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Design and Solid-Phase Synthesis of Multiple Muramyl Dipeptide (MMD)

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Abstract: As a non-specific modulator of macrophage, multiplied muramyl dipeptide (MMD) is solid-phase synthesized by application of standard Fmoc chemistry strategy. Tam's multiple antigen system (MAS) is used as our four branched-linker on Lysine.

Keywords: Multiplied muramyl dipeptide, multiple antigen system, macrophage, solid-phase synthesis

As a non-specific modulator of macrophage, muramyl dipeptide elicits potentially multiple biological activities[1], such as adjuvancy, antitumor, antiinfection, *etc*. Particularly, its adjuvancy has been studied by application for HIV vaccine. However, muramyl dipeptide can not serve as clinical agent due to its short lifetime *in vivo*, low immunogenicity, toxicity, and side effect such as pyrogenicity. Therefore, multiplied muramyl dipeptide (MMD) might increase its immunogenicity, decrease its hydrophilic property so that muramyl dipeptide could penetrate the membrane of macrophage. Tam[2] has developed a multiple antigen system (MAS) by application of Lysine as a linker. Here, we report a method of solid-phase synthesis of multiplied muramyl dipeptide on MAS branched-linker of Lysine.

Experimental

Crowns from Chiron Mimotopes of Australia are selected as solid support of synthesis. By a substitution of 5-8 μmol/pin, Fmoc-Lys(Fmoc)-OH is attached onto crown by using of 450 μL of 80 μM BOP as coupling reagent and HOBt, NMM as additives in DMF for 2 hours at room temperature. After thoroughly washing by a suspension in DMF, methanol, and DMF for three times individually (each for 5 minutes), the Fmoc protected groups of both α- and ω- side chain of Lysine are removed by treatment of 20 % piperidine/DMF for 20 minutes at r.t. Repeating the washing steps as above describes, second Fmoc-Lys(Fmoc)-OH attachment and Fmoc removal are sequentially finished under same conditions. The following peptide assembly is performed as synthetic

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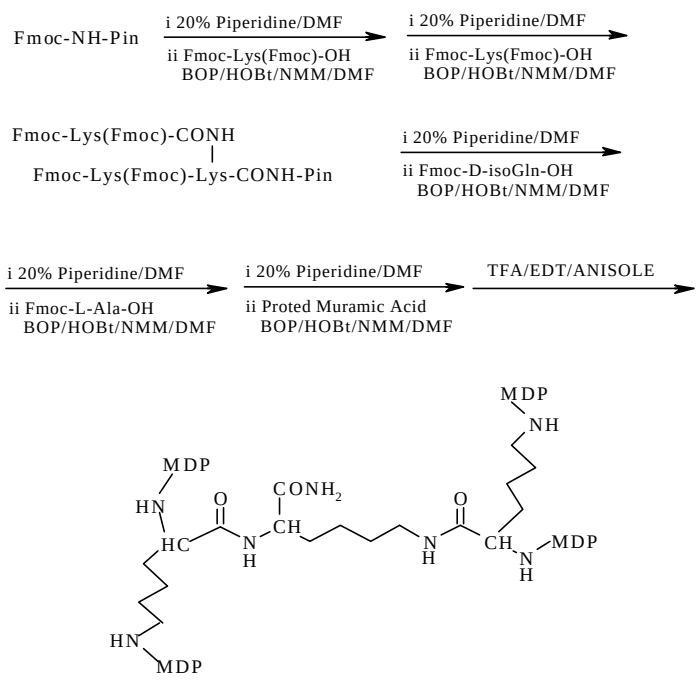
scheme 1. The final product is released off solid support by treatment of TFA cleavage cocktail in water. 4, 6-Benzylidene protected group of muramic acid is removed off simultaneously, however, 1-benzyl group of muramic acid is remaining. After lyophilization, crude product of the multiplied muramyl dipeptide is analyzed by HPLC and FAB mass system. All results show correct molecular weight and purity over 90% (data does not show here. Please requiring corresponding author if it is necessary).

Scheme 1 Four molecules of muramyl dipeptide are chemically covalent attached onto multiple antigen system (MAS).



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