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Accessory Publication

Lipid Peptide Core Nanoparticles as Multivalent Vaccine Candidates against *Streptococcus pyogenes*

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Protected L-amino acids were obtained from Novabiochem (Läufelfingen, Switzerland). pMBHA resin was purchased from Peptides International Inc. (Kentucky, USA). Rink amide MBHA resin, dichloromethane (DCM), methanol, *N*,*N*-diisopropylethylamine (DIPEA), piperidine and trifluoroacetic acid (TFA) were purchased from Merck (Hohenbrunn, Germany). Cu wires and 1-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Aldrich (Steinheim, Germany). HPLC grade acetonitrile was obtained from Labscan (Bangkok, Thailand). All other reagents were obtained at the highest available purity from Sigma-Aldrich (Castle Hill, NSW, Australia). Microwave assisted Fmoc SPPS was carried out by using a SPS mode CEM Discovery reactor (CME Corporation, Matthews, NC, USA). A Kel-F HF apparatus (Peptide Institute, Osaka, Japan) was used for HF cleavage. ESI-MS was performed on a Perkin-Elmer-Sciex API3000 instrument with Analyst 1.4 (Applied Biosystems/MDS Sciex, Toronto, Canada) software. Analytical RP-HPLC was performed on Agilent instrument with a 1 mL/min flow rate and detection at 214 nm. Separation was achieved by running gradient mode of 0-100%

solvent B over 40 min with 0.1% TFA/H_2O as solvent A and 90% MeCN/0.1% TFA/H_2O as solvent B on either a Vydac analytical C4 column (214TP54; 5 μm, 4.6 mm x 250 mm) or a Vydac analytical C18 column (218TP54; 5 μm, 4.6 mm x 250 mm). Purification was carried out by preparative RP-HPLC using a Waters Delta 600 system with a 10 mL/min flow rate. Compounds were detected at 230 nm. Separations were performed with solvent A and solvent B on either a Vydac preparative C4 column (214TP1022; 10 μm, 22 mm x 250 mm) or a Vydac preparative C18 column (218TP1022; 10 μm, 22 mm x 250 mm). A Zetasizer Nano ZP instrument (Malvern Instruments, UK) with DTS software was used for particle size measurements. Sizes were analysed using a non-invasive backscatter system. Measurements were taken at 25 °C with scattering angle of 173° using disposable cuvettes. Transmission electron microscopy was performed on a JEM-1010 transmission electron microscope (JEOL Ltd., Japan) operated at 100 kV. The sample images were taken and analyzed using the AnalySIS® software (Soft Imaging Systems, Megaview III, Munster, Germany).

Peptide azide 1

Azide derivative of FNBR-B epitope (VETEDTKEPG VLMGGQSESV EFTKDTQTGM) was prepared using HBTU/DIPEA Fmoc-SPPS. Peptide **1** was synthesized on rink amide *p*MBHA resin (0.56 mmol of NH2/g, 0.2 mmol scale) using Fmoc-chemistry by Microwave-Assisted SPPS (70 ºC, 20 Watt). Amino acid (0.64 mmol, 3.2 equiv) activated with 0.5M HBTU/DMF solution (1.2 mL, 0.60 mmol, 3.0 equiv) DIPEA (110 µL, 0.68 mmol, 3.4 equiv) was coupled to the resin (2 x 15 min) after deprotection of Fmoc group (twice, 2 and 5 min). The azido acetic acid was coupled using HBTU/DIPEA at room temperature (2 x 2 hours). The cleavage of **1** from the resin was carried out in the solution of TFA/triisopropylsilane/water in ratio 95/2.5/2.5 for 4 hours. The product was purified by preparative RP-HPLC on C18 column with solvent gradient 0%-50% solvent B over 50 min. HPLC analysis (C18 column): *tR* = 16.3 min, purity > 95%. ESI-MS: $[M+2H]^{2+}$ m/z 1658.3 (calc 1657.8); $[M+3H]^{3+}$ m/z 1105.9 (calc 1105.5); MW 3313.54. Yield: 12 %.

Peptide azide 2

Azide derivative of FNBR-BT epitope (EFTKDTQTGMS GQTTPQVETE DTKEPGVLM) was prepared in similar manner as above. HPLC analysis (C18 column): t_R = 18.3 min, purity > 95%. ESI-MS: $[M+2H]^{2+}$ m/z 1686.2 (calc 1685.3); $[M+3H]^{3+}$ m/z 1123.5 (calc 1123.9); MW3368.62. Yield: 10 %.

Peptide alkyne 3

Peptide **3** was synthesized using Boc-chemistry on p-MBHA resin (0.22 g, 0.1 mmol, 0.45 mequiv/g substitution) by SPPS method. Amino acids (0.42 mmol, 4.2 equiv) activated with HBTU (0.8 mL, 0.4 mmol, 4.0 equiv) and DIPEA (0.11 mL, 0.62 mmol, 6.2 equiv) were coupled to the peptide resin (2 x 60 min) after Boc deprotection with TFA (2 x 1 min). The attachment of alkyne moiety was achieved by double coupling with 4.0 equiv of propiolic acid mixed with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 4.0 equiv, 198 mg) in DMF (2 x 60 min) after Fmoc-deprotection of the lysine side chain. The cleavage of **3** from the resin was carried out with HF. The resulted compound was purified by preparative HPLC using C4 column with gradient 50-100% solvent B over 50 min. HPLC analysis (C4 column): t_R = 30.4 min, purity > 95%. ESI-MS: $[M+3H]^{3+}$ m/z 1466.9 (calc 1466.4); $[M+4H]^{4+}$ m/z 1100.0 (calc 1100.0); $[M+5H]^{5+}$ m/z 880.5 (calc 880.2); $[M+6H]^{6+}$ m/z 734.1 (calc 733.7); $[M+7H]^{7+}$ m/z 629.3 (calc 629.0); $[M+8H]^{8+}$ m/z 550.9 (calc 550.5); MW 4396.22. Yield: 19 %.

Peptide alkyne 4

Peptide **4** was prepared in similar manner as above. HPLC analysis (C4 column): t_R = 35.0 min, purity > 95%. ESI-MS: $[M+1H]^{1+}$ m/z 1060.0 (calc 1059.7); MW 1058.75. Yield: 20 %.

LCP construct 5

Compound **5** was synthesized according to the procedure described in the main text. HPLC analysis (C4 column): t_R = 27.2 min, purity > 95%. ESI-MS: $[M+4H]^{4+}$ m/z 1928.6 (calc 1928.4); $[M+5H]^{5+}$ m/z 1543.5 (calc 1543.0); $[M+6H]^{6+}$ m/z 1286.1 (calc 1286.0); $[M+7H]^{7+}$ m/z 1102.2 (calc 1102.4); $[M+8H]^{8+}$ m/z 964.6 (calc 964.7); $[M+9H]^{9+}$ m/z 858.2 (calc 857.6); $[M+10H]^{10+}$ m/z 772.1 (calc 772.0); MW 7709.76. Yield: 51%.

LCP construct 6

Compound **6** was synthesized according to the procedure described in the main text. HPLC analysis (C4 column): t_R = 27.2 min, purity > 95%. ESI-MS: $[M+5H]^{5+}$ m/z 1554.1 (calc 1554.0); $[M+6H]^{6+}$ m/z 1295.5 (calc 1295.1); $[M+7H]^{7+}$ m/z 1110.8 (calc 1110.3); $[M+8H]^{8+}$ m/z 971.5 (calc 971.6); MW 7764.84. Yield: 38 %.

LCP construct 7

Compound **7** was synthesized according to the procedure described in the main text. HPLC analysis (C4 column): t_R = 30.5 min, purity > 95%. ESI-MS: $[M+2H]^{2+}$ m/z 2187.5 (calc 2187.1); $[M+3H]^{3+}$ m/z 1458.7 (calc 1458.4); MW 4372.29. Yield: 43 %.

LCP construct 8

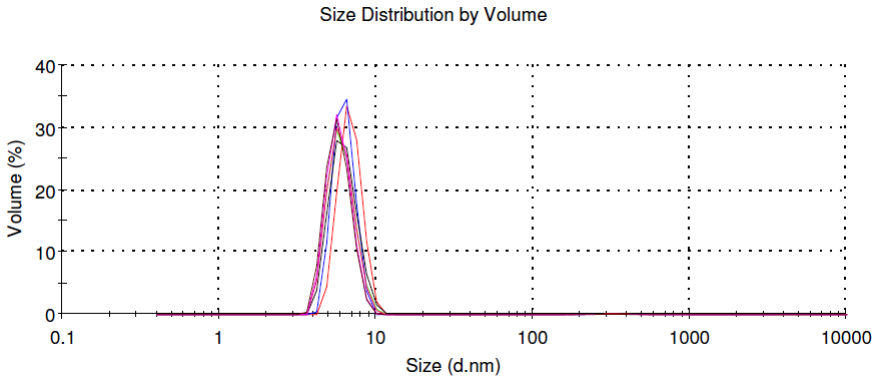
Compound **8** was synthesized according to the procedure described in the main text. HPLC analysis (C4 column): t_R = 30.3 min, purity > 95%. ESI-MS: $[M+2H]^{2+}$ m/z 2215.2 (calc 2215.0); $[M+3H]^{3+}$ m/z 1477.0 (calc 1477.0); $[M+4H]^{4+}$ m/z 1108.4 (calc 1108.0); $[M+5H]^{5+}$ m/z 886.6 (calc 887.4); MW : 4428.04. Yield: 32 %.

LCP construct 9

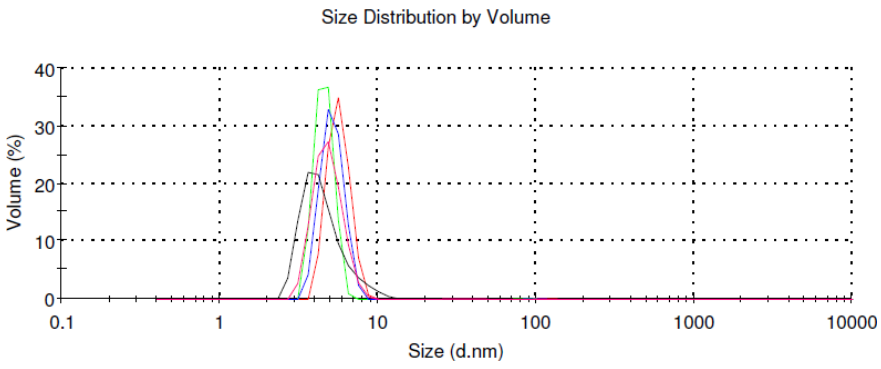
As mentioned in the main text compound **9** was synthesized by stepwise Boc-SPPS. HPLC analysis (C4 column): t_R = 28.5 min, purity > 95%. ESI-MS: $[M+3H]^{3+}$ m/z 1449.2 (calc 1449.1); $[M+4H]^{4+}$ m/z 1187.4 (calc 1087.0); $[M+5H]^{5+}$ m/z 869.9 (calc 869.8); $[M+6H]^{6+}$ m/z 725.3 (calc 725.0); $[M+7H]^{7+}$ m/z 622.1 (calc 621.6); MW 4344.19.

Figure 1s. Dynamic light scaterring analysis of nanoparticles (multiplied measurements).

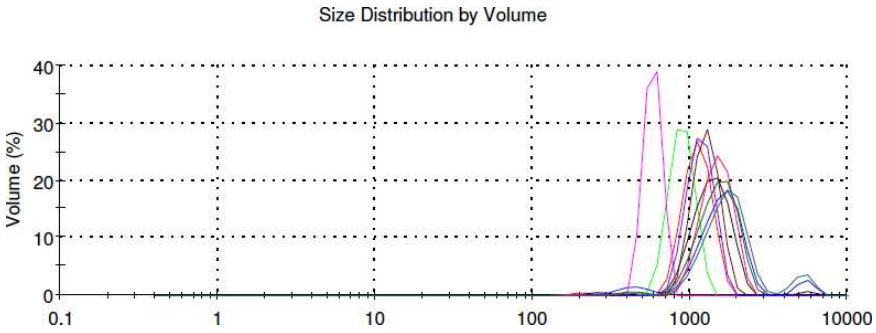
Compound **5**



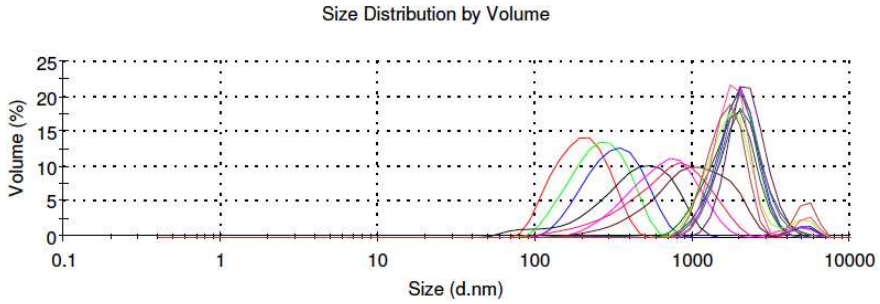
Compound **6**



Compound **7**



Compound **8**



Compound **9**