

## Supporting Information

### **Physical Mixture of a Cyclic Lipopeptide Vaccine Induced High Titres of Opsonic IgG Antibodies against Group A *Streptococcus***

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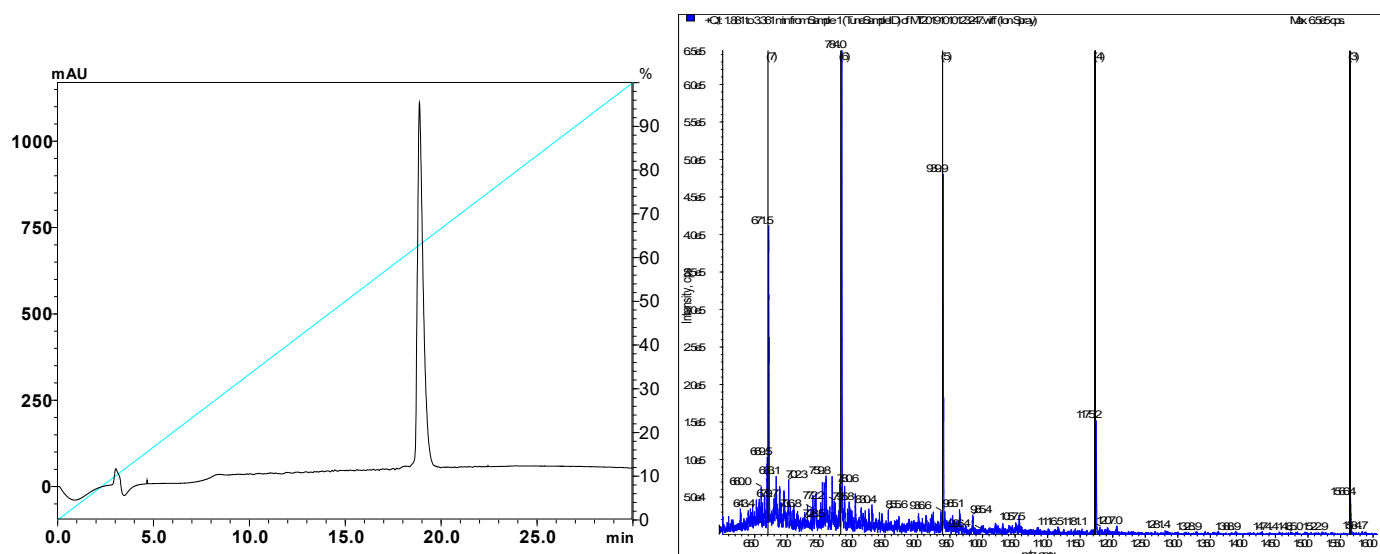
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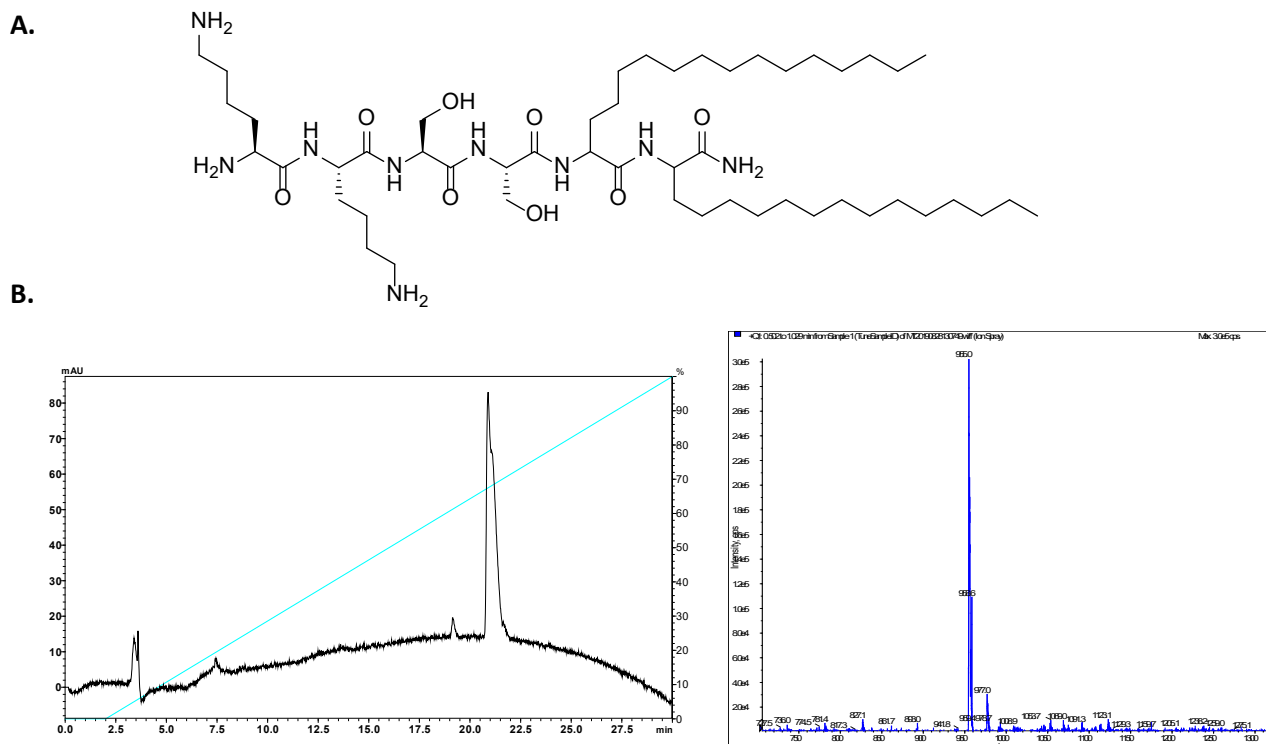
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## Azido-functionalised J8-PADRE



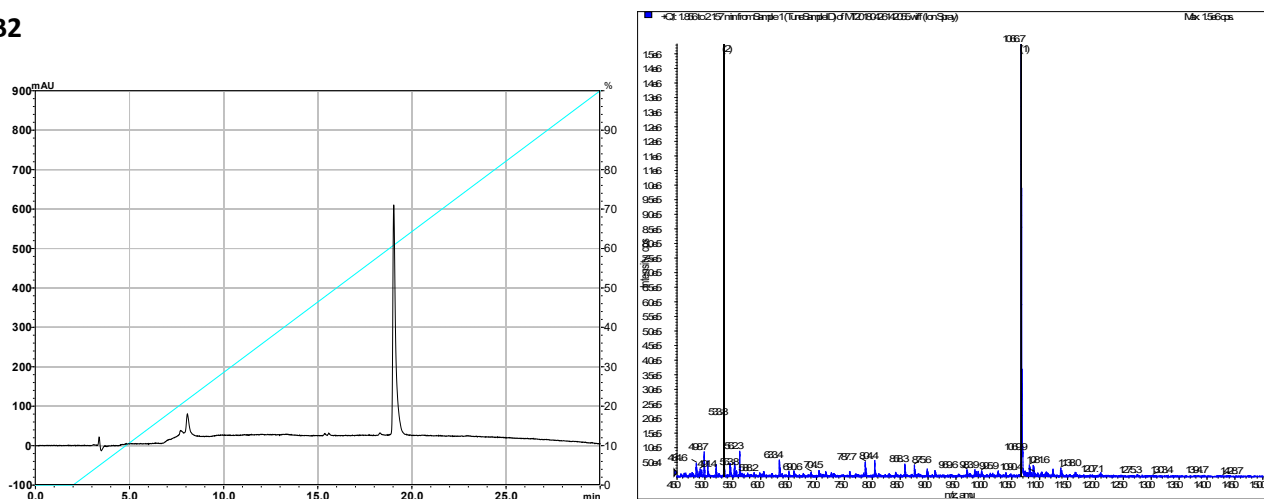
**Figure S1.** RP-HPLC trace and mass spectra for **Azido functionalised J8-PADRE** (165.7 mg, 33%).  $R_t = 18.8$  min ( $C_{18}$  column, 0-100% solvent B, 30 min). Molecular weight ( $C_{207}H_{347}N_{63}O_{61}$ ): 4694.43 g/mol. ESI-MS:  $[M + 3H]^{+3}$  m/z 1566.4 (calcd: 1565.8),  $[M + 4H]^{+4}$  m/z 1175.2 (calcd: 1174.6),  $[M + 5H]^{+5}$  m/z 939.9 (calcd: 939.9),  $[M + 6H]^{+6}$  m/z 784.0 (calcd: 784.0),  $[M + 7H]^{+7}$  m/z 671.5 (calcd: 671.5).

## BB1 (KKSS-C16-C16-NH<sub>2</sub>)

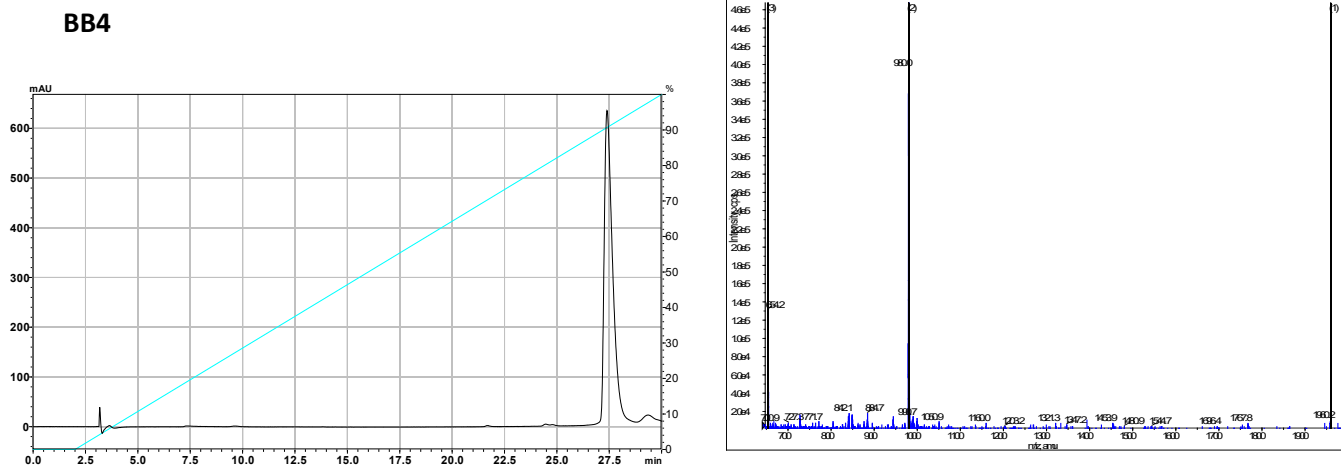


**Figure S2.** A) KKSS-C16-C16-NH<sub>2</sub> Structure. B) RP-HPLC trace and mass spectra for **KKSS-C16-C16-NH<sub>2</sub>** (10.6 mg, 48%).  $R_t = 20.9$  min (C<sub>4</sub> column, 0-100% solvent B, 30 min). Molecular weight (C<sub>50</sub>H<sub>99</sub>N<sub>9</sub>O<sub>8</sub>): 954.4 g/mol. ESI-MS:  $[M + 1H]^{+1}$  m/z 955.0 (calcd: 955.4).

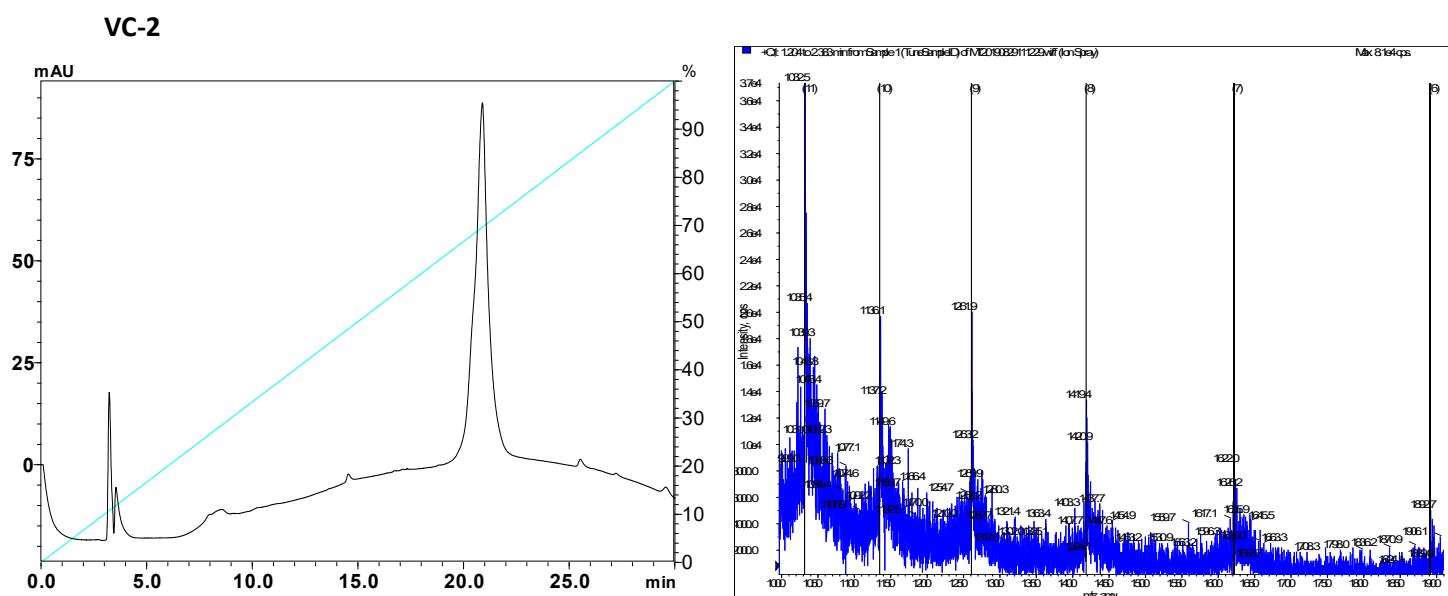
## BB2



**Figure S3.** RP-HPLC trace and mass spectra for **BB2** (5.4 mg, 32%).  $R_t = 19.0$  min (C<sub>18</sub> column, 0-100% solvent B, 30 min). Molecular weight (C<sub>51</sub>H<sub>79</sub>N<sub>13</sub>O<sub>12</sub>): 1066.27 g/mol. ESI-MS:  $[M + 1H]^{+1}$  m/z 1066.7 (calcd: 1067.27),  $[M + 2H]^{+2}$  m/z 533.8 (calcd: 534.1).

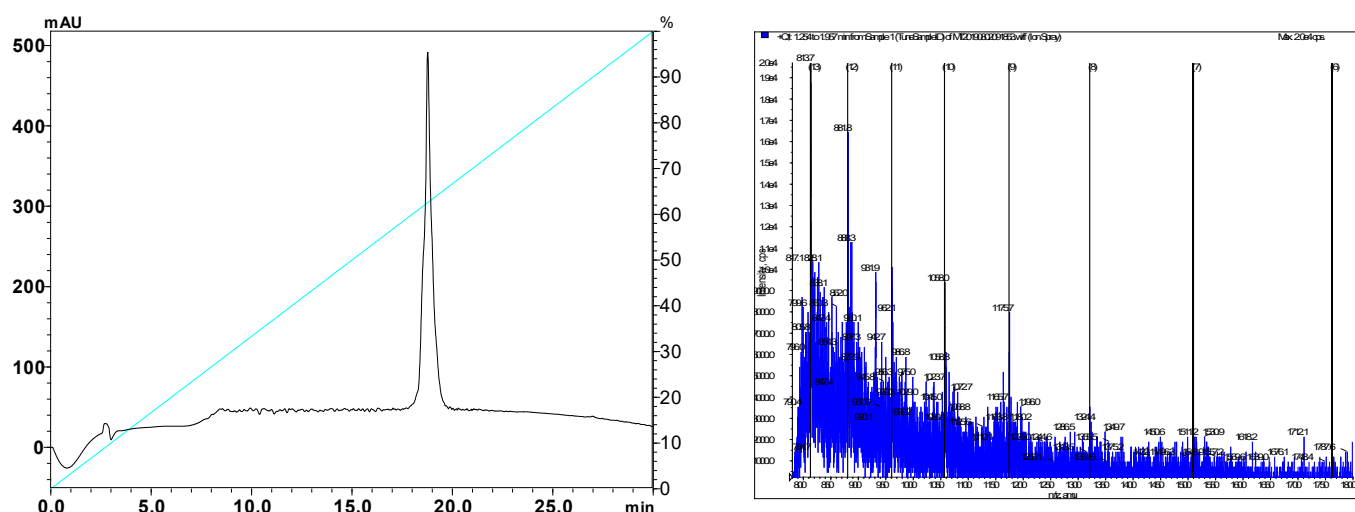


**Figure S4.** RP-HPLC trace and mass spectra for **BB4** (4.8 mg, 16%).  $R_t = 27.3$  min ( $C_4$  column, 0-100% solvent B, 30 min). Molecular weight ( $C_{106}H_{172}N_{16}O_{18}$ ): 1958.64 g/mol. ESI-MS:  $[M + 1H]^{+1}$  m/z 1960.2 (calcd: 1959.64),  $[M + 2H]^{+2}$  m/z 980.0 (calcd: 980.32),  $[M + 3H]^{+3}$  m/z 654.2 (calcd: 653.9).



**Figure S5.** RP-HPLC trace and mass spectra for **VC-2** (2.3 mg, 14%).  $R_t = 21.0$  min ( $C_4$  column, 0-100% solvent B, 30 min). Molecular weight ( $C_{520}H_{866}N_{142}O_{140}$ ): 11347.50 g/mol. ESI-MS:  $[M + 6H]^{+6}$  m/z 1892.7 (calcd: 1892.3),  $[M + 7H]^{+7}$  m/z 1622.0 (calcd: 1622.1),  $[M + 8H]^{+8}$  m/z 1419.4 (calcd: 1419.4),  $[M + 9H]^{+9}$  m/z 1261.9 (calcd: 1261.8),  $[M + 10H]^{+10}$  m/z 1136.1 (calcd: 1135.8),  $[M + 11H]^{+11}$  m/z 1032.5 (calcd: 1032.6).

## VC-0



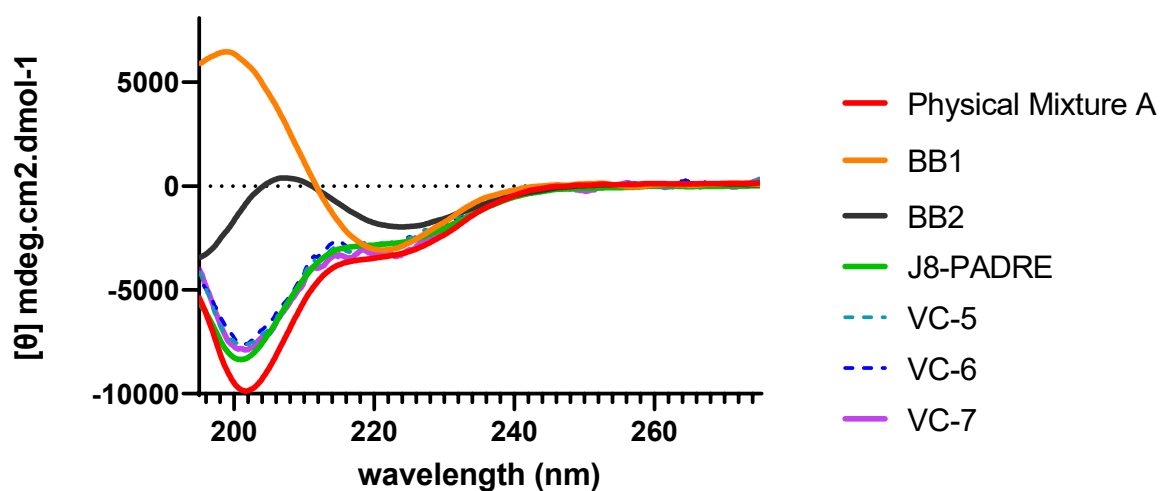
**Figure S6.** RP-HPLC trace and mass spectra for **VC-0** (3.5 mg, 19%).  $R_t = 19.6$  min ( $C_{18}$  column, 0-100% solvent B, 30 min). Molecular weight ( $C_{471}H_{787}N_{141}O_{134}$ ): 10569.33 g/mol. ESI-MS:  $[M + 8H]^{+8}$  m/z 1324.4 (calcd: 1322.2),  $[M + 9H]^{+9}$  m/z 1175.7 (calcd: 1175.4),  $[M + 10H]^{+10}$  m/z 1058.0 (calcd: 1057.9),  $[M + 11H]^{+11}$  m/z 962.1 (calcd: 961.8),  $[M + 12H]^{+12}$  m/z 881.8 (calcd: 881.8),  $[M + 13H]^{+13}$  m/z 813.7 (calcd: 814.0).

**Table S1.** Vaccine building block dose for conjugated and physically mixed vaccine candidates per mouse.

| Vaccine Groups             | VC-2  | VC-0    | J8-PADRE | BB1    | BB2    | BB4    |
|----------------------------|-------|---------|----------|--------|--------|--------|
| Physical Mixture A         | -     | -       | 24.8 ug  | 2.5 ug | 2.7 ug | -      |
| VC-0                       | -     | 27.3 ug | -        | -      | -      | -      |
| VC-2                       | 30 ug | -       | -        | -      | -      | -      |
| VC-4                       | -     | 27.3 ug | -        | 2.5 ug | -      | -      |
| VC-5                       | -     | -       | 24.8 ug  | -      | -      | 5.2 ug |
| VC-6                       | -     | -       | 24.8 ug  | 2.5 ug | -      | -      |
| VC-7                       | -     | -       | 24.8 ug  | -      | 2.7 ug | -      |
| Cyclic Peptide Alone (BB2) | -     | -       | -        | -      | 2.7 ug | -      |

**Table S2.** DLS measurement for all vaccine compounds and Physical Mixtures.

| Vaccine Groups             | Particle size (nm)                           | PDI           |
|----------------------------|--|---------------|
| Physical Mixture A         | 857.1 ± 56.9 (100%)                          | 0.29 ± 0.034  |
| VC-5                       | 249.3 ± 8.1 (100%)                           | 0.456 ± 0.03  |
| VC-6                       | 228.6 ± 17.3 (100%)                          | 0.636 ± 0.167 |
| VC-7                       | 200.3 ± 8.4 (100%)                           | 0.471 ± 0.69  |
| BB1                        | 1713 ± 210.7 (100%)                          | 0.393 ± 0.067 |
| Cyclic Peptide Alone (BB2) | 242.6 ± 14.7 (83.4%)<br>5138 ± 198.9 (16.6%) | 0.586 ± 0.092 |
| BB4                        | 184.4 ± 16.1 (85.7%)<br>5324 ± 196.2 (14.3%) | 0.544 ± 0.077 |
| J8-PADRE                   | 310.5 ± 29.9 (68.7%)<br>92.2 ± 9.2 (31.3%)   | 0.56 ± 0.045  |



**Figure S7.** Circular dichroism of individual vaccine building blocks (J8-PADRE, BB1 [KKSS-C16-C16-NH<sub>2</sub>], Cyclic Peptide Alone [BB2]) and Physical Mixture A, VC-5, VC-6, and VC-7.