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4-Amino- and 4-Hydroxycyclohexane-1,1-dicarboxylic Acid Peptides

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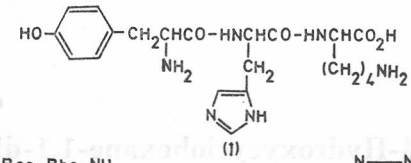
4-Amino-, 4-oxo-, and 4-hydroxy-cyclohexane-1,1-dicarboxylic acids have been inserted into di- and tri-peptides containing glycine, L-phenylalanine, L-cyclohexylalanine, and L-tyrosine. The geometries of 4-*c*-(IX A) and 4-*t*-(IX B) benzamidocyclohexane-r-1-carboxy-1-carbonyl-L-phenylalanine methyl esters and 4-*c*-(XXIII A) and 4-*t*-(XXIII B) benzoxycyclohexane-r-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine methyl esters were confirmed by their NMR spectra and intramolecular cyclisations of the cis isomers. The ring opening of 2-oxabicyclo[2.2.2]octan-3-one-4-carbonyl-L-cyclohexylalanine methyl ester (XXIV) in methanolic hydrochloric acid afforded 4-*c*-hydroxycyclohexane-r-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine methyl ester (XXI A) which on benzoylation was converted into the product identical with the isomer (XXIII A).

The thyrotropin releasing hormone (TRH) and certain analogous peptides of low molecular weights[1,2], appart from their endocrine functions, may have roles in controlling neuronal excitabilities in the central nervous system[3]. The synthesis of L-pyroglutamyl-L-histidyl-L-proline amide[4,5] confirmed the structure of TRH[6], isolated from ovine hypothalamic extracts[7].

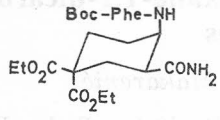
We considered the possibility that low molecular peptides, containing various cyclic systems[8] and carbamoyl group, may increase their biological potencies. Thus, as has already been reported[9,10] the polyfunctional L-tyrosyl-L-hystidyl-L-lysine (I), containing heterocyclic systems, stimulated the incorporation of the labelled sulphate into an embryonic chick cartilage. On the other hand our synthesis of diethyl 2-*N*-(*N*-*t*-butoxycarbonyl-L-phenylalanyl)c-2-amino-r-1-carbamoylcyclohexane-5,5-dicarboxylate[11] (II) by reductive ring opening of diethyl 4,5,6,7-tetrahydro-3-oxo-2H-indazole-5,5-dicarboxylate[12] (III) and coupling of the resulting aminocyclohexane dicarboxylate with the succinimide ester of *N*-*t*-butoxycarbonyl-L-phenylalanine could be regarded as an example of the carbamoyl group containing peptides.

As part of our continuing programme on the synthesis of unusual peptides containing cyclic systems, we decided to study the stereoisomeric 4-substituted cyclohexane-1,1-dicarboxylic acid peptides. To our knowledge only the stereochemistry of 2-[13,14], 3-[15], and 4-[16,17] aminocyclohexane carboxylic acids have been investigated in more details, the latter being coupled with several amino acid derivatives. Furthermore, in a previous article[18] we described cis- and

trans-3- and 4-amino-, hydroxymethyl-, and chloromethyl-cyclohexane-1-carboxylic acids inserted into di- and tri-peptides containing L-phenylalanine and L-cysteine. In contrast with these results no systematic data are available in



(I)



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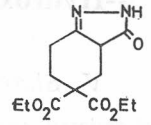
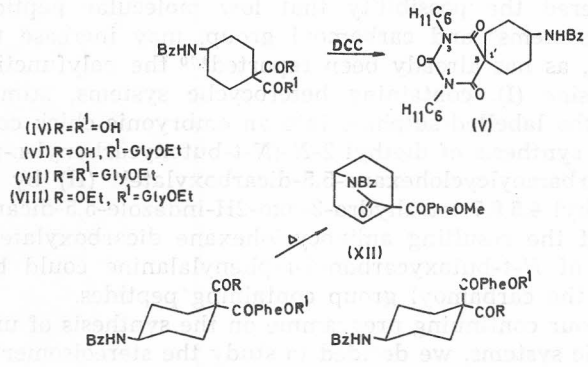


(III)

Boc-Phe = *t*-butoxycarbonyl- -phenylalanine

cyclohexane-dicarboxylic acids area. During this study it was found that the attachments of amino acid derivatives to the equatorial or axial *C*-terminus of 4-amino-cyclohexane-1,1-dicarboxylic acid could provide hitherto unknown chains and also adequate models for better understanding of their conformational features.

In order to circumvent undesirable reactions of 4-benzamidocyclohexane--1,1-dicarboxylic acid (IV) into 1,3-dicyclohexyl-2,4,6-trioxopyrimidine-5-spiro--1'(4'-benzamidocyclohexane) (V), when dicyclohexylcarbodi-imide was used as the condensing agent[19–22], a modified triphenyphosphine-carbon tetrachloride method[23–26] was shown to be the most convenient one for the synthesis of the stereoisomeric 4-benzamidocyclohexane-1-carboxy-1-carbonylglicine ethyl ester (VI). It is noteworthy that the synthesis of the peptide (VI), isolated as one of the two possible isomers, was completed at room temperature within 2.5 h in 87% yield, and that the prolonged time of reaction (19 h) did not facilitate the formation of 4-benzamidocyclohexane-1,1-dicarbonylglycine diethyl ester (VII), isolated in only 29% yield. The glycine-dipeptide (VI) was characterized as



(IV) R=R^1=OH
(VI) R=OH, R^1=GlyOEt
(VII) R=R^1=GlyOEt
(VIII) R=OEt, R^1=GlyOEt

(V)

(XII)

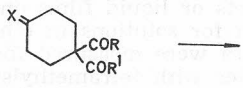
A | (IX) R=OH, R^1=Me; (X) R=OH, R^1=Bzl; (XI) R=OMe, R^1=Me | B

GlyOEt = glycine ethyl ester

4-benzamidocyclohexane-1-carbethoxy-1-carbonylglycine ethyl ester (VIII); its stereoisomeric purity was established by NMR data.

In contrast to the isolation of only one of the two possible glycine-dipeptides (VI) as isomer, L-phenylalanine-dipeptides, obtained from the reaction of dicarboxylic acid (IV) with L-phenylalanine methyl or benzyl ester, separated as 4-c- (IX A) and 4-t- (IX B) benzamidocyclohexane-1-carboxy-1-carbonyl-L--phenylalanine methyl and benzyl (X A) and (X B) esters, respectively. The isomeric carboxylic acid peptides (IX A) and (IX B), formed in ratio 1.7 : 1.0, were esterified to the corresponding cis- (XI A) and trans- (XI B) carbomethoxy derivatives. The cis-geometry of (XI A) could be proved by an intramolecular cyclisation leading to 2-benzoyl-2-azabicyclo[2.2.2]octan-3-one-4-carbonyl-L--phenylalanine methyl ester (XII).

To exemplify a peptide chain elongation at the *N*- and *C*-terminus 4-oxocyclohexane-1,1-dicarboxylic acid[27] (XIII) was first converted into 4-oxocyclohexane-1-carboxy-1-carbonyl-L-phenylalanine methyl ester (XIV) and then esterified to 4-oxocyclohexane-1-carbomethoxy-1-carbonyl-L-phenylalanine methyl ester (XV). The latter on oximation[28], followed by hydrogenation of the resulting oxime (XVI) over 5% Rh/Al_2O_3[29], gave the desired 4-aminocyclohexane-1-carbomethoxy-1-carbonyl-L-phenylalanine methyl ester (XVII) characterized as *N*-benzoyl derivative (XVIII). The *C*-dipeptide (XVII), obtained in high overall yield, was finally elongated at the *N*-terminus in a reaction with *N*-hydroxysuccinimide ester of *N*-*t*-butoxycarbonyl-*O*-benzyl-L-tyrosine



(XIII) X = O, R = R¹ = OH
(XIV) X = O, R = OH, R¹ = PheOMe
(XV) X = O, R = OMe, R¹ = PheOMe
(XVI) X = NOH, R = OMe, R¹ = PheOMe

(XVII) R = H
(XVIII) R = Bz
(XIX) R = Boc-Tyr(OBzl)

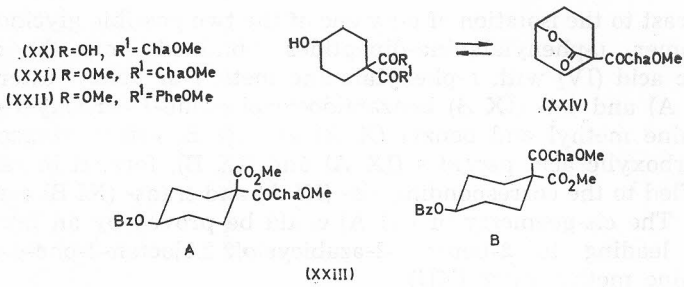
Boc-Tyr(OBzl) = *t*-butoxycarbonyl-O-benzyl-L-tyrosyl; ChaOMe = L-cyclohexylalanine methyl ester

giving 4-*N*-(*N*-*t*-butoxycarbonyl-*O*-benzyl-L-tyrosyl) 4-aminocyclohexane-1--carbomethoxy-1-carbonyl-L-cyclohexylalanine methyl ester (XIX). It should be mentioned that the stereochemistry of these peptides remains to be solved and will be described in a forthcoming paper.

The stereoisomeric 4-hydroxycyclohexane-1-carboxy-1-carbonyl-L-cyclohexylalanine methyl ester (XX) and their 1-carbomethoxy derivatives (XXI) were prepared by the hydrogenations of 4-oxocyclohexanes (XIV) and (XV), respectively, over PtO_2 as catalyst. A treatment of 4-oxocyclohexane-1-carbomethoxy peptide (XV) with sodium borohydride in anhydrous methanol, however, afforded 4-hydroxycyclohexane-1-carbomethoxy-1-carbonyl-L-phenylalanine methyl esters (XXII) with preserved aromatic ring.

In the successful separation of the stereoisomeric 4-hydroxycyclohexane--1,1-dicarboxylic acid peptides their benzoylations were shown to be decisive. Thus 4-benzoxycyclohexane-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine methyl esters (XXIII) were separated by preparative TLC providing *cis*-

(XXIII A) and *trans*-(XXIII B) isomer at R_F ca. 0.24 and 0.21 in 47.9 and 28.4% yields, respectively.



An intramolecular cyclisation of a mixture of the stereoisomeric 4-hydroxycyclohexane-1-carboxy dipeptides (XX) proceeded into 2-oxabicyclo[2.2.2]octan--3-one-4-carbonyl-L-cyclohexylalanine methyl ester (XXIV) in 41.8% yield. This bicyclic product (XXIV) was unambiguously reopened by treatment with 3% methanolic hydrochloric acid into 4-*c*-hydroxycyclohexane-r-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine methyl ester (XXI A), which on benzoylation yielded the *cis*-isomer (XXIII A).

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. The IR spectra were obtained for potassium bromide pellets or liquid films on a Perkin-Elmer 297 spectrophotometer. UV spectra were taken for solutions in ethanol with a Perkin--Elmer 124 spectrophotometer. NMR spectra were measured for solutions in deuteriochloroform on a Varian A60 spectrometer with tetramethylsilane as the internal standard for organic solutions. Mass spectra were measured with a Varian MAT CH7 spectrometer and optical rotations in methanol (1 = 1 dm) (unless otherwise stated) using a Zeiss-Winkel 179707 apparatus. The silica gel (Merck 0.08 mm) which was used for column chromatography and (Merck HF_{254}, type 60) for TLC and for preparative TLC was activated at 110 °C for 60 min. The products were developed in methylene chloride — methanol (9 : 1) unless otherwise stated and rendered visible by u. v. illumination and by a ninhydrin spray.

4-Benzamidocyclohexane-1,1-dicarboxylic Acid (IV)

A solution of diethyl 4-benzamidocyclohexane-,1,1dicarboxylate (209 mg, 0.602 mmol), obtained by benzoylation of the corresponding 4-aminocyclohexane derivative[30], was treated with 20% methanolic potassium hydroxide (10 ml) and heated under reflux for 50 min. The solvent was removed, the residue diluted with water, and then acidified with hydrochloric acid (to pH 3). The ethyl acetate extract afforded the produtc (173 mg, 98.7%), m. p. 227—229 °C from methanol-ether-*n*-hexane), R_F ca. 0.1.

Anal. $C_{15}H_{17}NO_5$ (291.30) calc'd.: C 61.85; H 5.88; N 4.81%
found: C 61.75; H 6.15; N 5.06%

UV spectrum: λ_{max} 228 nm (log ε 4.02). IR spectrum: ν_{max} 3390, 1730, 1675, 772, and 693 cm^{-1}.

1,3-Dicyclohexyl-2,4,6-trioxopyrimidine-5-spiro-1'-(4'-benzamidocyclohexane) (V)

To a suspension of 4-benzamidocyclohexane-1,1-dicarboxylic acid (IV) (146 mg, 0.5 mmol) in tetrahydrofurane (4 ml), cooled at 0 °C, dicyclohexylcarbodi-imide (221 mg, 1.1 mmol) was added and then stirred at room temperature for 3 days. A preci-

pitate was removed by suction and the filtrate evaporated to dryness. The residue was chromatographed on a silica gel column. The product, R_F ca. 0.72, 41% (100 mg), m. p. 209—210 °C (from chloroform-*n*-hexane).

Anal. $C_{28}H_{37}N_3O_4$ (479.60) calc'd.: C 70.12; H 7.78; N 8.76%
found: C 70.28; H 7.60; N 8.53%

Mass spectrum: *m/e* (M^+) 479, requires 479.60. UV spectrum: λ_{max} 229 nm (log ε 3.99). IR spectrum: ν_{max} 3416, 1743, 1684, 1648, 757, and 710 cm^{-1}. NMR spectrum: τ 2.12—2.43 and 2.45—2.68 (2H and 3H,2m,aromatic protons), 3.80 (1H,d,CONH; *J* 7.9 Hz), 5.17—6.33 (3H,m,3CH).

4-Benzamidocyclohexane-1-carboxyl-1-carbonylglycine Ethyl Ester (VI)

a) A suspension of 4-benzamidocyclohexane-1,1-dicarboxylic acid (IV) (50 mg, 0.172 mmol), hydrochloride of glycine ethyl ester (48 mg, 0.344 mmol), triphenylphosphine (90 mg, 0.344 mmol), triethylamine (0.072 ml, 0.514 mmol), and carbon tetrachloride (0.035 ml, 0.35 mmol) in acetonitrile (2 ml) was stirred at room temperature for 2.5 h. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate, washed with 5% citric acid, and then extracted with 10% $NaHCO_3$. The sodium hydrocarbonate solution was acidified and reextracted with ethyl acetate. The organic layer was evaporated to the crystalline product (56 mg, 86.9%), m. p. 166—167 °C (from ethyl acetate), R_F ca. 0.11.

Anal. $C_{19}H_{24}N_2O_6$ (376.40) calc'd.: C 60.62; H 6.43; N 7.44%
found: C 60.81; H 6.40; N 7.56%

UV spectrum: λ_{max} 228 nm (log ε 3.95). IR spectrum: ν_{max} 3389, 3348, 1751, 1749, 1703, 1672, 1654, 1624, and 716 cm^{-1}. NMR spectrum (DMSO-d_6): τ 1.78 (1H, d, CONH; *J* 7.9 Hz), 1.92—2.25 (2H and 1H, m, aromatic protons and CONH), 2.37—2.67 (3H, m, aromatic protons), 5.90 (2H, q, CH_2; J_{Et} 7.2 Hz), 6.2 (2H, d, N—CH_2; *J* 5.5 Hz), 8.80 (3H, t, CH_3; J_{Et} 7.2 Hz).

b) A suspension of dicarboxylic acid (IV) (225 mg, 0.772 mmol), hydrochloride of glycine ethyl ester (215 mg, 1.545 mmol), and triphenylphosphine (486 mg, 1.854 mmol), triethylamine (0.432 ml, 3.08 mmol) and carbon tetrachloride (0.155 ml, 1.55 mmol) in acetonitrile (3 ml) was stirred for 19 h. From the $NaHCO_3$ layer the acidic product (VI) was extracted (70 mg, 24%), and from ethylacetate solution 4-benzamidocyclohexane-1,1-dicarbonylglycine diethyl ester (VII), the latter being purified on a silica gel column by elution with methylene chloride (103 mg 28.9%), m. p.145—146 °C (from ethyl acetate), R_F ca. 0.652.

Anal. $C_{23}H_{31}N_3O_7$ (461.50) calc'd.: C 59.85; H 6.77; N 9.11%
found: C 59.86; H 6.50; N 9.34%

UV spectrum: λ_{max} 228 nm (log ε 4.11). IR spectrum: ν_{max} 3310 br, 1747, 1732, 1721, 1658, 1643, 717, and 694 cm^{-1}. NMR spectrum (DMSO-d_6): τ 1.61—1.98 (2H, m, 2CONH), 2.02—2.36 (2H and 1H, m, aromatic protons and CONH), 2.42—2.75 (3H, m, aromatic protons), 5.91 and 5.93 (each 2H, 2q, CH_2; J_{Et} 7.1 Hz), 6.23 (4H, d, 2N—CH_2; *J* 5.0 Hz), 8.82 (6H, t, 2 CH_3; J_{Et} 7.1 Hz).

4-Benzamidocyclohexane-1-carbethoxy-1-carbonylglycine Ethyl Ester (VIII)

Cyclohexane carboxylic acid (VI) (54 mg, 0.144 mmol) was dissolved in 3% ethanolic hydrochloric acid (11 ml), heated under reflux for 19 h, and then evaporated to drynes. Preparative TLC separated a glassy product (43 mg, 74.09%), R_F ca. 0.69, purified by precipitation from chloroform-*n*-hexane.

Anal. $C_{21}H_{28}N_2O_6$ (404.45) calc'd.: C 62.36; H 6.98; N 6.93%
found: C 62.22; H 6.69; N 6.69%

UV spectrum: λ_{max} 227 nm (log ε 4.04). IR spectrum: ν_{max} 3338 br, 1751 sh, 1735, 1665 sh, 1635, 713, and 693 cm^{-1}. NMR spectrum: τ 2.15—2.38 and 2.41—2.67 (2H and 3H,2m, aromatic protons), 3.20—3.75 (1H, m, CONH), 4.03 (1H, d, CONH; *J* 7.9 Hz),

5.72 and 5.78 (each 2H, 2q, 2 CH_2; J_{Et} 7.2 Hz), 6.04 (2H, d, N—CH_2; J 5.3 Hz), 8.70 and 8.74 (each 3H, t, 2 CH_3; J_{Et} 7.2 Hz).

Stereoisomeric 4-Benzamidocyclohexane-1-carboxy-1-carbonyl-L--phenylalanine Methyl Esters (IX A and B)

A suspension of cyclohexane dicarboxylic acid (IV) (187 mg, 0.642 mmol), hydrochloride of L-phenylalanine metyl ester (277 mg, 1.284 mmol), triethylamine (0.36 ml, 2.57 mmol), carbon tetrachloride (0.128 ml, 1.28 mmol), and triphenylphosphine (337 mg, 1.284 mmol) in acetonitrile (5 ml), following the procedure described for the preparation of the compound (VI), afforded a mixture of stereoisomers (257 mg, 88.6%), separated by preparative TLC (two developments) at R_F ca. 0.43 and ca. 0.39 (ratio 1.17 : 1.0). The stereoisomer A (R_F ca. 0.43) was recrystallized from chloroform-ether-*n*-hexane, m. p. 115—117 °C, $[\alpha]_D^{23} = 13.7^0$ (c 1.1).

Anal. $C_{25}H_{28}N_2O_6$ (452.49) calc'd.: C 66.36; H 6.24; N 6.19%
found: C 66.33; H 5.98; N 5.86%

UV spectrum: λ_{max} 215 sh and 226 sh nm (log ε 3.85 and 3.80). IR spectrum ν_{max} 3404 br, 1740 br, 1660 br, 1637, 756, 715, and 701 cm^{-1}. NMR spectrum (DMSO-d_6): τ 1.76 (1H, d, CONH; J 6.8 Hz), 2.10—2.24 (2H and 1H, m, aromatic protons and CONH), 2.49—2.56 (3H, m, aromatic protons), 2.76 (5H, m, aromatic protons), 5.45—5.67 (1H, m, CH—Phe), 6.23 br (1H, m, H-4, obscured by those of OCH_3), 6.38 (3H, s, OCH_3), 6.96 (2H, d, CH_2-Phe; J 6.8 Hz).

The stereoisomer B (R_F ca. 0.39) was recrystallized as hydrate from chloroform--*n*-hexane, m. p. 94—96 °C, $[\alpha]_D^{26} = -5.3^0$ (c 1.23).

Anal. $C_{25}H_{28}N_2O_6 \cdot H_2O$ (470.49) calc'd.: C 63.81; H 6.43; N 5.95%
found: C 64.13; H 6.56; N 5.85%

UV spectrum: λ_{max} 215 sh and 225 sh nm (log ε 3.87 and 3.81). IR spectrum: ν_{max} 3340 br, 1745 br, 1666, 1633, 754, 714, and 701 cm^{-1}. NMR spectrum: τ 1.36 (1H, d, CONH; J 6.8 Hz), 1.87 (1H, d, CONH; J 6.8 Hz), 2.11—2.24, 2.44—2.65, and 2.77 (2H, 3H, and 5H; 2m and s; aromatic protons), 5.33—5.63 (1H, m, CH-phe), 5.69—5.94 (1H, m, H-4). 6.43 (3H, s, OCH_3), 7.03 (2H, d, CH_2-phe; J 6.8 Hz).

Stereoisomeric 4-Benzamidocyclohexane-1-carboxyl-1-carbonyl-L--phenylalanine Benzyl Esters (X A and B)

A suspension of cyclohexane dicarboxylic acid (IV) (53 mg, 0.182 mmol), L-phenylalanine benzyl ester hydrochloride (106 mg, 0.364 mmol), trietylamine (0.088 ml, 0.63 mmol), carbon tetrachloride (0.036 ml, 0.36 mmol), and triphenylphosphine (105 mg, 0.4 mmol) in acetonitrile (3 ml), following the above described procedure, afforded a mixture of stereoisomers (33 mg, 34.3%). Crystallization from chloroform--ether-*n*-hexane yielded predominantly *cis*-isomer A, R_F ca. 0.21. From the mother liquor the *trans*-isomer B was isolated on preparative TLC at R_F ca. 0.13 (recovery with methanol). The stereoisomer A (R_F ca. 0.21) had m. p. 177—178 °C, $[\alpha]_D^{23} = -25^0$ (c 1).

Anal. $C_{31}H_{32}N_2O_6$ (528.58) calc'd.: C 70.45; H 6.10; N 5.30%
found: C 70.30; H 5.83; N 5.02%

UV spectrum: λ_{max} 203 and 223 sh nm (log ε 4.39 and 4.11). IR spectrum: ν_{max} 3339, 1745, 1733, 1667, 763, 732, and 700 cm^{-1}. NMR spectrum: τ —2.69 (1H, s, COOH), 1.76 and 2.0 (2H, 2d, CONH; J 6.8 Hz), 2.01—2.21 and 2.47—2.60 (2H, and 3H, 2m, N-benzoyl protons), 2.65 and 2.77 (10 H, 2s, 2 phenyl protons), 4.9 br (2H, s, OCH_2-ph), 5.34—5.70 (1H, m, N—CH—CO), 6.03—6.47 (1H, m, 4-H), 6.93 (2H, d, CH_2-ph; J 6.8 Hz).

The stereoisomer B (R_F ca. 0.13) showed m. p. 120—121 °C (from methanol--ether-*n*-hexane, exhibited τ — 2.6 br (1H, s, COOH), 1.82—2.01 (1H, m, 2CONH), 2.09—2.28 and 2.43—2.61 (5H, 2m, N-benzoyl protons) 2.71 and 2.80 (10 H, 2s, 2 phenyl protons), 4.92 br (2H, s, OCH_2-phe), 5.32—5.63 (1H, m, N—CH—CO), 6.21—6.55 (1H, m, 4-H), 6.94 (2H, d, CH_2-ph; J 6.6 Hz).

4-c-Benzamidocyclohexane-r-1-carbomethoxy-1-carbonyl-L-phenylalanine Methyl Ester (XI A)

A solution of *cis*-benzamidocyclohexane carboxylic acid (IX A) (75 mg, 0.166 mmol) in 3% methanolic hydrochloric acid (5 ml) was heated under reflux for 18 h, and then evaporated to dryness. The residue was dissolved in ethyl acetate, washed with 2% $NaHCO_3$, 5% citric acid, and water. The organic layer was dried and evaporated to a product which was purified by preparative TLC (65 mg, 84%), R_F ca. 0.56 (CH_2Cl_2—MeOH, 95 : 5), and then precipitated from methylene chloride--ether-*n*-hexane.

Anal. $C_{26}H_{30}N_2O_6$ (466.52) calc'd.: C 66.93; H 6.48; N 6.01%
found: C 67.26; H 6.68; N 6.20%

UV spectrum: λ_{max} 219 sh and 228 nm (log ε 4.10 and 4.04). IR spectrum: ν_{max} 3347 br, 1745 sh, 1738, 1657 sh, 1640, 715, and 702 cm^{-1}. NMR spectrum: τ 2.23—2.99 (10 H, m, aromatic protons), 3.62 (1H, d, CONH; *J* 7.6 Hz), 4.03 (1H, d, HNCO; *J* 7.6 Hz), 5.08—5.28 (1H, m, CH-Phe), ca. 5.84—6.22 (1H, m, H-4, partly obscured by those of OCH_3), 6.25 and 6.30 (each 3H, 2s, $2CH_3O$), 6.68—7.08 (2H, m, CH_2-Phe).

4-t-Benzamidocyclohexane-r-1-carbomethoxy-1-carbonyl-L-phenylalanine Methyl Ester (XI B)

A solution of *trans*-benzamido derivative (IX B) (82 mg, 0.181 mmol) in 3% methanolic hydrochloric acid (8 ml) was treated and worked up as described for isomer (XI A). Yield 72 mg (85.3%), m. p. 138—139 °C (from methylene chloride-ether-*n*--hexane), R_F ca. 0.56 (CH_2Cl_2-MeOH, 95 : 5).

Anal. $C_{26}H_{30}N_2O_6$ (466.52) calc'd.: C 66.93; H 6.48; N 6.01%
found: C 67.02; H 6.35; N 6.23%

UV spectrum: λ_{max} 218 sh and 226 nm (log ε 4.08 and 4.01). IR spectrum: ν_{max} 3337, 1738, 1732 sh, 1655, 1640, 717, and 707 cm^{-1}. NMR spectrum: τ 2.23—2.95 (10 H, m, aromatic protons), 3.59 (1H, d,CONH; *J* 7.8 Hz), 4.06 (1H, d, NHCO; *J* 7.8 Hz), 5.01—5.21 (1H, m, CH-Phe), ca. 5.84—6.20 (1H, m, H-4, partly obscured by those of OCH_3), 6.25 and 6.35 (each 3H, 2s, $2CH_3O$), 6.66—7.09 (2H, m, CH_2-Phe).

Cyclisation of 4-c-Benzamidocyclohexane-r-1-carbomethoxy-1-carbonyl--L-phenylalanine Methyl Ester (XI A)

Formation of 2-*N*-benzoyl-2-azabicyclo[2.2.2]octan-3-one-4-carbonyl-L-phenylalanine Methyl Ester XII). The pure *cis*-isomer XI A (38.5 mg, 0.083 mmol) was heated at 250 °C (N_2 atmosphere) for 1 h. The product was separated by preparative TLC in 27.9% (10 mg) yield, R_F ca. 0.75 (CH_2Cl_2—MeOH, 95 : 5). IR spectrum: ν_{max} 3204 br, 2948, 1756, 1743, 1686 br, 1661, 753, 702, and 696 cm^{-1}. NMR spectrum: τ 1.3 (1H, d, NH; *J* 6.4 Hz), 2.3—2.57 and 2.64—2.96 (2H and 3H, 2m, aromatic protons), 5.01—5.34 (1H, m, 4-H and N—CH—CO), 6.33 (3H, s, OMe), 6.74—7.16 (2H, h, CH_2-ph).

4-Oxocyclohexane-1-carboxy-1-carbonyl-L-phenylalanine Methyl Ester (XIV)

A suspension of 4-oxocyclohexane-1,1-dicarboxylic acid[27] (XIII) (372 mg, 2 mmol), hydrochloride of L-phenylalanine methyl ester (431 mg, 2 mmol), triphenylphosphine (525 mg, 2 mmol), triethylamine (0.56 ml, 4 mmol), and carbon tetrachloride (0.4 ml, 4 mmol) in acetonitrile (7 ml) was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue partitioned between ethyl acetate and 10% $NaHCO_3$. The $NaHCO_3$ layer was acidified with hydrochloric acid and reextracted with ethylacetate. The ethyl acetate layer was washed with water, dried, and evaporated to a foamy product (506 mg, 72.8%), which precipitated from chloroform-*n*-hexane, R_F ca. 0.23, $[\alpha]_D^{22} = -11.57°$ (c 2.61).

Anal. $C_{18}H_{21}NO_6$ (347.36) calc'd.: C 62.24; H 6.10; N 4.03%
found: C 62.22; H 6.35; N 3.86%

UV spectrum: λ_{max} 239 nm ($\log\varepsilon$ 3.35). IR spectrum: ν_{max} 3370 br, 1740 br, 1675 br, 750, and 709 cm^{-1}. NMR spectrum: τ — 0.68—0.0 (1H, m, COOH), 2.72—3.50 (5H, and 1H, m, aromatic protons and CONH), 4.90—5.42 (1H, m, CH-Phe), 6.33 (3H, s, OCH_3), 6.73—7.05 (2H, m, CH_2-Phe).

4-Oxocyclohexane-1-carbomethoxy-1-carbonyl-L-phenylalanine Methyl Ester (XV)

A solution of cyclohexane carboxylic acid (XIV) (289 mg, 0.858 mmol) in 3% methanolic hydrochloric acid (20 ml) was heated under reflux for 19 h and then evaporated to dryness. The residue was dissolved in ethyl acetate, washed with water, dried, and evaporated to the product (309 mg, 99.6%), m. p. 89—90 °C (from chloroform-*n*-hexane), R_F ca. 0.69, $[\alpha]_D^{26} = -28.6^0$ (*c* 1.1).

Anal. $C_{19}H_{23}NO_6$ (361.38) calc'd.: C 63.14; H 6.42; N 3.88%
found: C 63.12; H 6.48; N 4.16%

IR spectrum: ν_{max} 3344, 1744, 1727, 1716, 1656, 1620, 751, and 705 cm^{-1}. NMR spectrum: τ 2.58—3.06 (5H, aromatic protons), 3.47 (1H, d, CONH; *J* 7.7 Hz), 4.95—5.38 (1H, m, CH-Phe), 6.30 and 6.33 (each 3H, 2s, $2CH_3O$), 6.75—7.03 (2H, m, CH_2-Phe).

4-Oxyiminocyclohexane-1-carbomethoxy-1-carbonyl-L-phenylalanine Methyl Ester (XVI)

To a solution of 4-oxocyclohexane (XV) (492 mg, 1.361 mmol) in ethanol (6 ml) hydroxylamine hydrochloride (111 mg, 1.6 mmol) dissolved in water (2.5 ml) was added. The mixture was stirred and treated portionwise with Na_2CO_3 (85 mg, 0.8 mmol) in water (2.5 ml) during a time interval of 15 min. After 5 h water (20 ml) was added and extracted with ether. The extract was dried and evaporated to a foamy residue (483 mg, 94.2%), which precipitated from chloroform-*n*-hexane, R_F ca 0.67, $[\alpha]_D^{25} = -29.03^0$ (*c* 3.1).

Anal. $C_{19}H_{24}N_2O_6$ (376.40) calc'd.: C 60.62; H 6.43; N 7.44%
found: C 60.33; H 6.62; N 7.17%

IR spectrum: ν_{max} 3384 br, 1737 br, 1664 br, 1621 br, 742 br, and 700 cm^{-1}. NMR spectrum: τ 2.60—3.03 (5H, m, aromatic protons), 3.50 (1H, d, CONH; *J* 7.8 Hz), 4.95—5.33 (1H, m, CH-Phe), 6.28 and 6.33 (each 3H, 2s, $2CH_3O$), 6.76—6.96 (2H, m, CH_2-Phe).

Stereoisomeric 4-Aminocyclohexane-1-carbomethoxy-1-carbonyl-L--cyclohexylalanine Methyl Esters (XVII)

A solution of 4-oxyiminocyclohexane (XVI) (367 mg, 0.975 mmol) in glacial acetic acid (36 ml) was hydrogenated at room temperature and 0.34 MPa for 29 h over 5% Rh/Al_2O_3 (283 mg). The catalyst was filtered off and the filtrate evaporated to dryness. The residue was dissolved in dilute ammonia and extracted with ethyl acetate. From the ethyl acetate layer the product separated (204 mg, 56.7%), which precipitated from chloroform-ether-*n*-hexane as a foam, R_F ca. 0.1, $[\alpha]_D^{25} = -26.1^0$ (*c* 0.69).

Anal. $C_{19}H_{32}N_2O_5$ (368.46) calc'd.: C 61.93; H 8.75; N 7.60%
found: C 61.77; H 8.67; N 7.83%

IR spectrum: ν_{max} 3364, 1749 br, 1683 br, and 1650 br cm^{-1}. NMR spectrum: τ 3.70 (1H, d, CONH; *J* 7.9 Hz), 5.17—5.67 (1H, m, CH-Cha), 5.72 to region of OCH_3 (1H, m, H-4), 6.30 (6H, s, $2CH_3O$).

Stereoisomeric 4-Benzamidocyclohexane-1-carbomethoxy-1-carbonyl-L--cyclohexylalanine Methyl Esters (XVIII)

To a solution of 4-aminocyclohexane (XVII) (70 mg, 0.19 mmol) in pyridine (5 ml) benzoic anhydride (52 mg, 0.23 mmol) was added and the mixture stirred at

room temperature for 16 h. The residue obtained from ethylacetate extract was purified by preparative TLC to a foamy product (54 mg, 60.2%), R_F ca. 0.73, precipitated from ether-*n*-hexane, $[\alpha]_D^{27} = -20.5^0$ (c 2).

Anal. $C_{26}H_{36}N_2O_6$ (472.56) calc'd.: C 66.08; H 7.68; N 5.93%
found: C 66.08; H 7.60; N 5.95%

UV spectrum: λ_{max} 223 nm (log ε 4.16). IR spectrum: ν_{max} 3334, 1742, 1658, 1651, 1640, 718, and 698 cm^{-1}. NMR spectrum: τ 2.18—2.45 and 2.48—2.70 (2H and 3H, 2m, aromatic protons), 3.63 and 3.86 (each 1H, 2d, *J* 8.0 and 8.0 Hz), 5.18—5.68 (1H, m, CH-Cha), 5.69 to region of OCH_3 (1H, m, H-4), 6.17, 6.28, and 6.32 (6H, 3s, $2CH_3O$).

Stereoisomeric 4-N-(N-t-Butoxycarbonyl-O-benzyl-L-tyrosyl) 4-aminocyclohexane-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine Methyl Esters (XIX)

To a solution of *N*-hydroxysuccinimide ester of *N*-*t*-butoxycarbonyl-*O*-benzyl--L-tyrosine[31] (211 mg, 0.451 mmol) in 1,2-dimethoxyethane (5 ml) 4-aminocyclohexane (XVII) (166 mg, 0.451 mmol) was added and stirred at room temperature for 19 h. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate, washed with 5% citric acid, 0.2 mol dm^{-3} $NaHCO_3$, and water to be evaporated to dryness and purified by preparative TLC, R_F ca. 0.66. The product (110 mg, 33.8%) was precipitated from chloroform-ether-*n*-hexane, $[\alpha]_D^{25} = -5.3^0$ (c 2).

Anal. $C_{40}H_{55}N_3O_9$ (721.86) calc'd.: C 66.55; H 7.68; N 5.82%
found: C 66.79; H 7.74; N 5.93%

UV spectrum: λ_{max} 228 nm (log ε 4.00). IR spectrum: ν_{max} 3330 br, 1736, 1731 sh, 1651 br, 735, and 695 cm^{-1}. NMR spectrum: τ 2.68 (5H, s, aromatic protons), 2.83—3.35 (4H, m, Tyr-aromatic protons), 3.36 (1H, d, CONH; *J* 8.1 Hz), 4.98 (1H, d, CONH; *J* 7.9 Hz), 5.02 (2H, s, OCH_2), 6.34 (6H, s, $2CH_3O$), 7.10 (2H, d, CH_2-Tyr; *J* 6.0 Hz), 8.61 (9H, s, $C(CH_3)_3$).

Stereoisomeric 4-Hydroxycyclohexane-1-carboxy-1-carbonyl-L--cyclohexylalanine Methyl Esters (XX)

A solution of 4-oxocyclohexane-1-carboxyl-1-carbonyl-L-phenylalanine methyl ester (XIV) (1.795 mg, 5.168 mmol) in glacial acetic acid (60 ml) containing PtO_2 (359 mg) was stirred in hydrogen atmosphere under 0.34 MPa for 40 h. The catalyst was filtered off and the filtrate evaporated to an unseparable foam (1.7 g, 92.6%), R_F ca. 0.25, used for further experiments.

Stereoisomeric 4-Hydroxycyclohexane-1-carbomethoxy-1-carbonyl-L--cyclohexylalanine Methyl Esters (XXI)

A solution of 4-oxocyclohexane-1-carbomethoxyl-1-carbonyl-L-phenylalanine methyl ester (XV) (389 mg, 1.076 mmol) in glacial acetic acid (15 ml) containing PtO_2 (76 mg) was hydrogenated as described above. The product (368 mg, 92.6%), as a foamy mixture of stereoisomers, was purified by preparative TLC, R_F ca. 0.6, and used for further experiments.

Stereoisomeric 4-Hydroxycyclohexane-1-carbomethoxy-1-carbonyl-L--phenylalanine Methyl Esters (XXII)

To a solution of 4-oxocyclohexane derivative (XV) (275 mg, 0.761 mmol) in anhydrous methanol (5 ml) sodium borohydride (29 mg, 0.761 mmol) was added and the mixture set aside for 15 min. Acetone (6 ml) was then added and the solvents removed at the reduced pressure. The residue was dissolved in ethylacetate, washed with water, dried, and then evaporated to an oily product (240 mg, 86.7%), distilled at 130 °C and 10^{-2} mmHg, R_F ca. 0.58, $[\alpha]_D^{27} = -12.21^0$ (c 2.3).

Anal. $C_{19}H_{25}NO_6$ (363.40) calc'd.: C 62.79; H 6.93; N 3.85%
found: C 62.50; H 6.95; N 4.11%

UV spectrum: λ_{max} 238 nm (log ε 3.30). IR spectrum: ν_{max} 3410 br, 1738, 1664, 1640, 718, and 706 cm^{-1}. NMR spectrum: τ 2.64—3.13 (5H, m, aromatic protons), 3.62 (1H, d, CONH; J 8.0 Hz), 5.00—5.50 (1H, m, CH-Phe), 6.33, 6.43, and 6.47 (6H, 3s, $2CH_3O$), to 6.79 (1H, m, H-4, obscured by those of OCH_3), 6.80—7.10 (2H, m, CH_2-Phe), 7.47 (1H, s, OH).

4-c- *(XXIII A) and* 4-t- *(XXIII B) Benzoxycyclohexane-r-1-carbomethoxy--1-carbomethoxy-1-carbonyl-*L*-cyclohexylalanine Methyl Esters*

To a solution of a mixture of stereoisomeric 4-hydroxycyclohexane-1-carbomethoxy derivatives (XXI) (440 mg, 1.19 mmol) in anhydrous pyridine (30 ml) benzoyl chloride (0.16 ml, 1.39 mmol) was added and stirred at room temperature for 20 h. The solvent was removed under reduced pressure and a mixture of stereoisomers separated by preparative TLC (ether-*n*-hexane, 1 : 1). The foamy *cis*-isomer A (R_F ca. 0.24), $[\alpha]_D^{24} = -21.2^0$ (c 2.1) was separated in 47.9% (270 mg) yield.

Anal. $C_{26}H_{35}NO_7$ (473.55) calc'd.: C 65.94; H 7.45; N 2.96%
found: C 66.16; H 7.74; N 3.03%

UV spectrum: λ_{max} 229.5 nm (log ε 4.11). IR spectrum: ν_{max} 3376 br, 1740, 1719, 1715 sh, 1678 br, 753, and 684 cm^{-1}. NMR spectrum: τ 1.82—2.13 and 2.32—2.69 (2H and 3H, 2m, aromatic protons), 3.71 (1H, d, CONH; J 8.1 Hz). 4.79—5.14 (1H, m, H-4), 5.27—5.49 (1H, m, CH-Cha), 6.18 and 6.27 (each 3H, 2s, $2CH_3O$).

The *trans*-isomer B (R_F ca. 0.21), m. p. 87.5—88.5 °C (from ether-*n*-hexane), $[\alpha]_D^{23} = -20.6^0$ (c 2.1) was separated in 28.4% (160 mg) yield.

Anal. $C_{26}H_{35}NO_7$ (473.55) calc'd.: C 65.94; H 7.45; N 2.96%
found: C 66.21; H 7.73; N 3.18%

UV spectrum: λ_{max} 230 nm (log ε 4.08). IR spectrum: ν_{max} 3353, 1745, 1738, 1712, 1641 br, 748, 717, and 691 cm^{-1}. NMR spectrum: τ 1.80—2.10 and 2.40—2.70 (2H and 3H, 2m, aromatic protons), 3.69 (1H, d, CONH; J 8.1 Hz), 4.69—5.02 (1H, m, H-4), 5.23—5.46 (1H, m, CH-Cha), 6.20 and 6.27 (each 3H, 2s, $2CH_3O$).

*2-Oxabicyclo[2.2.2]octan-3-one-4-carbonyl-*L*-cyclohexylalanine Methyl Ester (XXIV)*

A mixture of stereoisomeric 4-oxocyclohexane-1-carboxyl-1-carbonyl-L-cyclohexylalanine methyl ester (XX) (202 mg, 0.569 mmol) and *p*-toluenesuplhonic acid (37 mg, 0.215 mmol) were suspended in anhydrous benzene (5 ml) and heated under reflux for 4 h and left at room temperature for additional 1 h. The solvent was removed, and the residue dissolved in ethyl acetate, washed with 10% $NaHCO_3$ water, dried, and evaporated to dryness. Preparative TLC separated an oily product (80 mg, 41.8%), R_F ca. 0.81 (CH_2Cl_2-MeOH, 95 : 5), distilled at 135—140 °C and 4.0 Pa $[\alpha]_D^{25} = -13.5^0$ (c 1.52).

Anal. $C_{18}H_{27}NO_5$ (337.90) calc'd.: C 64.07; H 8.06; N 4.15%
found: C 64.23; H 8.30; N 4.03%

IR spectrum (EtOH): ν_{max} 3430, 2920 br, 1754, 1722, 1691 sh, and 1681 cm^{-1}. NMR spectrum: τ 1.22 (1H, d, CONH-; J 7.8 Hz), 5.20 br (1H, s, H-1), 5.29—5.51 (1H, m, CH-Cha), 6.28 (3H, s, OCH_3).

Anal. $C_{19}H_{31}NO_6$ (369.45) calc'd.: C 61.76; H 8.46; N 3.79%
found: C 61.78; H 8.57; N 3.65%

IR spectrum: λ_{max} 3474, 2924, 1746, 1737, 1732, 1718, and 1641 cm^{-1}. NMR spectrum: τ 3.69 (1H, d, CONH; J 8.1 Hz), 5.31—5.53 (1H, m, CH-Cha), 6.21 and 6.29 (each 3H, 2s, $2CH_3O$), 7.68 br (1H, s, OH, disappearing in D_2O).

4-c-Benzoxycyclohexane-r-1-carbomethoxy-1-carbonyl-L-cyclohexylalanine Methyl Ester (XXIII A)

A solution of 4-*c*-hydroxycyclohexane-*r*-1-carbomethoxy dipeptide (XXII A) (45 mg, 0.122 mmol) in anhydrous pyridine (1 ml) treated with the equimolar amount of benzoylchloride and was stirred at room temperature for 17 h. The solvent was removed under reduced pressure and the residue separated on a preparative TLC. It afforded a foamy product (48 mg, 83.2%), R_F ca. 0.24 (ether-*n*-hexane, 1 : 1), identical IR, UV, and NMR spectra) with the product obtained from the benzoylation of 4-hydroxycyclohexane derivative XXI.

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SAŽETAK

Peptidi 4-amino- i 4-hidroksicikloheksan-1,1-dikarboksilnih kiselina

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4-Amino, 4-okso- i 4-hidroksicikloheksan-1,1-dikarboksilne kiseline su ugrađene u di- i tri-peptide koji sadrže glicin, L-fenilalanin, L-cikloheksilalanin i L-tirozin. Geometrije 4-*c*- (IX A) i 4-*t*- (IX B) benzamidocikloheksan-r-1-karboksi-1-karbonil-L-fenilalanin metil estera i 4-*c*- (XXIII A) i 4-*t*- (XXIII B) benzoksicikloheksan-r-1-karbometoksi-1-karbonil-L-cikloheksilalanin metil estera su potvrđene NMR spektrima i intramolekularnim ciklizacijama cis-izomera. Otvaranje prstena 2-oksabiciklo[2.2.2]oktan-3-on-4-karbonil-L-cikloheksilalanin metil estera (XXIV) u metanolnoj klorovodičnoj kiselini daje 4-*c*-hidroksicikloheksan-r-1-karbometoksi-1-karbonil-L-cikloheksilalanin metil ester (XXI A) koji benzoiliranjem prelazi u produkt identičan izomeru (XXIII A).

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