Supporting Information

© Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2005
Supporting Information

for

Rapid and Quantitative Cyclization of Multiple Peptide Loops onto Synthetic Scaffolds for Structural Mimicry of Protein Surfaces.

Peter Timmerman, Joris Beld, Wouter C. Puijk, Rob H. Meloen

FSH-β peptide microarray: Peptides were synthesized on an amino-functionalized solid support (polypropylene grafted with polyacrylic acid and treated with Boc-HMDA/DCC/HOBt followed by removal of the Boc groups with TFA) using standard Fmoc-peptide chemistry. The peptides were fully deprotected following standard procedures using TFA with scavengers. The deprotected peptides on the solid support were washed with excess of water and sonicated in disrupt-buffer (1% SDS/0.1% β-mercaptoethanol (BME) in phosphate-buffered saline (PBS, pH 7.2) at 70 °C for 30 min. followed
by sonication in water for another 45 min and subsequently used for screening in an ELISA-assay.

**ELISA:** microarrays were pretreated with PBS for 30 min. followed by precoating with incubation buffer (PBS containing 5% ovalbumin, 5% horse serum and 1% Tween-80) for 1h. Then, the microarrays were incubated with mAb 2 (10 ug/mL, diluted in incubation buffer) overnight at 4°C. After washing (3x10 min.) with PBS/Tween-80 (0.05%), the peptides were incubated with peroxidase-labeled rabbit anti-mouse antibody for 1h at 25°C (rampo, 1/1000; Dako, Glostrup, Denmark) and subsequently, after washing again (3 x 10 min.) with PBS/Tween-80 (0.05%), incubated with the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS; 50 mg in 100 mL 0.1 M citric acid-sodium phosphate (McIlvaine) buffer (pH 4.0) containing 20 uL 30% H₂O₂). After 1h the absorbance (at 405 nm) was measured using a CCD-camera (XC-77RR, Sony, Japan). Bound mAb was removed by sonication in disrupt-buffer as described above.
Supplementary Information (1a)

Reaction of 24-mer peptide 1f with 1.05 equiv. of o-T2

after 1 min.

after 5 min.

after 15 min.

after 45 min.

Supplementary Information (1b)

ES-MS of cyclic peptide o-2f

M^2+ (calc. 1398.7)

M^3+ (calc. 932.8)
Supplementary Information (2a)

Reaction of 24-mer peptide 1f with 1.05 equiv. of m-T2

- after 1 min.
- after 5 min.
- after 15 min.
- after 45 min.

* side product with the same MW as main product (most likely diastereomeric product)

Supplementary Information (2b)

ES-MS of cyclic peptide m-2f

M^+ (calc. 932.8)

M^2+ (calc. 1398.7)
Supplementary Information (3a)

Reaction of 24-mer peptide 1f with 1.05 equiv. of \( p\)-T2

* side product with the same MW as main product

Supplementary Information (3b)

ES-MS of cyclic peptide \( p\)-2f
Supplementary Information (4a)

Reaction of 31-mer peptide 5 with 1.05 equiv. of T3

* hydrolysis products formed upon reaction of excess T3 with TFA

Supplementary Information (4b)

ES-MS of bicyclic peptide 6
Supplementary Information (5a)

Reaction of 24-mer peptide 9 with 1.05 equiv. of T4

* hydrolysis products formed upon reaction of excess T4 with TFA/H₂O

Supplementary Information (5b)

ES-MS of tricyclic peptide 10

M⁺ (calc. 1226.8)

M⁺⁺ (calc. 920.3)