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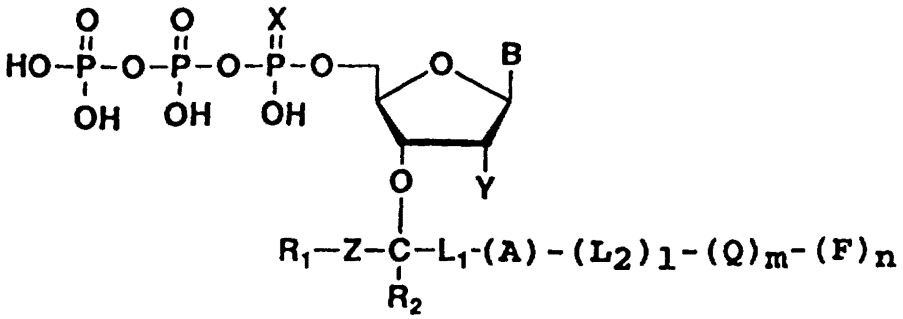
(54) Title: NOVEL CHAIN TERMINATORS, THE USE THEREOF FOR NUCLEIC ACID SEQUENCING AND SYNTHESIS AND A METHOD OF THEIR PREPARATION



(I)

(57) Abstract

The invention relates to compounds of general structure (I) or salts thereof, wherein B is a nucleobase, X and Z independently are oxygen or sulphur, Y is hydrogen or hydroxy, which optionally may be protected, R_1 is hydrocarbyl, which optionally is substituted with a functional group, R_2 is hydrogen or hydrocarbyl, which optionally is substituted with a functional group, A is an electron withdrawing or electron donating group capable of moderating the acetal stability of compound (I), L_1 and L_2 are hydrocarbon linkers, which may be the same or different, L_2, when present, being either (i) connected to L_1 via the group A, or (ii) directly connected to L_1, the group A then being connected to one of linkers L_1 and L_2, F is a dye label, Q is a coupling group for F, and 1, m and n independently are 0 or 1, with the proviso that 1 is 1 when m is 1, and 1 is 1 and m is 1 when n is 1. The compounds of formula (I) are useful as deactivatable chain extension terminators. The invention also relates to the use of the compounds (I) in [illegible] as well as to a method of preparing compounds of Formula (I).

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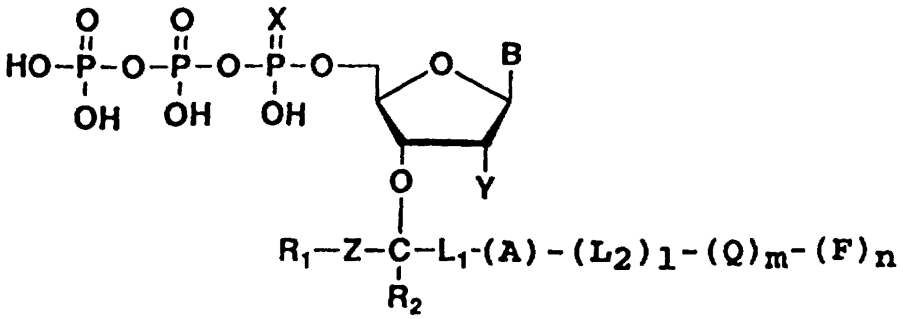

(I)

(57) Abstract

The invention relates to compounds of general structure (I) or salts thereof, wherein B is a nucleobase, X and Z independently are oxygen or sulphur, Y is hydrogen or hydroxy, which optionally may be protected, R_1 is hydrocarbyl, which optionally is substituted with a functional group, R_2 is hydrogen or hydrocarbyl, which optionally is substituted with a functional group, A is an electron withdrawing or electron donating group capable of moderating the acetal stability of compound (I), L_1 and L_2 are hydrocarbon linkers, which may be the same or different, L_2, when present, being either (i) connected to L_1 via the group A, or (ii) directly connected to L_1, the group A then being connected to one of linkers L_1 and L_2, F is a dye label, Q is a coupling group for F, and 1, m and n independently are 0 or 1, with the proviso that 1 is 1 when m is 1, and 1 is 1 and m is 1 when n is 1. The compounds of formula (I) are useful as deactivatable chain extension terminators. The invention also relates to the use of the compounds (I) in nucleic acid synthesis and nucleic acid sequencing as well as to a method of preparing compounds of Formula (I).

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NOVEL CHAIN TERMINATORS, THE USE THEREOF FOR NUCLEIC ACID SEQUENCING AND SYNTHESIS AND A METHOD OF THEIR PREPARATION

The present invention relates to novel nucleic acid chain extension terminators, their use in nucleic acid sequencing and synthesis, respectively, as well as a method for preparing such compounds.

Today, there are two predominant methods for DNA sequence determination: the chemical degradation method (Maxam and Gilbert, Proc. Natl. Acad. Sci. 74:560-564 (1977), and the dideoxy chain termination method (Sanger et al., Proc. Natl. Acad. Sci. 74:5463-5467 (1977)). Most automated sequencers are based on the chain termination method utilizing fluorescent detection of product formation. In these systems either primers to which deoxynucleotides and dideoxynucleotides are added are dye-labelled, or the added dideoxynucleotides are fluorescently labelled. As an alternative, dye labelled deoxynucleotides can be used in conjunction with unlabeled dideoxynucleotides. This chain termination method is based upon the ability of an enzyme to add specific nucleotides onto the 3' hydroxyl end of a primer annealed to a template. The base pairing property of nucleic acids determines the specificity of nucleotide addition. The extension products are then separated electrophoretically on a polyacrylamide gel and detected by an optical system utilizing laser excitation.

Although both the chemical degradation method and the dideoxy chain termination method are in widespread use, there are many associated disadvantages. For example, the methods require gel-electrophoretic separation. Typically, only 400-800 base pairs can be sequenced from a single clone. As a result, the systems are both time- and labor-intensive. Methods avoiding gel separation have been developed in attempts to increase the sequencing throughput.

Sequencing by hybridization (SBH) methods have been proposed by Crkvenjakov (Drmanac et al., Genomics 4:114

(1989); Strezoska et al., (Proc. Natl. Acad. Sci. USA 88:10089 (1991)), Bains and Smith (Bains and Smith, J. Theoretical Biol. 135:303 (1988)) and in US-A-5,202,231. This type of system utilizes the information obtained from multiple hybridizations of the polynucleotide of interest, using short oligonucleotides to determine the nucleic acid sequence. These methods potentially can increase the sequence throughput beacuse multiple hybridization reactions are performed simultaneously. To reconstruct the sequence, however, an extensive computer search algorithm is required to determine the most likely order of all fragments obtained from the multiple hybridizations.

The SBH methods are problematic in several respects. For example, the hybridization is dependent upon the sequence composition of the duplex of the oligonucleotide and the polynucleotide of interest, so that GC-rich regions are more stable than AT-rich regions. As a result, false positives and false negatives during hybridization detection are frequently present and complicate sequence determination. Furthermore, the sequence of the polynucleotide is not determined directly, but is inferred from the sequence of the known probe, which increases the possibility for error.

Methods have also been proposed which detect the addition or removal of single molecules from a DNA strand.

For example, Hyman E.D., Anal. Biochem., 174:423 (1988) discloses the addition of a nucleotide to a an immobilised DNA template/primer complex in the presence of a polymerase and determination of polymerisation reaction by detecting the pyrophosphate liberated as a result of the polymerisation.

Jett et al., J. Biomol. Struct. Dyn., I, p. 301, 1989 discloses a method wherein a single stranded DNA or RNA molecule of labelled nucleotides, complementary to the sequence to be determined, is suspended in a moving flow stream. Individual bases are then cleaved sequentially from the end of the suspended sequence and determined by a detector passed by the flow stream.

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