate (1×). The water phase was cooled, acidified with 1 M HCl and extracted with ethyl acetate (3×). The combined organic phases were washed with saline (3×), dried over $MgSO_4$ and evaporated to the brown glassy mass. It was dissolved in chloroform (20 ml), cooled and alkalized (pH 8) with dicyclohexylamine (about 7.8 ml). The brown oil was mixed with hexane (200 ml). After $\frac{1}{2}$ h at room temperature the brown mass of impurities was separated on the walls of the vessel. The hexane solution was poured into another vessel and new portion of cyclohexane (200 ml) was added. After $\frac{1}{2}$ h the hexane solution was again poured into another vessel and the next portion of hexane (300 ml) was added. The white solid of product gradually precipitated. Yield 12.1 g (24.0 mmol, 60%), m.p. 151–154 °C. TLC: R_F (A) 0.72, R_F (B) 0.62, R_F (C) 0.61. $[\alpha]_D^{20} = +16.6°$ (C = 1.3, EtOH). Found: C, 66.37; H, 8.98; N, 5.83%. $C_{28}H_{44}N_2O_6$ (504.66), requires C, 66.64; H, 8.79; N, 5.55%.

N-tert-Butyloxycarbonyl-*O*-methyl-3′-formyl-L-tyrosine (7)

A solution of NaCl (0.353 g, 604 mmol) in water (3.6 ml) was added into a solution of (**7DCHA**) (3.05 g, 6.04 mmol) in DMF (42 ml). After $\frac{1}{2}$ h of stirring and cooling with ice and water the precipitated dicyclohexylammonium hydrochloride was isolated by filtration and filtrate was evaporated. The semi-solid mass was dissolved in water (30 ml) and 20% citric acid was added in excess. The aqueous phase was extracted (3×) with ethyl acetate. The combined organic phase were washed with saline (3×), dried over $MgSO_4$ and evaporated to the glassy hygroscopic mass. Yield 1.7 g (5.26 mmol, 87%). TLC in the solvent systems A, B and C confirmed purity of the product. ^{1}H-NMR: 1.38 (9H,s,$C(CH_3)_3$), 2.94 (2H,m,CH_2), 3.88 (3H,s,OCH_3), 4.50 (1H,broad,CH), 5.05 (1H,broad,NH), 6.88–7.65 (3H,aromatic protons), 9.30 (1H,s,COOH), 10.43 (1H,s,CHO).

N-tert-Butyloxycarbonyl-*O*-methyl-3′-hydroxymethylphenyl-L-tyrosine (8)

Reduction of Aldehyde (7) using Meerwein-Ponndorf-Verley method. To a solution of 20 ml (20.0 mmol) of 1 M aluminium isopropoxide in isopropyl alcohol (prepared according to [10] 2.52 g (5.0 mmol) of dicyclohexylammonium salt of aldehyde (**7DCHA**) was added and heated in oil bath (temp. 130 °C). The mixture of *i*-PrOH + acetone was slowly distilled and collected. After distillation of 8 ml of liquid the distillate no longer gives a test for acetone (reaction with 2,4-dinitrophenylhydrazine failed to confirm the presence of acetone). After adding of excess of aluminium isopropoxide (5 ml) and heating, the 5 ml of distillate was collected. The reaction mixture was cooled and hydrolysed with HCl (1:1), and extracted with chloroform. The organic phase was washed with water, dried with $MgSO_4$ and evaporated to a glassy mass. The crude product was purified by dissolving in chloroform and extracting with 10 ml of 0.1 M KOH (4×). The alkaline solution was extracted with chloroform (1×) and acidified with 4 M HCl. After extraction of solution with chloroform (3×), the extract was washed with water, dried with $MgOSO_4$ and evaporated. The amorphous glassy mass was obtained (0.65 g, 1.89 mmol, 38%). TLC: R_F (A) 0.59, R_F (B) 0.60. $[\alpha]_D^{20} = +21.3°$ ($c = 1.0$, EtOH). Found: C, 55.94; H, 7.16; N, 3.90%. $C_{16}H_{23}NO_6 \times H_2O$ (343.38) requires C, 55.97; H, 7.33; N, 4.08%. ^{1}H-NMR: 1.37 (9H,s,C(CH_3)$_3$), 2.98 (2H,d,CH_2), 3.75 (3H,s,OCH_3), 4.4 (1H,broad,CH), 4.58 (2H,s,CH_2OH), 5.10 (1H,d,NH), 6.72-7.10 (5H,m,aromatic protons, CH_2OH, COOH).

Dicyclohexylammonium Salt of (8)

The solution of product (**8**) (0.16 g, 0.50 mmol) with chloroform (1 ml) was alkalized to pH 8.5 with dicyclohexylamine. After adding of petrol (20 ml) the solid precipitated. The solid was recrystallized from chloroform and petrol. The glassy hygroscopic solid was obtained, m.p. 50–60 °C, $[\alpha]_D^{20} = +24.9°$ ($C = 1.1$, EtOH). Found: C, 64.07; H, 9.17; N, 4.95%. $C_{28}H_{46}N_2O_6 \times H_2O$ (524.69) requires C, 64.10; H, 9.22; N, 5.34%.

Catalytic Reduction of Aldehyde (7). To a solution of aldehyde (**7**) (1.79, 5.25 mmol) in ethanol (40 ml) and water (10 ml), $FeCl_2 \times H_2O$) (20 mg) and $PtO_2 \times H_2O$ (150 mg) was added. The mixture was shaken at room temperature and hydrogenated (under pressure of 500 mm H_2O) until aldehyde could no longer be detected by TLC. The chromatogram was carried out in methanol or solvent system (A) and visualized with 2,4-dinitrophenylhydrazine or chlorine gas procedure. The reduction was completed in two to three days. The catalyst was separated and washed with ethanol. The combined solutions were collected and evaporated. The solution of crude product with a mixture of ethanol-benzene (1:1, 40 ml) was evaporated again to remove the water. The residue was solubilized with chloroform (30 ml) filtered and evaporated to the glassy mass. Yield 1.7 g (4.95 mmol, 94%) of chromatographically pure product with no sharp m.p. $[\alpha]_D^{20} = +22.9°$ ($C = 1.3$, EtOH). FIB-MS, *m/z* (+ve): 325 $(M)^+$, 651 $(2M+H)^+$; (− ve): 324 $(M\text{-}H)^-$, 649 $(2M\text{-}H)^-$.

N-tert-Butyloxycarbonyl-*o*-methyl-3′-benzyloxymethyl-L-tyrosine Dicyclohexylammonium Salt (9)

To a cooled (0 °C) and stirred solution of protected amino acid (**7**) (0.343 g, 1.0 mmol) in DMF (5 ml) sodium hydride (0.132 g, 60% suspension in oil, 3.3 mmol) was added; stirring continued for 1 h. Benzyl bromide (0.13 ml, 1.1 mmol) was added to the mixture and stirring continued for 1 h at 0 °C then at room temperature overnight. After adding methanol (1 ml), the solvents were removed and a crude oily product was redissolved with water (20 ml) and extracted with petrol ether (3×). The product was reextracted with saturated $NaHCO_3$ solution (3×). The alkaline extract was acidified with cold 1 M HCl and extracted with chloroform (3 × 70 ml). The collected extracts were washed with water and dried with $MgSO_4$. The solvent was removed and the residue oil dissolved in ethyl acetate (2 ml) and alkalized with dicyclohexylamine. Crystalline material formed after one day in the refrigerator. Yield 0.23 g (0.385 mmol, 38.5%), m.p. 141–143 °C. TLC: R_F (A) 0.81, $[\alpha]_D^{20} = +11.5°$ ($c = 1.5$, EtOH). Found: C, 69.29; H, 8.91; N, 4.66%. $C_{35}H_{52}N_2O_6$ (596.80) requires C, 70.49 H, 8.78; N, 4.69%. ^{1}H-NMR: 1.38 (9H,s,CH_3)$_3$C); 1.13–1.96 (22H,m,cyclohexyl protons); 2.83-3.23 (2H,m,CH_2); 3.77 (3H,s,OCH_3); 4.23 (1H,m,CH); 4.53, 4.58 (4H,2s,$C_6H_3CH_2O$ $CH_2C_6H_5$); 5.27, 5.31 (1H,d,NH); 6.70–7.40 (8H,m,aromatic protons). FIB-MS, *m/z* (− ve): 414 $(M\text{-}H)^-$.

N-tert-Butyloxycarbonyl-*O*-methyl-3′-(*E*)-hydroxyiminomethyl-L-tyrosine (10)

To a solution of *O-Me-3′*-(*E*)-CHNOH-L-Tyr-OH (1.19 g, 5.0 mmol) (obtained according to [13] in 1 M KOH (5 ml) and dioxane (12 ml) di-*tert*-butyldicarbonate (1.15 g, 5.0 mmol) was added. The mixture was stirred for 22 h at room temperature and evaporated. The semi-solid mass was diluted with water and alkalized to pH 9 with 1 M KOH. The solution was extracted with ethyl acetate (3×), cooled and acidified with citric acid. The precipitated mass and solution were extracted with ethyl acetate. The extract was washed with water, dried over $MgSO_4$ and evaporated. Yield 1.50 g (4.43 mmol, 89%), m.p. 165–166 °C, $[\alpha]_D^{20} = +19.7°$; ($c = 1.4$, EtOH). TLC: R_f (A) 0.87, R_f (B) 0.73. Found: C, 56.91; H, 6.80; N, 7.96%. $C_{16}H_{22}N_2O_6$ (338.36) requires C, 56.80, H, 6.55; N, 8.28%. ^{1}H-NMR: 1.40 (9H,s,$(CH_3)_3C$), 3.15 (2H,m,CH_2), 3.75 (3H,s,OCH_3), 4.60 (1H,s,CH), 5.13 (1H,broad,NH), 6.75–7.38 (3H,m,aromatic protons), 8.42 (1H,s,CH=NOH), 9.65 (1H,s,CH=NOH). ^{1}H-NMR (dioxane): 1.42 (9H,s,$(CH_3)_3C$), 2.59-2.60 (2H,m,CH_2), 3.88 (3H,s,OCH_3), 4.15 (1H,s,CH), 7.06–7.14 (3H,m,aromatic protons), 8.35 (1H,s,CH=NOH), 12.62 (1H,s,CH=NOH).

REFERENCES

1. Y. Kiso, M. Yamaguchi, T. Akita, H. Moritoki, M. Takei and H. Nakamura (1981). Super-active enkephalin analogues. *Naturwissenschaften 68*, 210–212.
2. L. Balaspiri, M.V. Toth, C. Somlai and K. Kovacs (1985). Preparation and analytical data of some unusual amino acid derivatives used in peptide syntheses (I). *Int. J. Peptide Protein Res. 26*, 1–4.
3. P. W. Schiller, C. F. Yam and M. Lis (1977). Evidence for topographical analogy between methionine enkephalin and morphine derivatives. *Biochemistry 16*, 1831–1838.
4. L. Terenius, A. Wahlsröm, G. Lindberg, S. Karlsson and U. Ragnarson (1976). Opiate receptor affinity of peptides related to Leu-enkephalin. *Biochem. Biophys. Res. Commun. 71*, 175–178.
5. K. Hsieh, I. C. Kiraly-Olah and E. C. Jorgensen (1979). Angiotensin II analogues. 13. Role of hydroxyl group of position 4 tyrosine in pressor activity. *J. Med. Chem. 22*, 1044–1047.
6. D. H. Coy and A. J. Kastin (1980). Tyrosine-modified analogs of methionine-enkephalin and their effects on the mouse vas deferens. *Peptides 1*, 175–177.
7. Z. Arnold and P. O. Larsen (1977). A new synthesis of 3-(3-carboxy-4-hydroxyphenyl)-L-alanine (3′-carboxy-L-tyrosine). *Acta Chemica Scand. B31*, 826–828.
8. Z. S. Arnold (1985). Optically active aromatic amino acids. Part IV. Synthesis and analgesic activity of four derivatives of D-Tyrosine. *Polish J. Chem. 59*, 837-843.
9. L. A. Kazicyna and N. B. Kupletska: *Spectroscopic Methods for Elucidation of Structure of Organic Compounds*, p. 93, PWN (Polish edition), Warsaw 1974.
10. A. l. Wilds in: *Organic Reactions 2*, R. Adams, W. E. Bachmann, L. F. Fieser, J. R. Johnson and H. R. Snyder, Eds., p. 178-223, John Wiley and Sons, Inc., New York 1957.
11. M. Bodanszky: *Principles of Peptide Synthesis*, p. 64, Springer-Verlag, Berlin Heidelberg 1993.
12. A. Turan and M. Manning (1977). Synthesis and some pharmacological properties of [4-homoserine]oxytocin. *J. Med. Chem. 20*, 1169–1172.
13. Z. Arnold (1982). Optically active aromatic amino acids. Part II. Synthesis of 3(*E*)-hydroxyiminomethyl derivatives of L-tyrosine. *Polish J. Chem. 56*, 1021–1025.
14. J. M. Stewart and J. D. Young: *Solid Phase Peptide Synthesis*, p. 120, Pierce Chemical Company, Rockford, Illinois 1984.
15. A. Kaiser, W. Koch, M. Scheer and Wölke (1972). 4-Methoxy-L-tyrosine from its esters. *Ger. Offen 2, 153,803; Chem Abstr. 77*: 75488.
16. A. Kaiser, K. Hohenlohe Oehringer and H. Bretschneider (1972). Antihipertensive and anorectic phenylalanine derivatives. *Ger. Offen 2, 122,485, Chem. Abstr. 76*: 60075.