Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Structural properties of cyclic peptides containing cis- or trans-2-aminocyclohexane carboxylic acid

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^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX 500 (^{1}H: 500.1 MHz, ^{13}C: 125.8 MHz) with $Si(CH_3)_4$ as internal standard. Chemical shifts are reported in δ and the coupling constants J are given in Hz. Infrared spectra were obtained with an ATI Mattson Genesis Series FTIR. MALDI-ToF MS analyses were performed by a Voyager-DE (Perseptive Biosystems) using 2,5-dihydrobenzoic acid as the matrix. Preparative RP-HPLC was performed on a Thermo Separation Products system equipped with a Vydac 218 TP 1022 efficiency column (22 * 250 mm) using water/acetonitrile as eluent and UV detection at 220 nm.

Amino acid syntheses:

Syntheses of cis- and trans-2-aminocyclohexane carboxylic acid have been done according to the procedures described by the Davies group.[1]

Fmoc-cis-2-aminocyclohexane carboxylic acid[2]

6.60 g (26.0 mmol) cis-2-aminocyclohexane carboxylic acid were dissolved in 30.0 mL water with 1 equiv. (2.63 g) triethylamine to which 8.10 g (24.0 mmol) 9-fluorenylmethyl succinimidyl carbonate in 25.0 mL acetonitrile was added. The pH of the solution was adjusted to 8.5 – 9.0 with triethylamine and maintained until no further drop in the pH was detectable over 15 minutes. The reaction mixture was then filtered and evaporated under reduced pressure to give a viscous oil which was slowly added to 1.5 M HCl (100 mL). The solution was extracted with 100 mL ethyl acetate. The organic layer was filtered through a sintered funnel (porosity 4) to remove residual flocculate, washed three times with 100 mL volumes of 1.5 M HCl, water and saturated NaCl before drying over anhydrous $MgSO_4$. After

evaporation recrystallization from ethanol gave 6.50 g (17.8 mmol, 74%) of the desired product.

^{1}H-NMR (500 MHz, DMSO): 0.80 – 0.92 (m, 2H, -CH_2-), 1.10 – 1.90 (m, 7H, $(CH_2)_3$, CH(COO)), 2.09 (m, 1H, CH-NH), 4.21 (t, ^{3}J = 6.8 Hz, 1H, Fluorenyl-C<u>H</u>-CH_2-O), 4.36 (d, ^{3}J = 6.8 Hz, 2H, Fluorenyl-CH-C<u>H_2</u>-O), 5.61 (m, 1H, NH), 7.30 (m, 2H, arom), 7.38 (m, 2H, arom), 7.57 (m, 2H, arom), 7.74 (m, 2H, arom). ^{13}C-NMR (500 MHz, DMSO): 22.3 (s, CH_2), 24.0 (s, CH_2), 25.4 (s, CH_2), 29.6 (s, CH_2), 44.4 (t, Fluorenyl-<u>C</u>H- CH_2), 47.2 (t, CH-COOH), 49.7 (t, CH-NH), 66.8 (s, Fluorenyl-CH-<u>C</u>H_2-O-), 119.9 (t, arom.), 125.1 (t, arom.), 127.0 (t, arom.), 127.4 (t, arom.), 142.4 (q, arom.), 144.0 (q, arom.), 155.8 (q, NH-CO-O), 179.0 (q, COOH). IR (KBr): 3417 br, 3332 br, 3070 m, 2931 s, 2858 m, 1708 vs, 1511 s, 1450 s, 1338 m, 1249 s, 1222 s, 1103 s, 1045 s, 740 s.

<u>Fmoc-trans-2-aminocyclohexane carboxylic acid[2]</u>

This compound was prepared according to the above mentioned procedure using trans-2-aminocyclohexane carboxylic acid (0.5 g, 3.5 mmol) in a yield of 1.10 g (3.0 mmol, 86%) of Fmoc-trans-2-aminocyclohexane carboxylic acid.

^{1}H-NMR (500 MHz, DMSO): 0.80 – 0.92 (m, 2H, -CH_2-), 1.10 – 1.87 (m, 7H, $(CH_2)_3$, CH(COO)), 2.24 (m, 1H, CH-NH), 4.21 (t, ^{3}J = 5.3 Hz, 1H, Fluorenyl-C<u>H</u>-CH_2-O), 4.23 (d, ^{3}J = 5.3 Hz, 2H, Fluorenyl-CH-C<u>H_2</u>-O), 7,31 (m, 2H, arom), 7.40 (m, 2H, arom), 7.68 (m, 2H, arom), 7.87 (m, 2H, arom). ^{13}C-NMR (500 MHz, DMSO): 24.8 (s, CH_2), 25.2 (s, CH_2), 28.8 (s, CH_2), 32.2 (s, CH_2), 46.7 (t, Fluorenyl-<u>C</u>H- CH_2), 48.4 (t, CH-COOH), 50.8 (t, CH-NH), 65.2 (s, Fluorenyl-CH-<u>C</u>H_2-O-), 120.1 (t, arom.), 125.3 (t, arom.), 127.1 (t, arom.), 127.6 (t, arom.), 143.8 (q, arom.), 144.0 (q, arom.), 155.2 (q, NH-CO-O), 175.4 (q, COOH). IR (KBr): 3307 s, 3043 m, 2935 m, 2856 m, 1705 s, 1693 s, 1533 s, 1236 m, 740 m.

Peptide Syntheses

Fmoc-α-amino acids were purchased from IRIS, Marktredwitz, Germany and ACT, Cambridgeshire, UK. Linear peptides were synthesized on an AdvancedChemTech 496 MOS peptide synthesizer according to an Fmoc-protocol using 2-chloro trityl resin as solid support pre-loaded with leucine.

Coupling was performed in duplicate with 1.5 equiv. Fmoc-amino acid (0.3 M in DMF), 1.5 equiv. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU; 0.3 M in DMF) and 3 equiv. diisopropylethylamine (DIPEA; 0.6 M in DMF) each. After washing with DMF the Fmoc group was cleaved with a solution of 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF.

The linear peptide was cleaved from the resin using a solution of 1% trifluoroacetic acid (TFA) in dichloromethane. The resin was treated with this solution 10 times for 5 minutes each.

The linear peptide is cyclized using a dual syringe pump with 3 equiv. 1-[(dimethylamino)-(dimethyliminium)methyl]-1H-1,2,3-triazolo[4,5-b]pyridine-3-oxide hexafluorophosphate (HATU) and 6 equiv. DIPEA in DMF.

Finally the permanent protecting groups were removed with a solution of 95% TFA, 2.5% triisopropyl silane and 2.5% water.

The peptides were purified with preparative RP-HPLC and characterized with MALDI-ToF MS and NMR.

Experimental Details for the NMR studies:

The samples were prepared by dissolving 4 – 10 mmol of the peptide in DMSO-D_6. The NMR spectra were recorded at 300 K and processed using XWINNMR software (Bruker). Chemical shifts were calibrated with the solvent signal for ^{1}H 2.49 ppm and ^{13}C 39.6 ppm. The temperature dependent proton spectra were measured between 300 and 330 K in 5 K steps. The crosspeak volumes of ROESY spectra were extracted using the program XWINNMR (Bruker). ^{13}C-Signals were assigned using inverse techniques (HMQC, HMBC). For conformational analysis of the peptides the following NMR experiments were used:

Homonuclear experiments:

Experiment	td in F2	ns	Mixing time [ms]	Processing
^{1}H		64		Exponential function
COSY	2048	24		Sinebell function in F1 und F2
TOCSY	2048	24	40 - 80	Squared sinebell function shifted by $+\pi/2$ in F1 und F2
ROESY	1024	16	140 - 200	Squared sinebell function shifted by $\dot{+}\pi/2$ in F1 und F2

Bruker Avance 600 (sf [^{1}H]: 600.1 MHz; sw 9 – 11 ppm; td in F1: 256; p1 [90°]: 10.5 µs, pL1: 4 dB; d1: 1.5 – 2 s)

Heteronuclear experiments:

Experiment	td in F2	ns	d2 [ms] J_{CH} = 145 Hz	d6 [ms]	Processing
HMQC	2048	32	3.45		Squared sinebell function shifted by $\dot{+}\pi/2$ in F1 und F2
HMBC	2048	32	3.45	70	Squared sinebell function shifted by $\dot{+}\pi/2$ in F1 und F2

Bruker Avance 600 (sf [^{1}H]: 600.1 MHz, sf [^{13}C]: 150.9 MHz; sw 160 – 200 ppm in F1, sw 9 – 11 ppm in F2; td in F1: 256; p1 [90°]: 10.5 µs, pL1: 4 dB; pL2: -3 dB; d1: 1.5 – 2 s)

Theoretical investigations:

All structural calculations and molecular dynamics simulations were carried out using the InsightII Software package (MSI) and the GROMACS[3] software package on a Silicon Graphics O_2 workstation and a Linux PC with two AMD Athlon processors. The simulations were performed using the force field with automatic evaluation and cluster analysis of the trajectories. Starting structures were generated by torsion angle variation under experimental constraints. The structures were simulated in a cubic solvent box with periodic boundary conditions (a = 3.5 Å, ≈300 DMSO molecules) for 10 ps with constraints and for 1.2 ns in a free molecular dynamics simulation. Snapshots of the trajectory were clustered. All structures correspond to the investigated proton-proton distances within an error margin of about 10%.

NMR data:

P1: MALDI-ToF MS: $[M+Na]^+$ 577.28 (calcd.: 577.26).

^1H (600 MHz):

δ [ppm]	NH	CH^{α}	CH^{β}	CH^{γ}	CH^{δ}	H^{ω}	Δδ/ΔT (NH) [ppb/K]	$^3J_{NHH\alpha}$ [Hz]
Ser	8.05	3.95	3.72; 3.69				-1.9	7.2
cACHC	7.39	3.85	2.48 – 2.56	1.94 – 1.86	1.55- 1.24*	1.55- 1.24*	-0.5	5.7
Leu	8.21	3.71	1.86	1.56	0.89; 0.86		-4.4	5.3
Asn	8.05	4.41	2.81; 2.57			7.51; 7.04	-3.8	6.4
Asp	8.35	4.32	2.79; 2.64			12.3	-2.8	7.9

* Signal overlap

^{13}C (600 MHz):

δ [ppm]	CO	C^{α}	C^{β}	C^{γ}	C^{δ}	C^{ω}
Ser	171.3	59.3	62.0			
cACHC	169.9	48.9	45.0	39.3	25.3	23.4; 35.4
Leu	175.5	61.8	29.7	25.5	22.3; 24.2	
Asn	172.0	52.1	36.2			173.2
Asp	173.4	51.5	36.7			171.2

P2: MALDI-ToF MS: $[M+Na]^+$ 577.22 (calcd.: 577.26).

^1H-NMR (600 MHz):

δ [ppm]	NH	CH^{α}	CH^{β}	CH^{γ}	CH^{δ}	H^{ω}	Δδ/ΔT (NH) [ppb/K]	$^3J_{NHH\alpha}$ [Hz]
Ser	7.57	4.18	3.68; 3.54				-1.7	9.1
tACHC	7.18	3.41	2.47	1.94 – 1.80	1.57- 1.08*	1.57- 1.08*	-2.0	7.2
Leu	7.76	4.34	1.32 – 1.23	1.65	0.87; 0.83		-4.3	8.7
Asn	8.09	4.31	2.84; 2.72			7.41; 6.95	-3.1	7.9
Asp	8.54	4.15	2.64; 2.47*				-3.6	7.2

* Signal overlap

^{13}C-NMR (600 MHz):

δ [ppm]	CO	C^{α}	C^{β}	C^{γ}	C^{δ}	C^{ω}
Ser	170.2	57.2	62.0			
tACHC	169.1	52.3	48.7	30.5	23.2	24.3; 39.8
Leu	173.3	51.6	32.4	25.7	22.2; 24.2	
Asn	170.7	52.2	36.2			172.9
Asp	173.8	52.4	37.0			172.0

P3: MALDI-ToF MS: $[M+Na]^+$ 690.34 (calcd.: 690.13).

δ [ppm]	NH	CH^{α}	CH^{β}	CH^{γ}	CH^{δ}	H^{ω}	Δδ/ΔT (NH) [ppb/K]	$^3J_{NHH\alpha}$ [Hz]
Ser	7.82	4.04	3.64; 3.53				-2.4	7.9
cACHC	7.59	4.20	2.65	1.69 – 1.61	1.35; 1.25	1.50; 1.76	-1.6	8.9
Leu	8.48	3.59	1.54 – 1.46	1.71	0.90; 0.86		-4.4	n.d.
Asn	8.31	4.27	2.69; 2.52			7.58; 7.13	-3.0	5.4[#]
Ile	7.73	4.15	1.86	1.48 (CH_2); 0.81 (CH_3)	0.80		0	7.5[#]
Asp	7.95	4.15	2.99; 2.75				-1.2	5.8[#]

*Signal overlap

^{13}C (600 MHz):

δ [ppm]	CO	C^{α}	C^{β}	C^{γ}	C^{δ}	C^{ω}
Ser	169.4	56.3	60.8			
cACHC	171.9	46.6	45.8	24.4	30.3	22.5; 30.6
Leu	178.9	55.3	39.7	24.2	21.6; 23.6	
Asn	173.3	52.5	35.5			169.3
Ile	172.9	58.8	36.3	12.0 (CH_3); 30.3 (CH_2)	16.0	
Asp	173.0	51.9	35.9			169.1

P4: MALDI-ToF MS: $[M+Na]^+$ 690.34 (calcd.: 690.70).

^{1}H-NMR (600 MHz):

δ [ppm]	NH	CH^{α}	CH^{β}	CH^{γ}	CH^{δ}	H^{ω}	Δδ/ΔT (NH) [ppb/K]	$^{3}J_{NHH\alpha}$ [Hz]
Ser	7.63	4.09	3.56*				-2.7	8.9
tACHC	7.42	4.71	3.56*	1.82 – 1.73	1.69 – 1.61	1.42 – 1.32*; 2.65 – 2.56*	-2.3	7.4
Leu	7.83	4.02	1.52 – 1.44	1.18	0.88; 0.80		-4.6	n. d.
Asn	7.51	4.50	2.73; 2.61			7.66; 7.21	0.6	n. d.
Ile	8.34	3.84	1.75	1.21 (CH_2) 0.86 (CH_3)	0.85		-1.5	n. d.
Asp	8.45	4.36	2.87; 2.65				-4.7	6.9#

*Signal overlap

^{13}C-NMR (600 MHz):

δ [ppm]	CO	C^{α}	C^{β}	C^{γ}	C^{δ}	C^{ω}
Ser	169.4	56.3	61.7			
tACHC	172.5	50.1	50.9	29.9	30.9	24.3; 41.0
Leu	178.3	51.0	40.7	25.2	23.7; 21.6	
Asn	172.5	49.7	38.3			170.5
Ile	170.4	59.9	36.3	40.7 (CH_2); 11.9 (CH_3)	15.7	
Asp	172.8	51.4	36.3			170.3

Torsion Angles of Designed Peptides

	P1a		**P1b**		**P2**		**P3**		**P4**	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
Ser	73°	-59°	-121°	-120°	-116°	-64°	-95°	-88°	-73°	155°
ACHC	-120°	152°	-119°	-64°	-119°	-162°	-87°	105°	-104°	73°
Leu	-62°	-9°	-83°	-176°	-91°	-27°	-63°	-20°	-140°	95°
Asn	-100°	-64°	-53°	-39°	167°	-74°	-74°	-64°	-158°	179°
Ile							-126°	132°	-55°	-32°
Asp	-146°	114°	-84°	-39°	-100°	-59°	81°	-103°	-113°	-52°

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