**Supplementary Information**

**Conformationally restricted dipeptide-based self-assembled nanoparticles for efficient vancomycin delivery**

Nitin Yadav†,a,b, Utkarsh Kumar†,a, Virander Singh Chauhan\*,a

a Molecular Medicine Group, International Centre for Genetic Engineering & Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India.

b Delhi Institute of Pharmaceutical Sciences and Research, Mehrauli-Badarpur Road, Sector-3, Pushpvihar, New Delhi-110017, India

† Authors contributed equally

\* Correspondence: virander@icgeb.res.in

**Supplementary Text**

**S-1 Synthesis and characterization of the dipeptide**

The dipeptide synthesis was carried out at a 20 mM scale, where 10.54 g of Boc-Arg(Pbf)-OH was dissolved in minimum amount of distilled THF in a dry round bottom flask and kept at stirring in an ice-salt bath. For the activation of the carboxyl group of Boc-Arg(Pbf)-OH , 2 ml of of NMM followed by 2.73 ml of IBCF was added. An aqueous solution (at 4˚C) of DL-threo-β-phenylserine (3.986g) and NaOH (0.88g) was added to the round bottom flask and the entire mixture was stirred at room temperature for overnight. Next day, THF was evaporated followed by acidification using saturated citric acid solution. The precipitated product was dissolved in ethyl acetate (15-20 ml). The dissolved product was washed 3-4 times with water and was passed through sodium sulphate before evaporating ethyl acetate. The dried product was dissolved in anhydrous acetic anhydride and then 1.8 g of anhydrous sodium acetate was added. The solution was kept on stirring at room temperature for 24 hrs. The resulting yellow-colored product (azlactone) was collected using filtration, washed 3-4 times with cold water and dried under vaccume. The dried product was hydrolyzed using 1.1 molar excess of NaOH, which gave Boc-Arg(Pbf)∆F. The N-terminal and side-chain deprotection was achieved using a cocktail of TFA and water (19:1 v/v) for 4 hours. TFA was evaporated and anhydrous diethyl ether was added to precipitate the final product, which was filtered and washed multiple times (thrice) with cold diethyl ether. The resultant dipeptide was dissolved in 10% acetic acid and was lyophilized twice. The dipeptide was characterized using RP-HPLC (observed retention time – 19.5 min) and mass spectrometry [theoretical mass – 319.3 Da and observed mass- 320.2 Da (+1H)].

**Supplementary Tables**

**Table S-1:** RP-HPLC data for the calibration curve of Vancomycin at different concentration.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Amount (µg)** | **Sample name** | **Retention time (min)** | **Area** | **HT** | **Average Area** | **SD** |
| 40 | n=1 | 21.527 | 8674240 | 1148417 | 9,684217 | 80788.42 |
| 40 | n=2 | 22.171 | 11730578 | 1157877 |
| 40 | n=3 | 22.207 | 8647832 | 981964.4 |
| 30 | n=1 | 21.552 | 6653902 | 943198.6 | 7199368 | 24420.07 |
| 30 | n=2 | 22.166 | 7431158 | 886522.3 |
| 30 | n=3 | 22.16 | 7513045 | 898296.1 |
| 20 | n=1 | 21.61 | 4511462 | 689819.5 | 4804436 | 27795.15 |
| 20 | n=2 | 22.174 | 4653640 | 631256.3 |
| 20 | n=3 | 22.187 | 5248205 | 690610 |
| 10 | n=1 | 21.654 | 2262253 | 373649.7 | 2362983 | 5603.364 |
| 10 | n=2 | 22.193 | 2452802 | 373539.8 |
| 10 | n=3 | 22.212 | 2373895 | 361708.6 |
| 8 | n=1 | 21.658 | 1705924 | 289824.4 | 1715090 | 8273.075 |
| 8 | n=2 | 22.192 | 1732701 | 275002.4 |
| 8 | n=3 | 22.192 | 1706645 | 270445.6 |
| 6 | n=1 | 21.667 | 1367523 | 235920.7 | 1303849 | 14345.64 |
| 6 | n=2 | 22.242 | 1266877 | 204687.2 |
| 6 | n=3 | 22.21 | 1277148 | 206359.8 |
| 4 | n=1 | 21.673 | 862103.9 | 151153.2 | 841566.4 | 6695.267 |
| 4 | n=2 | 22.276 | 828437.3 | 136943.2 |
| 4 | n=3 | 22.283 | 834158 | 136957.6 |
| 2 | n=1 | 21.675 | 435876.8 | 76745.7 | 430976.8 | 2510.408 |
| 2 | n=2 | 22.291 | 432457.4 | 72129.1 |
| 2 | n=3 | 22.307 | 424596.1 | 70919.6 |
| 1 | n=1 | 21.661 | 217438.6 | 39204.7 | 226032.6 | 668.2148 |
| 1 | n=2 | 22.259 | 233807 | 39143.3 |
| 1 | n=3 | 22.285 | 226852.3 | 37757.5 |
| 0.5 | n=1 | 21.657 | 87147.3 | 15622.3 | 92649.5 | 1089.713 |
| 0.5 | n=2 | 22.219 | 87615.2 | 14686 |
| 0.5 | n=3 | 22.243 | 103186 | 17318.9 |
| 0.1 | n=1 | 21.594 | 20091.5 | 3736.4 | 21818.57 | 147.5781 |
| 0.1 | n=2 | 22.246 | 23656.5 | 4011 |
| 0.1 | n=3 | 22.234 | 21707.7 | 3670.1 |