SYNTHESIS OF IMMUNOADJUVANT CONJUGATES WITH HIV-DERIVED PEPTIDE INDUCING PEPTIDE-SPECIFIC ANTIBODY

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MDP and lipopeptide analog conjugates inducing HIV-derived peptide-specific antibody without adding further macromolecular carriers or adjuvants were synthesized.

KEYWORDS MDP; lipopeptide analog; HIV-derived peptide; synthetic vaccine

It has been known that a peptidoglycan of the bacterial cell walls exhibits several biological activities. Ellouz *et al.*[1] and Kotani *et al.*[2] had established that a minimum structure required for the immunoadjuvant activity of bacterial cell walls is N-acetylmuramyl-L-alanyl-D-isoglutamine(MDP). MDP can be substituted for mycobacterial cells in Freund's complete adjuvant(FCA) to enhance both B cell- and T cell-mediated immune responses.[3] It has also been known that a lipopeptide from the outer membrane of *Escherichia coli* is an active mitogen and polyclonal activator for B lymphocytes.[4-7] Kurimura *et al.* reported a new synthesis of chiral lipopeptide analog with higher activity than the native lipopeptide.[8]

We developed a completely synthetic virus peptide vaccine which consists of these synthetic immunoadjuvants, covalently coupled to low-molecular-weight antigen.[9] We selected a trimer of HIV-1 gp120-derived peptide(GPGRAF),[10] and described the synthesis of conjugates with synthetic immunoadjuvants at the N-terminus of this oligopeptide(Fig.1). We expected that the conjugates have introduced peptide-specific antibody in mice without adding further macromolecular carriers or adjuvants.

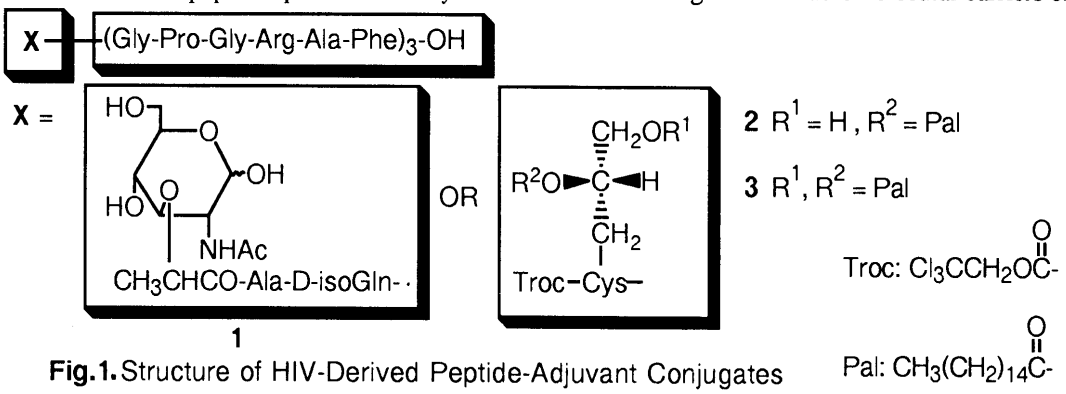

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Fig. 1. Structure of HIV-Derived Peptide-Adjuvant Conjugates

The protected MDP **9** was prepared according to the procedure in the literature.[3,11] Lipopeptide analog **11** was prepared according to the procedure of Kurimura *et al.*.[7] The trimer of HIV-derived peptide was synthesized according to the reaction sequence shown in Chart 1. For the synthesis of 18-residue polypeptide, a synthetic strategy based on fragment condensation method was adapted.

The assembly of the synthetic adjuvant-HIV-derived polypeptide conjugates is shown in Chart 2. The protected MDP **9** and lipopeptide analog **11** were introduced to the N-terminus free polypeptide **8** by the water-soluble carbodiimide(WSC)-1-hydroxy-1H-benzotriazole(HOBt) coupling method. All protecting groups in the conjugates **10**,**12** were removed with anhydrous HF in the usual way and the crude products were purified by HPLC to give **1**[12] and depalmitoyl product **2**.[13] Peptide **13**[14] was prepared from **7** by a similar procedure. Since the treatment with HF-anisole of **12** had caused removal of an acyl group, lipopeptide analog conjugate was constructed as shown in Chart 3. Lipopeptide analog **11** was introduced

directly to the deprotected peptide 13 by the *p*-nitrophenyl ester coupling method, and the product was purified by HPLC to give conjugate 3.[15)]

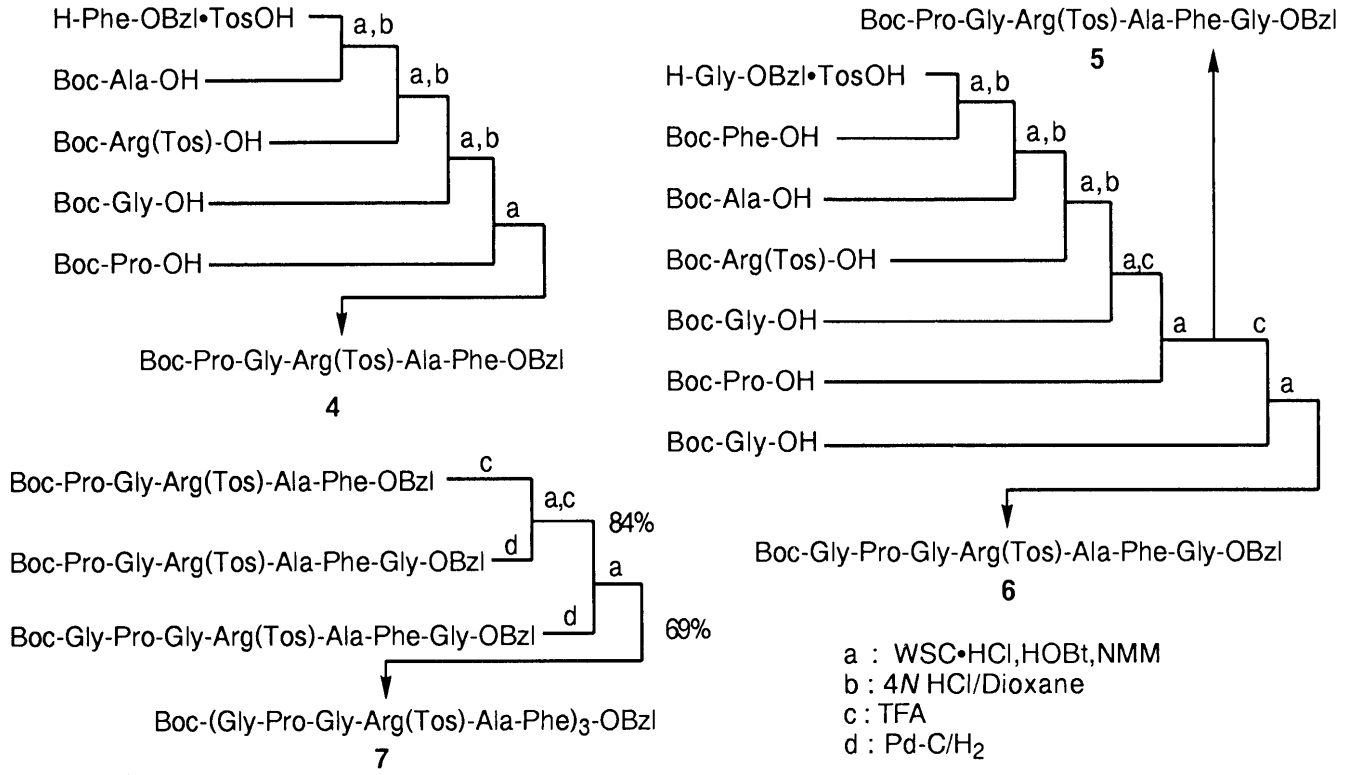

Chart 1

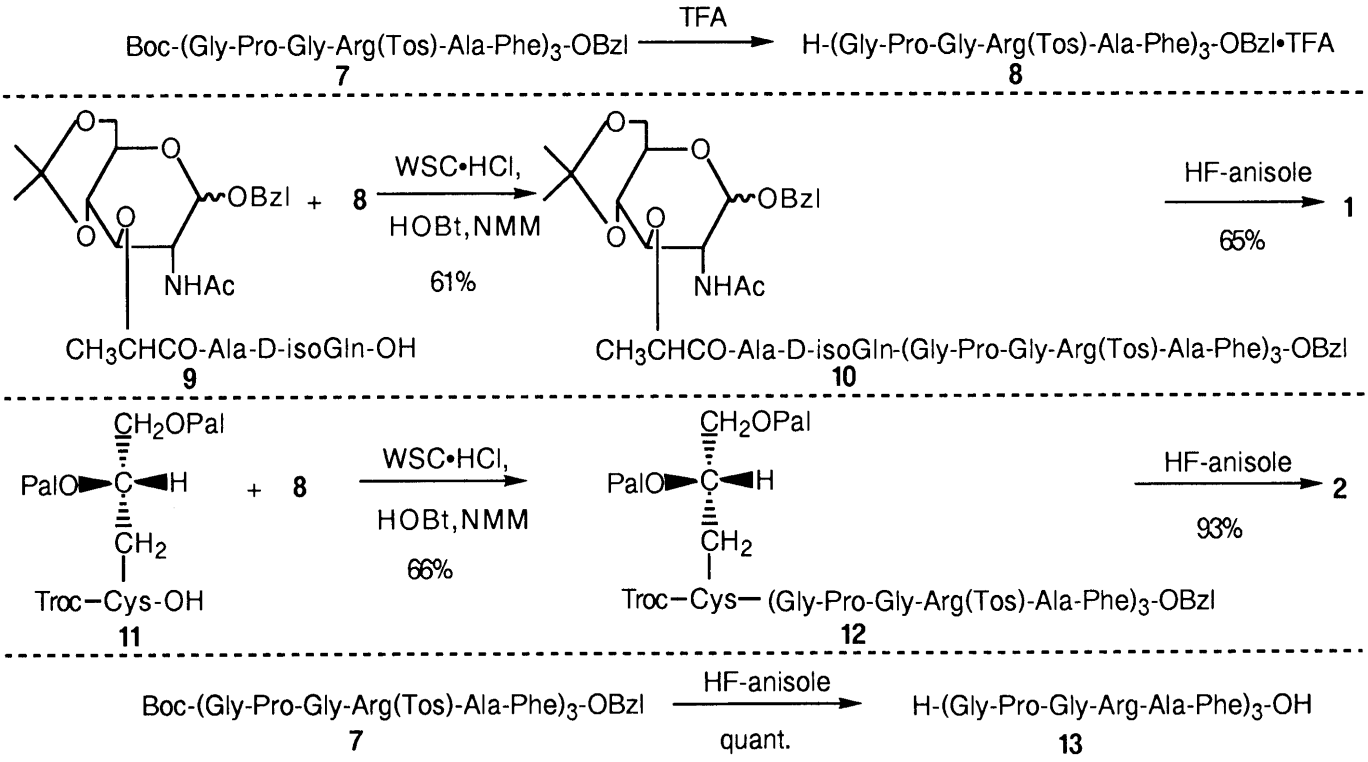

Chart 2

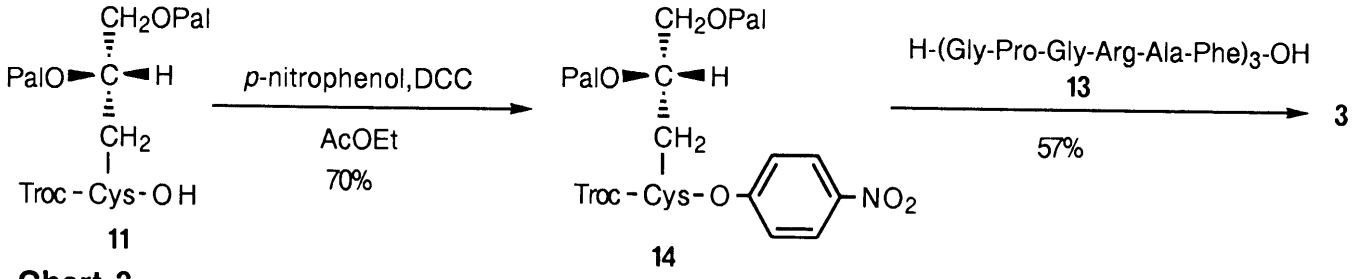

Chart 3

The conjugates 1,2 and 3 were examined for their ability to induce HIV-derived peptide-specific antibody in male BALB/c mice. The groups of animals were sensitized subcutaneously three times with each compound in a dose of 50 nmol. Blood was taken from the retroorbitalis several times after the sensitizations. The titers for the specific anti-peptide antibody of each serum were determined in a 96-microplate coated conjugate as the antigen using ELISA. The sera sensitized by compound 3 showed about twice as much anti-peptide antibody response as compared with those sensitized by the peptide 13. Further studies on the biological activities of the compounds are in progress.

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12) $[\alpha]_D^{21}$-35.2°(*c* 0.20, 3%AcOH), FABMS(*m/z*) 2250$(M+H)^+$.

13) $[\alpha]_D^{25}$-42.8°(*c* 0.22, 3%AcOH), FABMS(*m/z*) 2367$(M+H)^+$.

14) $[\alpha]_D^{21}$-57.3°(*c* 0.22, 3%AcOH), FABMS(*m/z*) 1775$(M+H)^+$, Amino acid analysis(6*N* HCl, 110°C, 24h): Gly 5.98(6), Ala 3.50(3), Phe 3.15(3), Arg 2.73(3), Pro 2.64(3).

15) $[\alpha]_D^{25}$-50.4°(*c* 0.25, 3%AcOH), FABMS(*m/z*) 2604$(M+H)^+$.

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