Thesis Title: Synthesis of C^5(2,4 dinitrophenyl-4-aminoallyl) Deoxyuridine 5′ Triphosphate for Nonisotopic Gene Detection

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Date of Graduation: 26 February B.E 2539 (1996)

ABSTRACT

C^5(2,4dinitrophenyl-4-aminoallyl)dUTP was synthesized for nonisotopic gene detection. The natural nucleotide dUTP was mercurated at C^5 position by mercuric acetate, and the mercurated dUTP intermediate was reacted with allylamine to yield the C^5(3-aminoallyl)dUTP (AA-dUTP). The reaction product was fractionated by DEAE cellulose column chromatography, and 67% yield identified as AA-dUTP was obtained. Dinitrophenylation reaction of amino end of AA-dUTP with FDNB gave dinitrophenyl derivative of dUTP. This reaction product was effectively purified by Sephadex G10 column, and 40% yield was obtained.

The rate of incorporation of DNP-dUTP by DNA polymerase I (Klenow enzyme) was 39% as effective as dTTP control in random primed

synthesis. With lower percentage of substitution of dTTP by DNP-dUTP analogue (50% or lower) the rate of incorporation of analogue was as effective as the control. Probe with 50% of the analogue substituted for dTTP was synthesized for BlueSEMBV, and target DNA of 1 ng could be detected.

Optimal conditions in detection procedure were determined: hybridized temperature was around 60°C, 16 hr, concentration of first antibody was 1.3 μg/ml and 760 mU/ml of enzyme in second antibody, and the percent substitution of the analogue in the probe was 35%. Target DNA of 5 pg could be detected.