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Synthesis and Use of New Digoxigenin- Meled Nucleotides in Non-Radioactive Labeling and Detection of Nucleic Acids

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SYNTHESIS AND USE OF NEW DIGOXIGENIN-LABELED NUCLEOTIDES IN NON-RADIO-ACTIVE LABELING AND DETECTION OF NUCLEIC ACIDS

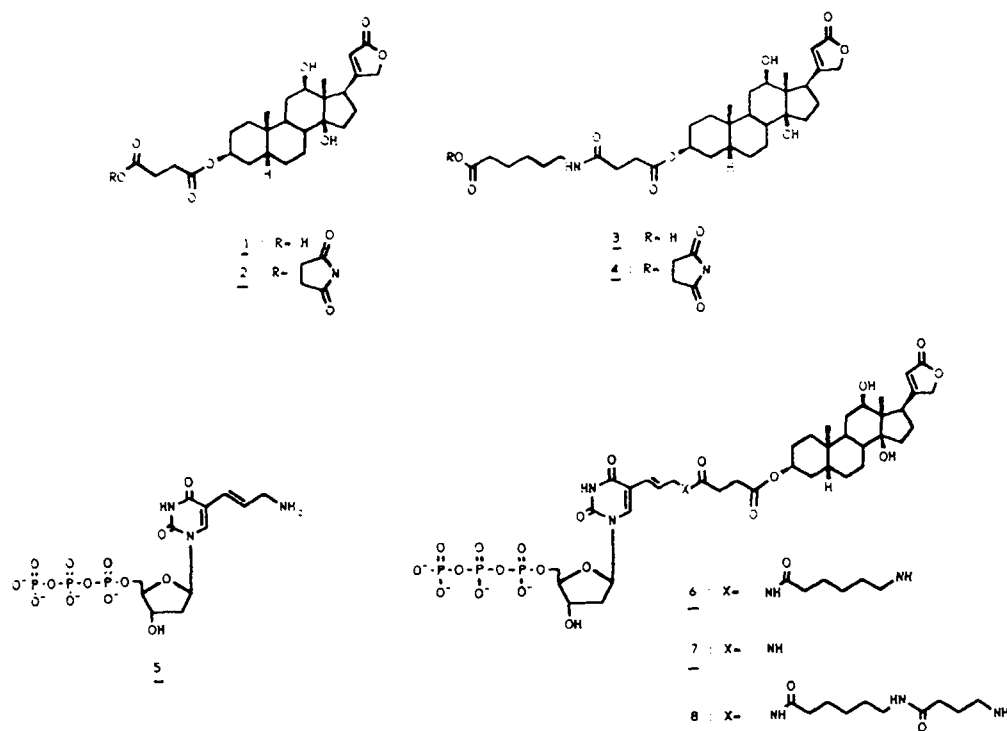
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Abstract: The chemical syntheses and use of novel digoxigenin-derivatized 5-aminoallyl-2'-deoxyuridine-5'-triphosphates are reported. The digoxigenin-modified deoxynucleotides were incorporated into DNA. Hybridization and following detection by ELISA technique allows the detection of homologous DNA down to 0,1 pg.

The technique of DNA hybridization and following sequence-specific DNA detection has become a powerful tool in analytical molecular biology. In order to circumvent labeling with radioactive isotopes a number of methods have been recently developed for non-radioactive labeling and detection [1, 2]. Because the well known biotin/(strept)avidin system [3] encounters problems with unspecific side reactions, our aim was the development of a non-radioactive DNA-labeling and detection system with equal sensitivity but without the inherent disadvantages of the biotin/(strept)avidin system.

The steroid-hemisuccinate 1 was prepared according to [4]. The N-hydroxy-succinimide ester 2 was isolated after a 20 h reaction of the hemisuccinate with N-hydroxy-succinimide (NHS) and dicyclohexylcarbodiimide (DCC) in tetrahydrofuran at room temperature (yield 85 %). Further treatment with 6-aminocaproic acid in dimethylformamide (DMF)/Et₃N at ambient temperature overnight afforded 3 (68 %), which was again converted by reaction with NHS and DCC in DMF into compound 4 (84 %). Subsequent reaction of this activated ester with 5-aminoallyl-2'-deoxyuridine-5'-triphosphate 5 (prepared by a modified procedure described by Langer [3]) in 0,1 M sodium borate buffer, pH 8,5/DMF gave after 15 h reaction time at room temperature and chromatographic work-up 6 (Dig-"11"-dUTP) in 45 % yield.



The derivatives 7 and 8 (Dig-"4"-dUTP and Dig-"16"-dUTP) have been synthesized in a similar way by reaction between 2 and 5 (37 %) resp. 2 and 4-aminobutyric acid, followed by the preparation of the NHS ester. Further derivatization was identical to the synthesis of 6 (overall yield 15 %).

Labeling with digoxigenin was performed by the incorporation of digoxigenin-substituted compounds 6, 7 and 8 into DNA. Random primed labeling was superior to that obtained by nick translation.

We observed an approx. 5-fold higher sensitivity of DNA labeled by the incorporation of 6, over that of 7. No more improvement in sensitivity was observed when 8 was used.

Hybridization was followed by detection with an ELISA technique, using the system anti digoxigenin antibodies/alkaline phosphatase/BCIP-NBT.

The digoxigenin system showed a sensitivity and specificity comparable to biotin but avoiding background problems obtained with the latter.

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