Synthesis of a chiral amino acid with bicyclo[1.1.1]pentane moiety and its incorporation into linear and cyclic antimicrobial peptides

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Peptide Synthesis and Purification

Synthesis of the peptides was performed manually by the solid-phase method using standard Fmoc chemistry. TentaGel SRAM resin (Rapp, 0.22 mmol g^{-1}) or 2-Chlorotrityl resin (Novabiochem, 1.23 mmol g^{-1}) were used for the linear peptides or for the linear precursors of the cyclic peptides, respectively. Couplings were carried out with 3 or 4 equiv of Fmoc-amino acid derivatives, 3-4 equiv of HBTU and 6-8 equiv of DIEA at ambient temperature. After final cleavage with 10% phenol in TFA crude linear peptides were purified by preparative HPLC on a PolyEncap A300 column, 10 µm, 250 × 20 mm i.d. (Bischoff Analysentechnik GmbH, Leonberg), using a LDC Analytical System: mobile phase A, 0.1% TFA/water: mobile phase B, 80% acetonitrile/water-0.1% TFA (v/v/v); linear gradient 20-60% B in 70 min, at a flow rate of 10 ml/min. Cyclisation of crude peptides was achieved by activation with 1.1 equiv. HAPyU in DMF (1.0 mM). Purification was done as described above.

HPLC analyses were carried out on a PolyEncap A300 column using an acetonitrile/water/TFA solvent system (eluent A: 0.1% TFA, eluent B: 80% acetonitrile/0.1% TFA, linear gradient 5-95% B in 40 min). Peptides were characterised by matrix-assisted laser desorption/ionisation mass spectrometry which gave the expected $[M+H]^+$ mass peaks.

Name	Sequence	mw calcd. $[M+H]^+$	mw found $[M+H]^+$	HPLC purity
10a	Ac-RRWWRF-NH_2	1047.58	1047.53	>99%[a]
11a	Ac-RRW**1**RF-NH_2	1040.63	1040.66	>99%[a]
12a	Ac-RR**11**RF-NH_2	1033.68	1033.60	96%[a]
	H-RRWWRF-OH	1006.54	1006.45	97%[a]
	H-RRW**1**RF-OH	999.59	999.60	70%
	H-RR**11**RF-OH	992.64	992.65	53%
10b	cyclo(RRWWRF)	988.54	988.47	97%[a]
11b	cyclo(RRW**1**RF)	981.59	981.39	85%[a]
12b	cyclo(RR**11**RF)	974.64	974.46	90%[a]

[a] Purified product.