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Fmoc Protected Peptide Mimetic Based on a Cyclohexane Framework and Incorporation into Angiotensin II

Christian Kühn[a], Gunnar Lindeberg[c], Adolf Gogoll[d], Anders Hallberg[b], Boris Schmidt*[a]

[a]Institut für Organische Chemie der Universität Hannover, Schneiderberg 1b, D-30167 Hannover, Germany

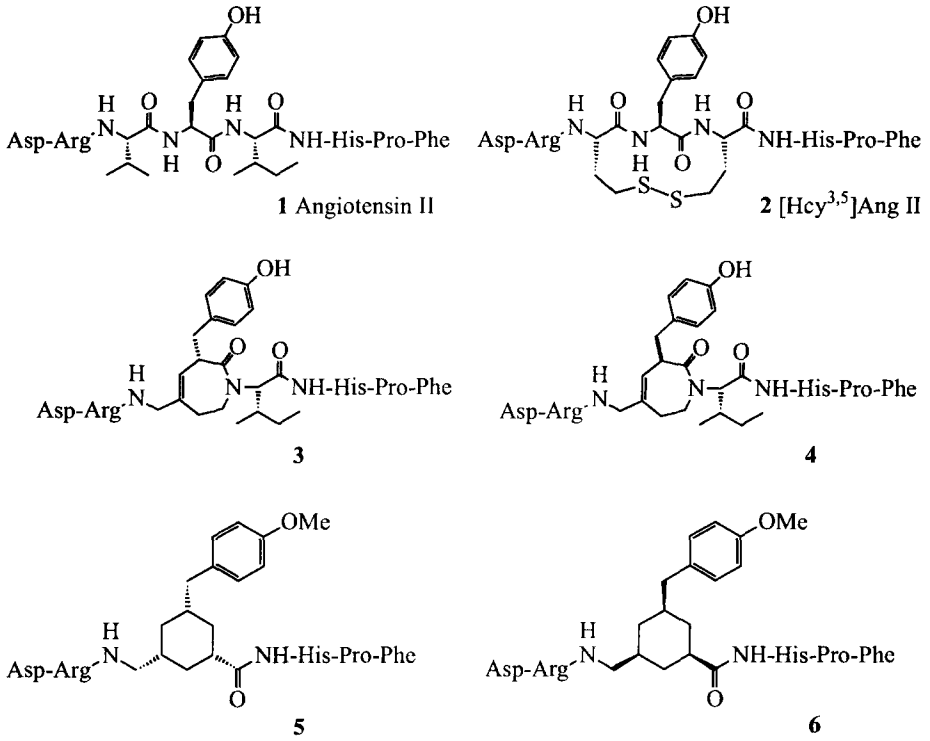
[b]Departments of Organic Pharmaceutical Chemistry, [c]Medical and Physiological Chemistry, Biomedical Centre, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden

[d]Department of Organic Chemistry, Uppsala University, SE-751 21 Uppsala, Sweden.

Abstract: 1,3,5-syn substituted cyclohexane based amino acids have been prepared and incorporated into synthetic peptides to serve as scaffold mimicking the Val-Tyr-Ile sequence of angiotensin II. The conformationally constrained tripeptide mimetic holds potential use as a γ-turn replacement.

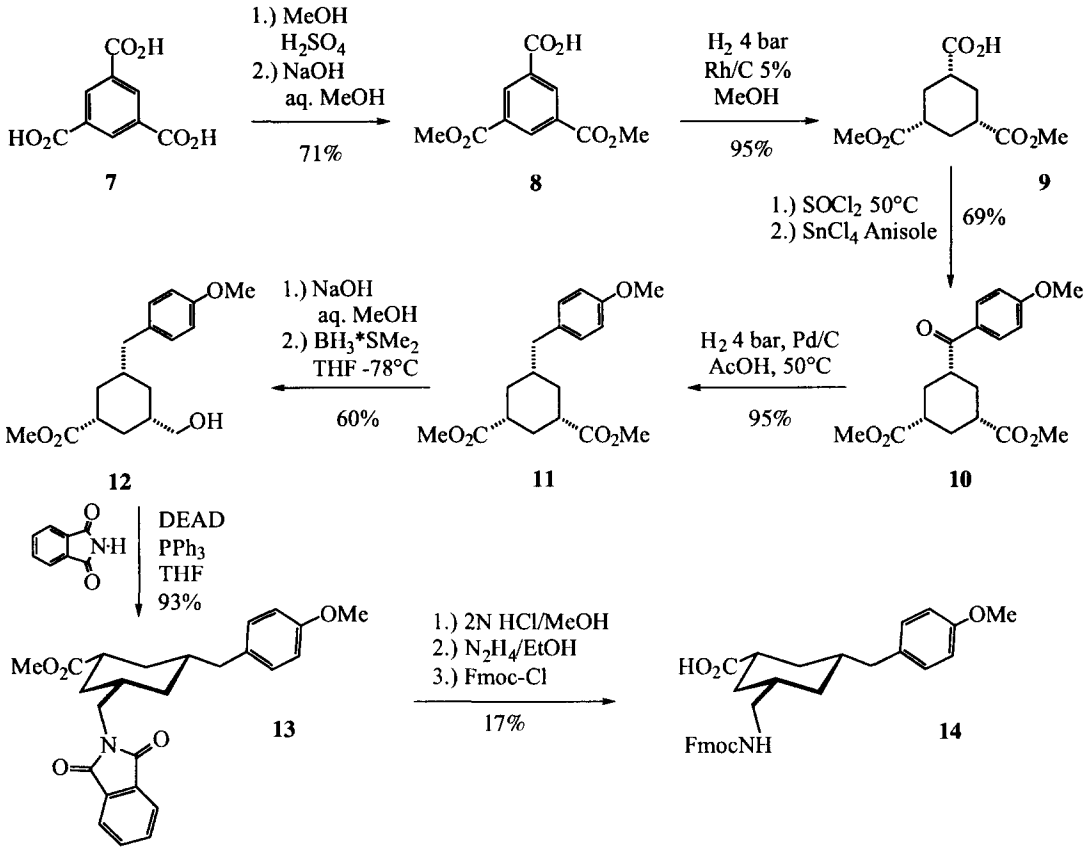
The octapeptide angiotensin II (**1**), plays an important role in blood pressure regulation. The well established structure-activity relationship[1] makes this peptide an attractive target to probe strategies for the design of peptidomimetic analogues[2-5] e.g. **2**. Generally, the problems caused by oral absorption and rapid metabolism of true peptides are reduced for non-peptidic analogues. Moreover, the restriction of conformational freedom may result in improved receptor affinity. Even reduced receptor affinity reveals information on the bioactive conformation and thereby improves design strategies. We recently found that c[Pen3,5]Ang II, which[6] induces a γ-turn formation, is an angiotensin II agonist, although weak.[13]

This prompted us to synthesise and incorporate potential γ-turns in the 3-5 region of Ang II. The number of reported γ-turn mimetics[7-12] is still limited, which lead us to use Huffman's 7-membered azepine for the design[13] of the potential γ-turn mimetic **4**, which displays rather promising binding affinities at the Ang II AT_1 receptor. However, problems in the solid phase peptide synthesis arose from benzyl protection and double bond instability under acidic conditions. This stimulated us to design a new γ-turn motive with higher rigidity and increased chemical stability. Contraction of the tripeptide Val-Tyr-Ile core to *all-syn*-1,3,5-substituted cyclohexanes **5** and **6** removes all inherent flexibility of the ε-lactams **3** and **4** and simultaneously gives derivatives with high chemical and conformational stability.



Scheme 1

Here we report the synthesis of the racemic Fmoc-amino acid **14** from inexpensive trimesic acid. The triacid was esterified according to the literature with MeOH, followed by charge controlled monohydrolysis to give the diester **8** in 71% yield after recrystallisation.[14,15] Hydrogenation at 4 bar over rhodium/C provided the all equatorially substituted cyclohexane **9** in 95% yield. We decided to use anisole in a Friedel-Crafts reaction to introduce the tyrosyl-mimicking subunit, because methylation of the tyrosyl hydroxy function is known to enhance Ang II antagonistic properties. A second catalytic hydrogenation, this time over palladium/C, required a reaction temperature of 50°C for deoxygenation of the ketone **10**. Stimulated by Hoffmann's desymmetrisation of *meso*-diesters[16] we planned a *meso trick* on the key intermediate **11** to provide enantiopure analogues. But interested in both enantiomers, as they might correspond to a γ- and an inverse γ-turn, we continued with the racemic synthesis. The hydrolysis by NaOH in aqueous MeOH and reduction of the crude product with BH_3*Me_2S in THF gave the alcohol **12** in 60% yield, accompanied by diol (20%) resulting from double hydrolysis. A modified Mitsunobu-Gabriel sequence yielded the phthalimide **13** (93%), which was converted to the free amino acid by 2 N HCl in aq. MeOH and subsequent treatment with hydrazine to release the free amine. Protection of the amino acid with Fmoc-chloride proceeded sluggishly and reduced the 3-step yield to 17%.



Scheme 2

The Fmoc-protected acid **14**, which resembles either β- or γ-turn[12,13] geometry, was incorporated as a Ψ[Val-Tyr-Ile] mimetic into the Ang II mimetics **5** and **6** by solid phase peptide synthesis as reported[13] for the lactams **3** and **4**. The diastereomeric peptides were readily separated by HPLC on a 218TP1010 VYDAC column (1 x 25 cm, 10 µm, 0.1 % TFA in H_2O/MeCN 20%–50%) and verified by amino acid analysis and Plasma Desorption Mass Spectroscopy (PDMS). The assignment of the absolute configuration of the diastereomers by NMR spectroscopy was not yet possible. The peptides will be evaluated for their binding properties in the rat pituitary AT_1 receptor binding essay.

EXPERIMENTAL SECTION

General. Melting points (uncorrected): open glass capillaries, Büchi apparatus – ^{1}H and ^{13}C NMR spectra: Bruker WP 200, Bruker AM 400, JEOL JNM-EX 270, Varian Unity 400 spectrometer at 200 (50.3) MHz, 270 (67.8) MHz and 400 (100.58) MHz. Chemical shifts are reported as δ values (ppm) downfield from Me_4Si. – IR spectra: Perkin Elmer 1710 FT and 1600 FT and Bruker IFS 25, recorded in ν_{max} (cm^{-1}). Mass spectroscopy: Applied Biosystems BIOION 20 plasma desorption mass spectrometer, Finnigan MAT 312, VG Autospec (FAB, HRMS). PDMS-samples were applied on aluminised mylar foils, coated with electro-sprayed nitrocellulose, by drying from EtOH/H_2O/TFA mixtures and removal of excess liquid by nitrogen. Elemental analysis: Elementar Vario EL. Column chromatography: Merck and Baker silica gel 60 (40-63 μm and 15-40 μm), Et_2O (ethyl ether), EE (ethyl acetate) PE (light petroleum, bp 40-60°C), MTB ($MeOBu^t$). TLC was carried out using aluminium sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). Chromatographic spots were visualised by UV and/or spraying with an acidic, ethanolic solution of *p*-anisaldehyde, or an ethanolic solution of ninhydrin, followed by heating. Analytical and preparative HPLC/MPLC were performed on a Merck-Hitachi L-6250, equipped with a variable wavelength detector L-4000, RP18 4 x 250 5μm LiChrocart®, Waters Radial Pak 10 μm μBondapak® RP18 (8 x 100 Cartridge and 25 x 100 Prep Pak), 218TP1010 VYDAC (10 μm, 10 x 250) and Sephadex® G10/G15/LH20 columns of various sizes. Amino acid analyses and peptide content were performed by Dr. M. Sundquist on 24 h hydrolysates with an LKB 4151 alpha plus analyser using ninhydrin detection. *Materials:* SPPS resins, amino acid derivatives e.g. Fmoc-His(Boc)-OPfp, Fmoc-Arg(Pmc)OH, Fmoc-Asp(O^tBu)OH and condensation reagents were bought from Fluka Chemie (Switzerland), NovaBiochem (Switzerland), Millipore and Bachem (Switzerland). DCM, DMSO, $CHCl_3$ and DMF were distilled and stored over 3Å molecular sieves. Organic phases were dried over $MgSO_4$.

***(3S*,5R*)-cis-3,5-Bis(methoxycarbonyl)cyclohexanecarboxylic acid* (9)**: A suspension of 4.20 g of benzene-tricarboxylic acid dimethylester[14,15] (17.6 mmol) **7** and 1.66 g Rh/C (5%) in 60 ml dry MeOH was stirred under 4 kPa H_2-atmosphere at 50°C for 14 h. The suspension was filtered and concentrated to leave 4.1 g of a colourless oil, which crystallised on standing: **9** (16.7 mmol, 95%). R_f = 0.28 (PE/EE/AcOH:10/10/0.5). mp. 65-66°C. IR($CHCl_3$): 3512 (w), 1732 (s), 1713 (s), 1436 (s), 1288 (s), 1260 (s), 1172 (s), 1132 (m). ^1H NMR (400MHz, $CDCl_3$): 1.57 (dq, J = 3.0 + 12.8, 3 H, $C\underline{H}_2$), 2.27 - 2.39 (m, 3 H), 2.40 - 2.51 (m, 3 H), 3.72 (s, 6 H, Me). ^{13}C NMR ($CDCl_3$): 30.1 (t, 2-$\underline{C}H_2$), 30.3 (t, 4-$\underline{C}H_2$), 41.4 (d, 1), 41.5 (d, 3+5), 51.9 (q, $CO_2\underline{C}H_3$), 174.4 (s, $\underline{C}O_2Me$), 179.8 (s, CO_2H). Anal.: calc. for $C_{11}H_{16}O_6$: C: 54.10, H: 6.56, found: C: 54.01, H: 6.53. MS (110°C): 227 (11, M^+-OH), 213 (19, M^+-CH_3O), 198 (9, M^+-CO_2H), 184 (6, M^+-CO_2CH_3), 79 (100).

***(1S*,3R*)-cis -5-(4-Methoxybenzoyl)-1,3-bis(methoxycarbonyl)cyclohexane* (10)**: A solution of 0.11 g diester **9** (0.45 mmol) in 0.1 ml DMF was treated with 0.1 ml $SOCl_2$ at 50°C for 3h. Excess $SOCl_2$ was removed in vacuum. The crude acid chloride was mixed with 0.243 g anisole (2.25 mmol), cooled to 0°C and treated with 1.4 ml 1 M $SnCl_4$ solution in DCM over 10 min. The deep red solution was stirred at rt for 14 h, quenched by 0.5 ml 2 M HCl and 1g of ice. Phase separation and extraction of the aqueous phase by 3 x 3 ml DCM gave a crude oil after drying and concentration. Purification by flash chromatography (PE/EE:2/1) gave 0.103 g of **10** as a colourless oil, which crystallised on standing (0.308 mmol, 69%). R_f = 0.28 (PE/EE: 2/1). mp. 75-78°C. IR($CHCl_3$): 2844 (w), 1732 (s), 1672 (s), 1600 (s), 1576 (m), 1256 (s), 1168 (s). ^{1}H NMR ($CDCl_3$): 1.64 (dq, J = 9.3 + 12.7, 3 H, CH_2), 2.15 - 2.22 (m, 2 H), 2.29 - 2.37 (m, 1 H), 2.55 (tt, J = 3.5 + 12.7, 2 H, 1+3), 3.34 (tt, J =3.3 + 12.2, 1 H, 5), 3.68 (s, 6 H, CO_2CH_3), 3.88 (s, 3 H, $PhOCH_3$), 6.95 (d, J = 9.0, 2 H, 3'), 7.92 (d, J = 9.0, 2 H, 2'). ^{13}C NMR ($CDCl_3$): 30.5 (t, 2), 31.0 (t, 4 + 6), 42.1 (d, 1 + 3), 43.4 (d, 5), 51.9 (q, $CO_2\underline{C}H_3$), 55.5 (q, $PhO\underline{C}H_3$), 114.0 (d, 3'), 128.6 (s, 1'), 130.6 (d, 2'), 163.6 (s, H-4'), 174.6 (s, $\underline{C}O_2CH_3$), 199.8 (s, $\underline{C}OPh$). Anal.: calc. for $C_{18}H_{22}O_6$: C: 64.67, H: 6.63, found: C: 64.72, H: 6.53. MS (100°C): 334 (3, M^+), 303 (4, M^+-CH_3O), 274 (2, M^+-CO_2CH_3), 197 (1), 149 (2), 135 (100).

***(1S*,3R*)-cis-5-(4-Methoxybenzyl)-1,3-bis(methoxycarbonyl)cyclohexane* (11)**: A suspension of 45 mg the ketone **10** (0.13 mmol) and 8 mg Pd/C (10%) in 1 ml HOAc was hydrogenated at 3.7 kPa at 50°C for 20 h in a Parr apparatus. The catalyst and HOAc were removed by filtration through silica gel with MTB. Concentration gave 40 mg of the pure product as a yellow oil (0.124 mmol, 95%). R_f = 0.43 (PE/EE:2/1). IR($CHCl_3$): 2860 (w), 1732 (s), 1512 (s), 1244 (s), 1176 (s). ^{1}H NMR($CDCl_3$): 1.10 (q, J = 12.6, 2 H), 1.52 (q, J = 12.7, 1 H), 1.92 - 2.00 (m, 2 H), 2.16 - 2.24 (m, 1 H), 2.33 (tt, J =3.5 + 12.4, 2 H, 1 + 3), 2.51 (d, J = 7.0, 2 H, $C\underline{H}_2Ph$), 3.65 (s, 6 H, $CO_2C\underline{H}_3$), 3.79 (s, 3 H, $PhOC\underline{H}_3$), 6.82 (d, J = 8.4, 2 H, 3'), 7.04 (d, J = 8.4, 2 H, 2'). ^{13}C NMR ($CDCl_3$): 31.1 (t, 2-$\underline{C}H_2$), 34.4 (t, 4-$\underline{C}H_2$), 38.6 (d, 5), 42.3 (d, 1 + 3), 42.4 (t, $\underline{C}H_2Ph$), 51.7 (q, $CO_2\underline{C}H_3$), 55.2 (q, $PhO\underline{C}H_3$), 113.7 (d, 3'), 130.0 (d, 2'), 131.9 (s, 1'), 157.9 (s, 4'), 175.4 (s, C=O). MS (120°C): 320 (12, M^+), 289 (3, M^+-CH_3O), 121 (100). HRMS: calc. for $C_{18}H_{24}O_5$: 320.162374, found: 320.16229.

***cis,cis-3-Hydroxymethyl-5-(4-methoxybenzyl)-1-methoxycarbonylcyclohexane* (12)**: 360 mg Diester **11** (1.13 mmol) and 89 mg potassium hydroxide (1.59 mmol) were refluxed in 18.8 ml MeOH and 1.2 ml H_2O for 36 h. The solution was concentrated, treated with 10 ml $NaHCO_3$ sol. and extracted by MTB. The aqueous phase was acidified by HCl and extracted by DCM. The latter extracts were dried and concentrated to leave 253 mg of two acids ($R_{f,1}$ = 0.15, $R_{f,2}$ = 0.34, PE/EE/AcOH:40/20/3). The crude mixture was reduced by BH_3*Me_2S 0.12 ml in 5 ml dry THF at -78°C, warmed to rt for 12 h and quenched by 3 ml NH_4Cl solution. Extraction by DCM, drying ($MgSO_4$) and concentration left a crude oil, which was purified by flash chromatography (PE/EE = 3/2) to give 193 mg of the hydroxyester **12** (0.66 mmol, 60%) and 62 mg of the diol (0.22 mmol, 20%). R_f = 0.34 (PE/EE:1/1). IR($CHCl_3$): 3328 (w), 3372 (b), 2928 (s), 2860 (m), 1724 (s), 1512 (s), 1244 (s). ^{1}H NMR ($CDCl_3$): 0.67 (q, J = 12.2, 1 H), 1.08 (qd, J = 4.8 + 12.3, 2 H), 1.49 - 1.66 (m, 2 H), 1.71 - 1.80 (m, 1 H), 1.91

- 2.06 (m, 2 H), 2.33 (tt, J = 3.5 + 12.4, 1 H, 1-H), 2.50 (d, J = 7.0, 2 H, $C\underline{H}_2Ph$), 3.47 (dd, J = 1.7 + 6.1, 2 H, $C\underline{H}_2OH$), 3.64 (s, 3 H, CO_2Me), 3.79 (s, 3 H, $PhOC\underline{H}_3$), 6.82 (d, J = 8.6, 2 H, 3'), 7.05 (d, J = 8.6, 2 H, 2'). ^{13}C NMR($CDCl_3$): 31.8 (t), 35.05 (t), 35.15 (t), 38.7 (d), 39.5 (d, 3), 42.66 (d, 1), 42.74 (t), 51.6 (q, $CO_2\underline{C}H_3$), 55.2 (q, PhO<u>Me</u>), 67.9 (t, $\underline{C}H_2OH$), 113.6 (d, 3'), 130.0 (d, 2'), 132.4 (s, 1'), 157.8 (s, 4'), 176.3 (s, C=O). MS (80°C): 292 (5, M^+), 261 (1, M^+-CH_3O), 84 (100). HRMS: calc. for $C_{17}H_{24}O_4$: 292.167460, found: 292.1675.

cis,cis-1,3-Bis(hydroxymethyl)-5-(4-methoxybenzyl)-cyclohexane: R_f = 0.20 (PE/EtOAc:1/1). IR($CHCl_3$): 3324 (m), 3428 (b), 2916 (s), 2844 (w), 1712 (m), 1672 (m), 1512 (s), 1244 (s). ^1H NMR($CDCl_3$): 0.62 (q, J = 12.1, 3 H), 1.44 - 1.67 (m, 3 H), 1.75 (d, J = 12.3, 2 H), 1.86 (d, J = 11.8, 1 H), 2.48 (d, J = 7.0, 2 H, CH_2Ph), 3.45 - 3.53 (m, 4 H, $C\underline{H}_2OH$), 3.79 (s, 3 H, PhOMe), 6.82 (d, J = 8.4, 2 H, 3'), 7.05 (d, J = 8.4, 2 H, 2'). MS (90°C): 264 (13, M^+), 122 (20), 121 (68), 91 (4), 84 (100). HRMS: calc. for $C_{16}H_{24}O_3$: 264.172545, found: 264.173.

cis,cis 5-(4-Methoxybenzyl)-1-methoxycarbonyl-3-phthalimidomethylcyclohexane (13): Hydroxyester **12** (80 mg, 0.27 mmol), phthalimide (45 mg, 0.30 mmol) and triphenylphosphine (79 mg, 0.30 mmol) were dissolved in 0.12 ml dry THF under argon atmosphere at rt and treated with diethylazodicarboxylate (52 mg, 0.30 mmol) over a period of 5 min. The mixture was stirred for 14 h, concentrated, and purified by flash chromatography (PE/EE:3/2) to yield **13** as a colourless wax (106 mg, 0.25 mmol, 93%). R_f = 0.49 (PE/EE:2/1). IR($CHCl_3$): 2932 (m), 2848 (w), 1772 (s), 1734 (s), 1712 (s), 1612 (w). ^1H NMR($CDCl_3$): 0.78 (q, J = 12.1, 1 H), 1.07 (q, J = 12.4, 1 H), 1.15 (q, J = 12.3, 1 H), 1.49 - 1.61 (m, 1 H), 1.69 - 1.77 (m, 1 H), 1.81 - 1.96 (m, 3 H), 2.26 (tt, J = 3.3 + 12.4, 1H, 1), 2.42 (dd, J = 7.6 + 13.6, 1 H, $C\underline{H}_2Ph$), 2.51 (dd, J = 6.4 + 13.5, 1 H, $C\underline{H}_2Ph$), 3.55 (dd, J = 1.2 + 6.6, 2 H), 3.61 (s, 3 H, $CO_2C\underline{H}_3$), 3.77 (s, 3 H, OMe), 6.80 (d, J = 8.6, 2 H, 3'), 7.02 (d, J = 8.6, 2 H, 2'), 7.69 - 7.74 (m, 2 H), 7.81 - 7.86 (m, 2 H). ^{13}C NMR ($CDCl_3$): 32.9 (t), 34.6 (t), 36.35 (d), 36.6 (t), 38.6 (d), 42.60 (t, $\underline{C}H_2Ph$), 42.63(d, 1), 43.6 (t), 51.6 (q, $CO_2\underline{C}H_3$), 55.2 (q, $O\underline{C}H_3$), 113.6 (d, 3'), 123.3 (d), 130.0 (d, 2'), 132.0 (s), 132.2 (s), 134.0 (d), 157.8 (s, 4'), 168.6 (s, $\underline{C}O$), 175.8 (s, $\underline{C}O_2Me$). MS (100°C): 422 (3), 421 (6, M^+), 147 (87), 104 (73), 91 (9), 76 (100). HRMS: calc. for $C_{25}H_{27}NO_5$: 421.1889079, found: 421.1883.

cis,cis-N-Fluorenylmethyloxycarbonyl-3-aminomethyl-5-(p-methoxybenzyl)-1-cyclohexanecarboxylic acid (14): Phthalimidoester **13** (144 mg, 0.343 mmol) was refluxed in 2.8 ml 2 N HCl (40 % MeOH) for 3 h. The mixture was concentrated, acidified by 3 drops conc. HCl and extracted by 5x 5ml $CHCl_3$. The pooled, dried organic phases were concentrated and heated to reflux with 1.3 ml EtOH and 26 mg (0.53 mmol) hydrazine hydrate for 4 h. The mixture was concentrated in vacuum, treated with 1 ml 2N HCl and heated to 50°C for 10 min. The precipitate was filtered off and washed by 2x 1.5 ml 2N HCl. The combined liquids were adjusted to pH 4 by Na_2CO_3 and extracted by 6x 8 ml $CHCl_3$. The dried, concentrated extracts were redissolved in 0.72 ml 10% Na_2CO_3 solution and 0.35 ml dioxane. A solution of Fmoc-Cl (69 mg, 0.26 mmol) in 0.53 ml dioxane was added over 30 min at 0°C. The solution was stirred at 0°C for 3 h and an additional h at rt prior to quenching by 14 ml of H_2O and acidification to pH 2 by conc. HCl. Concentration of the dried $CHCl_3$ extracts left a crude oil,

which was purified by flash chromatography (PE/EE/AcOH:50/50/1) to give **14** as yellow oil (30 mg, 0.06 mmol, 17%) R_f= 0.66 (PE/EE/AcOH:50/50/1). IR($CHCl_3$): 3454 (w), 2945 (m), 2857 (w), 1711 (m), 1660 (s), 1365 (m), 1092 (m). ^{1}H NMR ($CDCl_3$/MeOH-D_4): 0.54 (q, J = 12.1, 1 H), 0.74 - 1.06 (m, 2 H), 1.35 - 1.56 (m, 2 H), 1.58 - 1.70 (m, 1 H), 1.75 - 1.97 (m, 2 H), 2.19 (bt, J = 10.5, 1H, 1), 3.55 (bd, J = 2.40, 2 H, C$\underline{H}_2$N), 2.87 (dd, J = 7.1 + 13.7, 1 H, C$\underline{H}_2$Ph), 2.97 (dd, J = 6.4 + 13.7, 1 H, C$\underline{H}_2$Ph), 3.69 (s, 3 H, PhOC$\underline{Me}$), 4.11 (d, J = 7.2, 1 H, CH_{Fmoc}), 4.21 - 4.37 (m, 2 H, $CH_{2,Fmoc}$), 6.73 (bd, J = 8.6, 2 H, 3'), 6.96 (bd, J = 8.2, 2 H, 2'), 7.17 - 7.39 (m, 4 H, Fmoc), 7.43 - 7.73 (m, 4 H, Fmoc). ^{13}C NMR($CDCl_3$/MeOH-D_4): 32.5, 34.8, 36.0, 37.0, 38.5, 42.4, 46.5, 47.0, 54.9 (PhO$\underline{C}H_3$), 66.2 (CH_{Fmoc}), 70.8 (CH_2O), 113.6, 119.6, 124.7, 126.8, 127.4, 129.8 (2'), 133.9 (1'), 141.0, 143.7, 156.8 (C=O), 157.5 (4'), 178.4 (CO_2H). PDMS: 523 (M^++Na^++H^+).

***Asparagyl-arginyl-(cis,cis-3-aminomethyl-5-(p-methoxybenzyl)-1-cyclohexanecarbonyl)-histidyl-prolyl-phenylalanine* (5,6)**: 89.4 mg Fmoc-His(Trt)-Pro-Phe-Wang-resin (42.09 µmol, Nova) were suspended in 2 ml DMF, deprotected by 20% (v/v) piperidine-DMF solution (3x) and rinsed 6x by DMF. The resin is suspended in a mixture of 23.1 mg N-fluorenylmethyloxycarbonyl-3-aminomethyl-5-(p-methoxybenzyl)-1-cyclohexane-carboxylic acid (46.3 µmol), 36.1 mg PyBob (69.5 µmol), 17.9 mg DIEA (139 µmol) and 1 ml DMF and shaken for 22 h. The resin is collected, washed by DMF (3x), capped by 10% (v/v) Ac_2O-DMF and 15.9 mg (122.7 µmol) DIEA within 10 min, washed by DMF (6x), deprotected by 20% (v/v) piperidine-DMF solution (3x), and finally rinsed by DMF (6x). The elaboration to the full peptide was achieved by 96.5 mg (126.2 µmol) Fmoc-Arg(Pmc)-OH*(i-Pr)$_2$O and 51.9 mg Fmoc-Asp(^{t}Bu)-OH (126.2 µmol) in analogue procedures, yet in reduced coupling time (2h). Fmoc deprotection by 20% (v/v) piperidine-DMF solution (3x), washing by DMF (6x), DCM (6x) and drying left the resin ready for deprotection by 1.5 ml TFA-TESH-H_2O (90/5/5) within 80 min and a final rinse by TFA (3x 0.3 ml). The concentrated TFA solutions (1.5 ml) were diluted by Et_2O, cooled to 0°C for 20 min and centrifuged. The precipitated peptide was 5x resuspended in TFA and precipitated by Et_2O. The residue was dried *in vacuo* to leave 29.9 mg crude peptides, which were separated by C_{18}-RP chromatography on a 218TP1010 VYDAC column (1 cm x 25 cm, 10 µm, 0.1% TFA/H_2O, MeCN, gradient 20%-50% MeCN). Lyophilisation left the diastereomeric peptides **5** and **6** as amorphous powders. Assignment of the absolute stereochemistry of the cyclohexane subunit by NMR techniques was not yet possible. Diastereomer A: 7.8 mg, PDMS: 930.5 (M^++1), amino acid analysis: Asp: 1.00, Pro: 1.00, Phe: 0.98, His: 1.00, Arg: 1.01, peptide cont.: 77%. Diastereomer B: 9.0 mg PDMS: 930.7 (M^++1), amino acid analysis: Asp: 1.00, Pro: 0.98, Phe: 1.00, His: 1.03, Arg: 0.98, peptide cont.: 75%.

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