# SYNTHESIS OF DERIVATIVES OF N-ACETYLMURAMYL-ℓ-ALANYL-d-ISOGLUTAMINE CONTAINING ACTIVATED DISULFIDE BONDS

A. F. Sheval'e, P. I. Pozdnyakov, and V. V. Samukov

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N-Acetylmuramyl-ℓ-alanyl-d-isoglutaminyl-ℓ-S-(2-pyridylthio)cysteine and 1-(N-acetylmuramyl-ℓ-alanyl-d-isoglutaminamido)-6-[3-(2-pyridylthio)-propionamido]hexane, derivatives of muramyldipeptide each with an activated disulfide bond, have been synthesized. Disulfide conjugates of muramyldipeptide with peptide fragment 136-147 of the heavy chain of the hemagglutinin of influenza virus A/Aichi/2/68 have been obtained.

The concept of the creation of bifunctional vaccines presupposes the linkage in one molecule of an antigenic determinant and a modulator of the immune response [1, 2]. As such an immunomodulator it is proposed to use N-acetylmuramyl-ℓ-alanyl-d-isoglutamine (MDP), a synthetic peptidoglycan capable of replacing the mycobacterium in complete Freund's adjuvant [3]. Several conjugates of MDP with peptides having the amino acid sequences of antigenic determinants of various proteins have been described [4-6]. Such conjugation may cause the formation of specific antibodies in high titers on administration to animals in physiological solution or in an oil-water emulsion. To obtain MDP-peptide conjugates the condensation of the components by means of water-soluble carbodiimides is usually employed. As a rule, this leads to the formation of a complex mixture of products and to excessive modification of the peptide, distorting its antigenic properties.

In the present paper we report the synthesis of derivatives of MDP (I and II) bearing reactive dithiopyridyl groups capable through a thiol-disulfide exchange reaction of forming disulfide bonds with thiols such as the SH groups of cysteine residues in peptides or proteins. The structures of the derivatives are shown in Schemes 1 and 2. In both derivatives, the dithiopyridyl group was attached through a spacer to the γ-carboxy group of the d-glutamic acid residue. In a number of studies it has been shown that the γ-carboxy group of MDP can be esterified or amidated or added to another amino acid with no change in the biological activity of the MDP [7]. Consequently, it may be expected that the reactive MDP derivatives synthesized should retain their adjuvant activity.

The MDP was synthesized by a combination of known methods [8, 9]. The optical rotation of the material synthesized corresponded to that given in the literature, and its structure was confirmed by its mass spectrum and elementary analysis. The methods of synthesizing the MDP derivatives (I and II) are shown in Schemes 1 and 2. S-(2-Pyridylthio)cysteine (III) was synthesized by the reaction of cysteine with 2-pyridylsulfenyl chloride, obtained by the chlorination of di-2-pyridyl disulfide immediately before the synthesis. 6-[3-(2-Pyridyldithio)propionamido]hexylamine (V) was obtained by the reaction of the N-hydroxysuccinimide ester of 3-(2-pyridyldithio)propionic acid (IV) with an excess of hexamethylenediamine.

The MDP was activated with dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzothiazole (HBT) and, without isolation, was condensed with compounds (II) and (V). The desired products (I) and (II) were purified by column chromatography on Bio-Gel P-4 or on Toyopearl TSK 40 HW. The compounds obtained were homogeneous according to TLC, and their structures were confirmed by their mass spectra and elementary analyses, and also by qualitative reactions – the liberation of pyridine-2-thione on treatment with dithiothreitol.

We used the reactive MDP derivatives (I and II) for the syntheses of conjugates with the synthetic fragment 136-147 of the heavy chain of the hemagglutinin (HA) of the influenza

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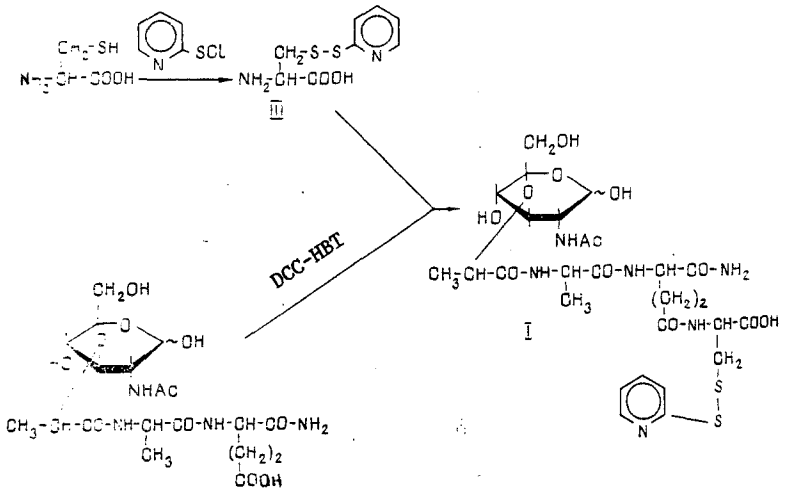
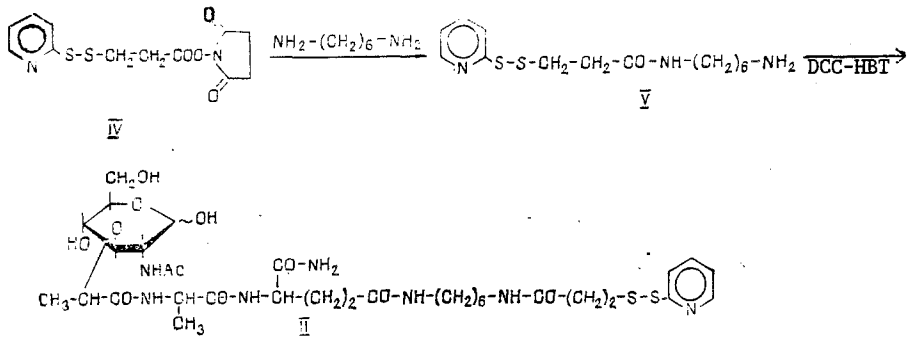
Scheme 1. Synthesis of N-acetylmuramyl-ℓ-alanyl-d-isoglutaminyl-ℓ-S-(2-pyridylthio)cysteine (I).



Scheme 2. Synthesis of 1-(N-acetylmuramyl-ℓ-alanyl-d-isoglutaminamido)-6-[3-(2-pyridyldithio)propionamido]hexane (II).



virus A/Aichi/2/68 (serosubtype H3N2). This peptide has the structure Ser-Asn-Ala-Cys(acm)-Lys-Arg-Gly-Pro-Gly-Ser-Gly-Phe and is part of the antigenic determinant A of the HA of subtype H3 [10].

To obtain conjugates with a peptide, the S-acetamidomethyl (acm) group was removed with mercury acetate, the Hg(II) ions were precipitated with hydrogen sulfide, and the peptide was rapidly used for conjugation with an excess of compound (I) or (II). The conjugation reaction was performed in 1 M acetic acid, and the products were isolated by chromatography on a column of TSK 40 HW. The conjugates obtained were homogeneous according to analytical reversed-phase high-performance liquid chromatography (HPLC), and the amino acid compositions of the preparations corresponded to those expected. The immunogenic properties of the conjugates are now being studied.

## EXPERIMENTAL

We used DCC, HBT, and dithiothreitol from Fluka and di-2-pyridyl disulfide from Merck. The N-hydroxysuccinimide ester of 3-(2-pyridyldithio)propionic acid was synthesized as in [11], and the synthesis of the HA (136-147) peptide has been described previously [12].

TLC was conducted on Kieselgel $60F_{254}$ plates (Merck) in the following solvent systems: 1) chloroform–acetone–water (4:1:0.1); 2) ethyl acetate–acetic acid–water–pyridine (70:8:6:16); and 3) butan-1-ol–pyridine–acetic acid–water (5:5:1:4). The column chromatography of the MDP derivatives and the conjugates was performed on 2 × 30 cm columns of Bio-Gel P-4 and 2.5 × 55 cm columns of Toyopearl TSK 40 HW (Toyo Soda) in 1 M acetic acid. Analytical HPLC was conducted on a LKB 2152 chromatograph using a 4 × 125 mm steel column filled with LiChrosorb RP18 (Merck) having a particle size of 5 μM. Eluting buffer: concentration gradient of acetonitrile (5-90%) in water containing 0.1% of trifluoroacetic acid.

The analysis of the amino acid compositions of the conjugates after hydrolysis in a mixture of trifluoroacetic acid and 6 N HCl (1:2) in evacuated sealed ampuls (160°C, 25 and 60 min) was carried out on a Biotronik LC 7000 analyzer. The optical rotations of the samples were measured on a DIP-360 polarimeter (JASCO) in a cell with a pathlength of 5 cm. Mass spectra were recorded on a MS 7070 HS instrument (VG Analytical) in the regime of bombardment with accelerated argon atoms at an accelerating voltage of 4 kV.

MDP was synthesized as described in [8, 9] $[\alpha]_D^{25}$ +43.7° (c 1.0; $CH_3COOH$), m/z 492 ($M + H^+$). According to the literature: $[\alpha]_D^{25}$ +44° [8]; +43.7° [9].

S-(2-Pyridyldithio)cysteine (III). A suspension of 5 g of di-2-pyridyl disulfide in 150 ml of dry petroleum ether was saturated with gaseous chlorine at 20°C for 1.5 h. The solvent was evaporated off in vacuum and the residue was dissolved in 100 ml of glacial acetic acid. To the resulting solution of pyridine-2-sulfenyl chloride was added a suspension of 2.5 g of ℓ-cysteine in 20 ml of acetic acid. The mixture was stirred for 1 h, and the solvent was distilled off in vacuum. The residue was dissolved in water and the solution was extracted with chloroform (3 × 60 ml). The aqueous layer was brought to pH 7 with 0.5 M $NaHCO_3$, and the precipitate that deposited was filtered off. The residue was dissolved in 0.05 N HCl, the solution was treated with carbon, the filtrate was brought with aqueous ammonia to pH 8, and the resulting precipitate was filtered off and dried in vacuum. Yield 3.6 g (70%), mp 213-215°C, $R_f$ 0.65 (system 2), m/z 231 ($M + H^+$).

6-[3-(2-Pyridyldithio)propionamido]hexylamine (V). A solution of 0.7 g of the N-hydroxysuccinimide ester of 3-(2-pyridyldithio)propionic acid (IV) in 2 ml of dimethylformamide was added dropwise to a solution of 1 g of hexamethylenediamine in 10 ml of water. After 1.5 h, the reaction mixture was diluted with water to 100 ml and was extracted with ethyl acetate (3 × 60 ml). The ethyl acetate extract was washed with saturated NaCl solution, dried with anhydrous $Na_2SO_4$, and evaporated in vacuum. The residue was treated with hexane and the product was filtered off. Yield 0.5 g (60%), mp 71-74°C, $R_f$ 0.3 (system 2).

Synthesis of Compound (I). A solution of 60 mg (0.12 mmole) of MDP and 25 mg (0.2 mmole) of HBT in 1 ml of acetonitrile was treated with 30 mg (0.15 mmole) of DCC. After 1 h, 46 mg (0.2 mmole) of compound (III) was added to the reaction mixture and it was incubated at room temperature for 18 h and was then evaporated in vacuum. The residue was dissolved in 0.4 ml of 1 M acetic acid and was chromatographed on a column of TSK 40 HW. The fractions containing the desired product were combined, evaporated in vacuum, and treated with ether. Yield 80 mg (86%), mp 191-193°C, m/z 705 ($M + H^+$). Found %: C 46.12; H 5.81; N 12.04. For $C_{27}H_{40}N_6O_{12}S_2$ (704.77) calculated %: C 46.02; H 5.68; N 11.93.

Synthesis of Compound (II). A solution of 60 mg (0.12 mmole) of MDP in 1 ml of acetonitrile–water (9:1) was treated with 65 mg (0.15 mmole) of compound (V), 27 mg (0.2 mmole) of HBT, and 15 μl of N-methylmorpholine, the resulting mixture was cooled to 0°C, and 30 mg (0.15 mmole) of DCC was added. After 16 h, the dicyclohexylurea that had deposited was filtered off, the filtrate was evaporated to the state of an oil, and it was treated with ether and the resulting precipitate was filtered off and washed with ether. Then it was dissolved in 1 ml of water and was chromatographed on a column of Bio-Gel B-4. The fractions containing the product were combined and evaporated in vacuum. The residue was dissolved in ethanol and precipitated with ether. Yield 65 mg (65%), mp 154-156°C. $R_f$ 0.15 (system 1), m/z 788 ($M + H^+$). Found %: C 50.14; H 6.35; N 12.61. For $C_{33}H_{53}N_4O_{11}S_2$ (787.95) calculated %: C 50.30; H 6.78; N 12.45.

Preparation of Conjugates. The acm group was eliminated from the peptide HA (136-147) as described previously [12]. The deblocked peptide (6 μmole) in 0.3 ml of 1 M acetic acid was mixed with a solution of compound (I) or (II) (10 μmole) in 0.2 ml of 1 M acetic acid. The course of the reaction was monitored spectrophotometrically from the accumulation of pyridine-2-thione ($\varepsilon_{343}$ 8800). After 18 h, the reaction mixture was deposited on a column of TSK 40 HW and chromatographed in 1 M acetic acid. The fractions were analyzed by the ninhydrin test [12] and by analytical HPLC, and the desired conjugate was collected and lyophilized. The yields of the conjugates were 80-85%. Amino acid analysis of the compounds showed the presence in them of additional alanine and glutamic acid residues (one each) as compared with the composition of the initial peptides.

## SUMMARY

1. Derivatives of N-acetylmuramyl-ℓ-alanyl-d-isoglutamine containing activated disulfide bonds have been synthesized.

2. Conjugates of N-acetylmuramyl-ℓ-alanyl-d-isoglutamine with a synthetic peptide fragment of influenza virus hemagglutinin have been obtained.

## LITERATURE CITED


1. R. Arnon and M. Sela, Ann. Inst. Pasteur Immunol., 136, No. 3, 271 (1985).
2. M. Sela, Biopolymers, 22, No. 3, 415 (1983).
3. R. Arnon, M. Sela, M. Parant, and L. Chedid, Proc. Natl. Acad. Sci. USA, 77, No. 11, 6769 (1980).
4. G. Werner, F. Floc'h, D. Migliore-Samour, and P. Jolles, Experientia, 42, No. 5, 521 (1986).
5. F. Audibert, M. Jolivet, L. Cherdid, R. Arnon, and M. Sela, Proc. Natl. Acad. Sci. USA, 79, No. 16, 5042 (1982).
6. V. Ivanov, T. Andronova, M. Berzukov, V. Rar, E. Makarov, S. Kozmin, M. Astapova, T. Barkova, and V. Nesmeyanov, Pure Appl. Chem., 59, No. 3, 317 (1987).
7. P. Lefrancier and E. Lederer, Pure Appl. Chem., 59, No. 3, 449 (1987).
8. C. Hiebert, W. Kopp, H. Richerson, and C. Barfknecht, J. Med. Chem., 26, No. 12, 1729 (1983).
9. A. Hasegawa, E. Tanahashi, and M. Kiso, Carbohydr. Res., 103, No. 2, 251 (1982).
10. D. Viley, I. Wilson, and J. Skehel, Nature (London), 289, No. 5796, 373 (1981).
11. A. F. Shval'e, V. I. Ofitserov, and V. V. Samukov, Zh. Obshch. Khim., 55, No. 9, 2152 (1985).
12. V. V. Samukov, V. V. Kalashnikov, V. I. Ofitserov, and A. F. Shval'e, Bioorg. Khim., 11, No. 8, 1037 (1985).


# PRINCIPLES OF MODELING IN THE STUDY OF CONFORMATIONS OF HISTONES


V. K. Burichenko, E. I. Ramm, N. I. Koryakina, L. I. Mar'yash, and R. R. Kamilova



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A study has been made by the CD method of the conformational potentialities of the polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$ and $(Ala\text{-}Lys\text{-}Ala)_n$ and fragments of the terminal sections of histones H4 (the sequence 1-16), H2B (1-21), and H1 (152-184), and they have been used as models of histones in complex formation with DNA under various conditions of the medium.


It is traditional to consider that in the majority of protein molecules there are two main types of regular secondary structure – α-helix and β-structure. As exceptions have been found proteins of the collagen type in which a unique left-handed helical conformation of the type of poly-L-proline-II is realized at the positions of accumulation of imino acids and glycine (projection of a residue on the axis of the helix of the order 3 Å, $3_1$ symmetry, mean values of the angles $\varphi$ and $\psi$: 77.2 and 145.9°). However, a conformation of this type was later detected in certain synthetic peptides in aqueous solutions [1, 2], in hormones [3], and in globular proteins [4].

It has become known that the left-handed helical conformation is also realized in an aqueous medium at fairly low temperatures when hydration is most effective [1, 2]. The absence of interpeptide hydrogen bonds in the chain in the organization of this structure and the possibility of its stabilization by water ensure its lability in the conformational respect, which is important for the organization of enzyme-substrate, hormone-receptor, and other functionally important interactions.


V. I. Nikitin Institute of Chemistry, Tadzhik SSR Academy of Sciences, Dushanbe.


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