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SYNTHESIS OF [6-^{3}H]-N-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE

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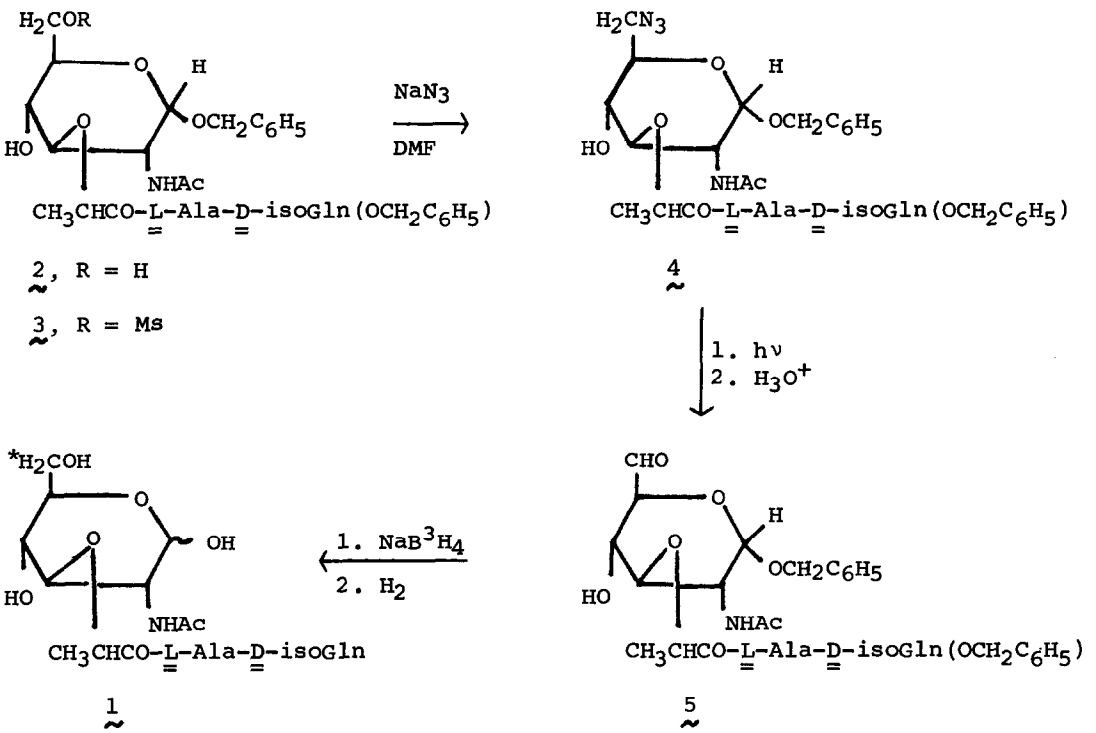
Of the known immunoadjuvants the most active is Freund's complete adjuvant (FCA), which is a water-in-oil emulsion containing the antigen in the aqueous phase and whole killed mycobacteria in paraffin oil.[1] The minimum structure necessary for the adjuvant activity of FCA has recently been identified[2] by chemical synthesis as a low molecular-weight, hydrosoluble peptidoglycan fragment of the bacterial cell wall, viz., N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide - MDP). Addition of synthetic MDP to an emulsion of Freund's incomplete adjuvant with an antigen increases levels of antibodies against the antigen (humoral response) and induces delayed hypersensitivity (cellular immunity).[1] Significantly for potential clinical application, MDP is devoid of immunogenicity and toxicity in normal and adrenalectomized mice and is also active when administered orally as an aqueous solution.[3]

The mechanism of action of MDP has recently been the subject of a number of investigations Macrophage appears to be the primary target cell for the adjuvant effects of MDP,[4] although enhancement of helper T cell function has also been observed.[5] To assist in the elucidation of the mode of action in the cellular and cell membrane systems, radiolabelled MDP was required. The synthesis of [^{14}C-alanine]-MDP has been reported.[6] However, the procedure[6] involved the handling of very hot materials at all stages without dilution in order to maintain sufficient radioactivity in the final product for biological experiments. In this communication we report the synthesis of [6-^{3}H]-N-acetylmuramyl-L-alanyl-D-isoglutamine (1), i.e., MDP specifically tritiated at the C-6 position of the muramic acid portion of the molecule. Since the label is incorporated at the penultimate step in the synthetic sequence, the present method for the preparation of radiolabelled MDP is considered more practical and should also be conveniently applicable to the preparation of labelled analogs.

The synthesis of 1 was accomplished by (a) regioselective introduction of a mesylate group at the C-6 position of an appropriately blocked MDP derivative 2; (b) nucleophilic displacement with azide anion to give the primary azide 4; (c) photolysis of the primary azide 4 and hydrolysis of the intermediate imine to the 6-aldehyde 5; (d) reduction of aldehyde 5 with NaB^3H_4 to the radiolabelled 4,6-diol; and (e) removal of the benzyl ester and benzyl glycoside blocking groups by hydrogenolysis. The method for generation of an aldehyde group in a protected sugar by photolysis of a primary azide has been developed by Horton and co-workers.[7] However, the synthesis of 1 represents the first application of this procedure for incorporation of tritium at a specific position of a sugar derivative.



The experimental details are as follows.[8] Regioselective mesylation of the 4,6-diol 2 with methanesulfonyl chloride in pyridine at 0° afforded the 6-mesylate 3 in 90% yield, m.p. 188-190° (dec.); $[\alpha]_D^{25}$ + 92° (c 1, DMF); n.m.r. (300-MHz, DMSO-d_6): δ 5.69 (d, $J_{4,OH}$ 6.5 Hz,

OH), 5.09 (s, $CO_2\underline{CH_2}Ph$), 4.82 (d, $\underline{J}_{1,2}$ 2.5 Hz, H-1), 4.69 (d, 1H, $\underline{J}$ 13 Hz, $\underline{OCH_2}Ph$), 4.48 (d, 1H, $\underline{OCH_2}Ph$), 3.22 (s, OMs), 2.36 (t, $\underline{CH_2}CH_2CO_2Bn$), 1.80 (s, NH$\underline{Ac}$), 1.26, 1.22 [2d, CH_3(ala) and CH_3(lac)]. Subsequent nucleophilic displacement with azide anion gave the 6-azide 4 in 73% yield, m.p. 221-225° (dec.); $[\alpha]_D^{25}$ + 98° (c 1, DMF); ir (Nujol) 2120 cm^{-1} (N_3). Photolysis[9] of 4 (360 mg, 0.52 mmol) in 5:1 2-methoxyethanol-benzene (145 ml) in an inert atmosphere at 15-20° for 4h followed by treatment with aqueous AG 50W (H^+) resin gave the 6-aldehydo derivative 5 as an amorphous solid (275 mg). A solution of 5 (111 mg, 0.16 mmol) in absolute ethanol (10 ml) at 0-5° was treated with NaB^3H_4 (140 mCi, 0.5 mg) for 1h and then with normal $NaBH_4$ (8 mg) for an additional hour. The mixture was then neutralized with 50% acetic acid, evaporated, and the residue was transferred with a little chloroform-methanol (10:1) to a silica gel column (10 g, E. Merck #7734; wet-packed in chloroform). The column was eluted with chloroform-methanol, and the eluate was monitored by count and tlc (Analtech silica gel, $CHCl_3$-MeOH 9:1). The product fractions were combined and evaporated, yielding 38 mCi of labelled 4,6-diol containing a trace of 5 by tlc. A solution of the 4,6-diol (38 mCi, about 30 mg) in acetic acid (2 ml) was hydrogenolyzed at 20 psi for 18 h in the presence of 10% Pd-C (50 mg). The reaction mixture was then filtered through Supercel and the filter was washed with water. The combined filtrates were evaporated under vacuum, flushed with toluene and then with ethanol. The residue of crude [6-^{3}H]-muramyl dipeptide was purified by preparative tlc on silica gel (4 x 250μm plates, 20 x 20 cm, Analtech; 60:40:10 $CHCl_3$-MeOH-H_2O). The product bands were removed with ethanol, and the chromatography was repeated using two plates. The final ethanol solution was evaporated, and the residue was dissolved in a little water and freeze-dried to a white solid (8.6 mg, 601 mCi/mmol, 10.4 mCi). The radioactive purity, determined by analysis of the material by scintillation counting of sections scraped from a tlc plate developed in either 60:40:10 chloroform-methanol-water or 7:3 n-propanol-water, was 95%, and the 300-MHz n.m.r. spectrum in D_2O was identical to that of an authentic cold sample.

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8. Compounds 3 and 4 gave correct microanalyses and exhibited n.m.r. spectral characteristics in agreement with their structures. Complete characterization data for compounds 2-5 will be reported elsewhere.

9. Irradiation was conducted with unfiltered light from a 450-w Hanovia Type L mercury-arc lamp Model 679A having a 4.5-in. arc. The lamp was placed in a water-cooled, quartz immersion-well, and the whole assembly mounted in a Pyrex reaction vessel. Conversion of the azide 4 to the slower-moving intermediate imine was followed by tlc (9:1 $CHCl_3$-MeOH).

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