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United States Patent [19]

Hiatt et al.

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[54] **3' PROTECTED NUCLEOTIDES FOR ENZYME CATALYZED TEMPLATE-INDEPENDENT CREATION OF PHOSPHODIESTER BONDS**

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Related U.S. Application Data

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[51] **Int. Cl.**[6] **C07H 21/00**; C07H 21/02; C07H 19/04; C12Q 1/68

[52] **U.S. Cl.** **536/25.3**; 536/25.1; 536/25.31; 536/25.32; 536/25.33; 536/25.34; 536/26.1; 435/6

[58] **Field of Search** 536/25.1, 26.1, 536/25.3, 25.31, 25.32, 25.33, 25.34; 435/6

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[57] **ABSTRACT**

A method for the stepwise creation of phosphodiester bonds between desired nucleosides resulting in the synthesis of polynucleotides having a predetermined nucleotide sequence by preparing an initiation substrate containing a free and unmodified 3'-hydroxyl group; attaching a mononucleotide selected according to the order of the predetermined nucleotide sequence to the 3'-hydroxyl of the initiating substrate in a solution containing a catalytic amount of an enzyme capable of catalyzing the 5' to 3' phosphodiester linkage of the 5'-phosphate of the mononucleotide to the 3'-hydroxyl of the initiating substrate, wherein the mononucleotide contains a protected 3'-hydroxyl group, whereby the protected mononucleotide is covalently linked to the initiating substrate and further additions are hindered by the 3'-hydroxyl protecting group. Methods in which a mononucleotide immobilized on a solid support is added to a free polynucleotide chain are also disclosed.

6 Claims, 4 Drawing Sheets

FIGURE 1.

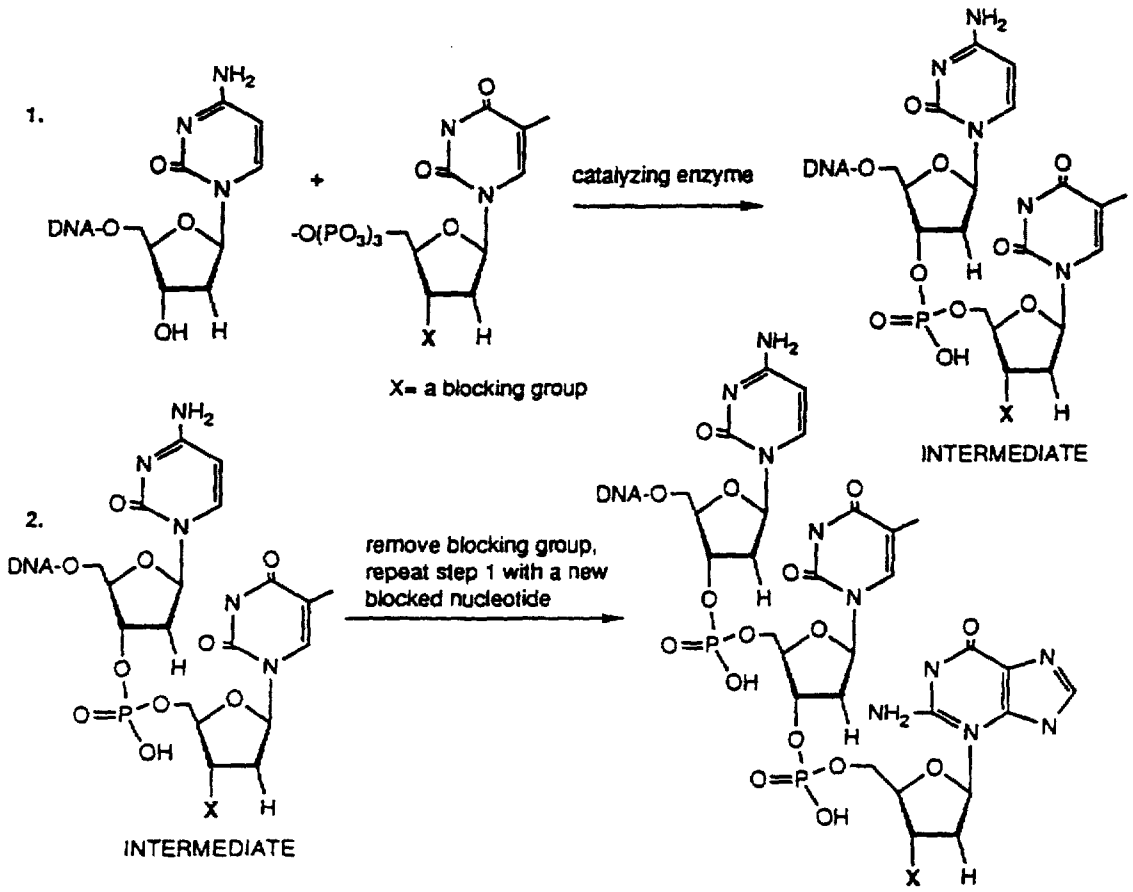
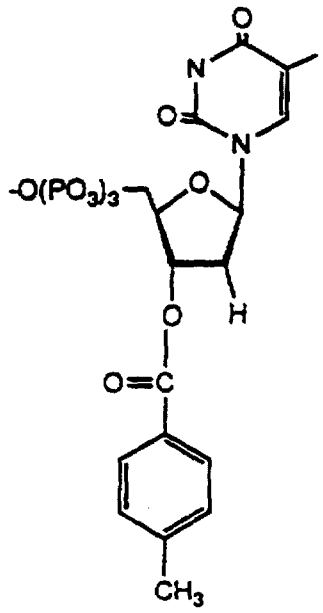
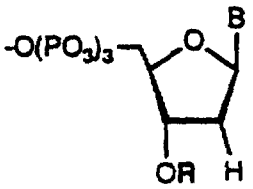


FIGURE 2.





3'- TOLUIC ACID ESTER OF THYMIDINE 5'-TRIPHOSPHATE

FIGURE 3.

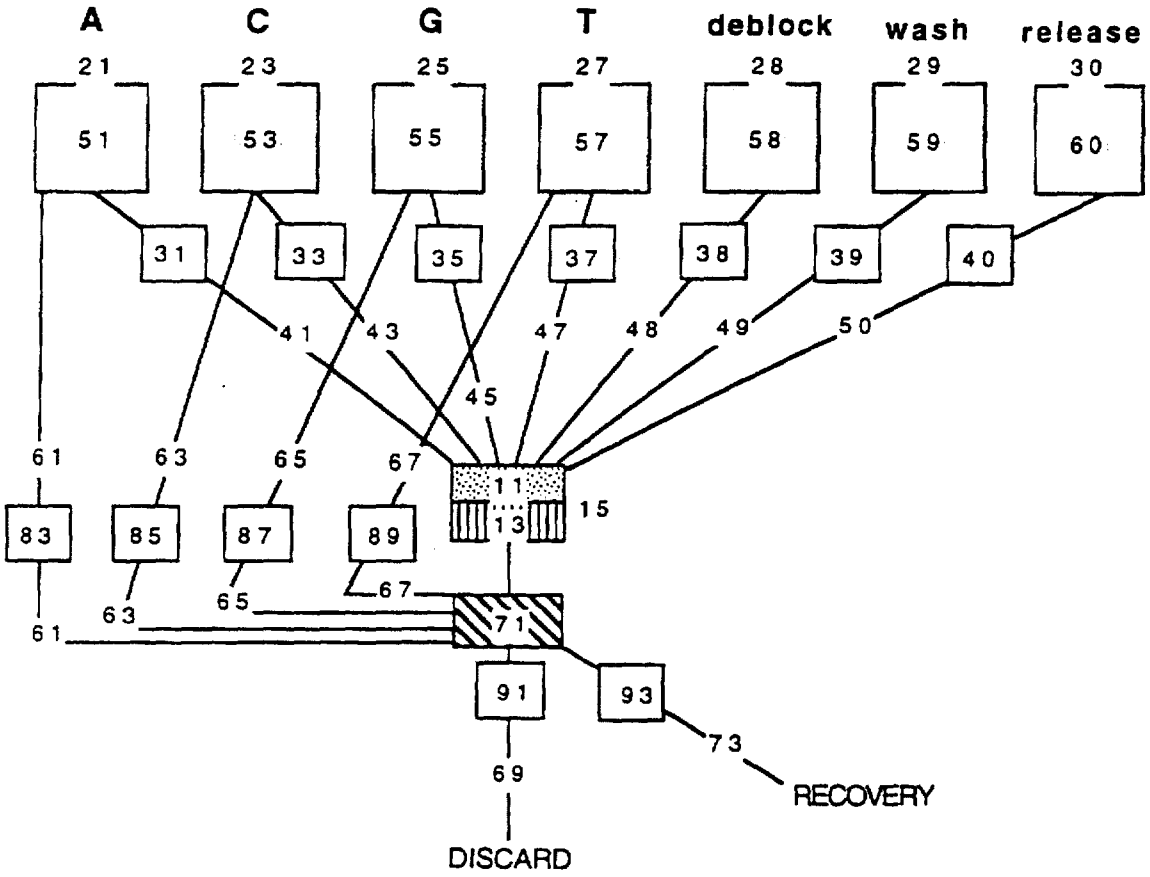
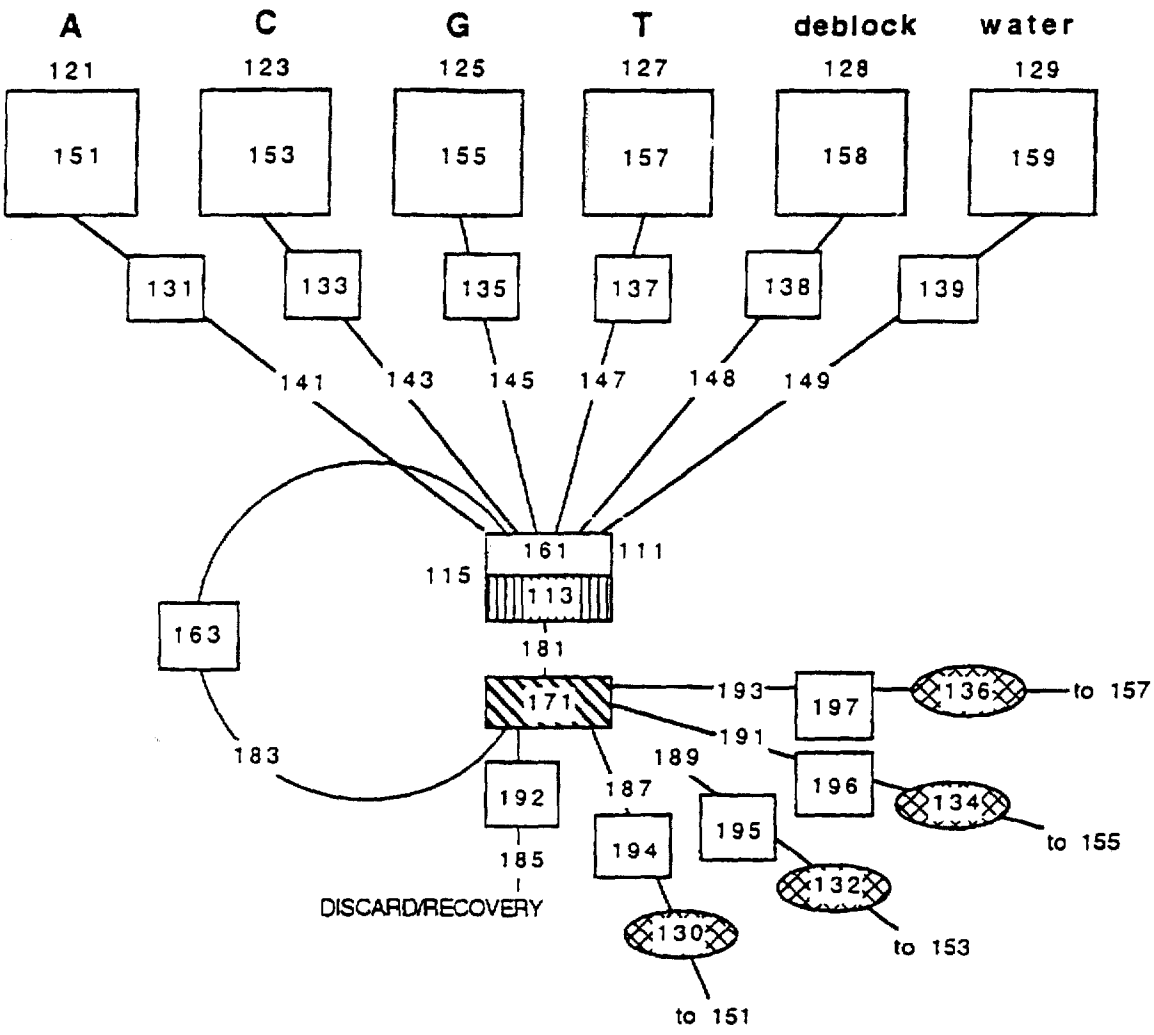


FIGURE 4.



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