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METHODS AND STRUCTURES EMPLOYING NON-RADIOACTIVE
CHEMICALLY-LABELED POLYNUCLEOTIDE PROBES

5 BACKGROUND OF THE DISCLOSURE

In the determination of the presence or the identity of genetic material, such as DNA genetic material, it has been proposed to denature the genetic material to form
10 single-stranded DNA or single-stranded genetic material. The single-stranded genetic material is then fixed to a solid support and contacted with a probe, such as a DNA probe, having in its make up bases complementary to the
15 make up of the fixed genetic material to be identified and/or determined. The contacting of the single-stranded genetic material along with the single-stranded probe is carried out under conditions to effect hybridization of the genetic material to be determined or identified and the probe.

20 Radioactively-labeled probes, such as radioactively-labeled single-stranded DNA probes, have been employed. U.S. Patent 4,358,535 discloses a method of identifying a pathogen present in the clinical sample by denaturing the
25 genetic material present in the clinical sample to form single-stranded genetic material thereof and to fix the resulting single-stranded genetic material characterizing the pathogen to an inert support or surface. The thus-fixed single-stranded genetic material characterizing or
30 identifying the pathogen is brought into contact with a radioactive single-stranded probe under hybridizing conditions to effect duplex form or double-strand formation of the genetic material derived from the pathogen and the probe. The presence of the resulting formed duplex
35 between the probe and the pathogen genetic material would then be detected and would confirm the presence and/or identity of the pathogen.

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The disadvantages of employing a radioactively-labeled probe, such as a radioactively-labeled DNA probe, for the identification of genetic material are well known to those skilled in the art. Such disadvantages include not only the precautions and hazards involved in handling the radioactive material but also the short life of such radioactive material and expense in connection with the handling and use of such radioactively-labeled DNA probes.

10 It is known to chemically-label nucleotides and polynucleotides to avoid the hazards and/or difficulties associated when such compounds are radioactively-labeled. For example in the article by P.R. Langer, A.A. Waldrop and D.C. Ward entitled "Enzymatic Synthesis of Biotin-

15 Labeled Polynucleotides: Novel Nucleic Acid Affinity Probes", in Proc. Natl. Acad. Sci., USA, Vol. 78, No. 11, pp. 6633-6637, November 1981, there are described analogs of dUTP and UTP that contain a biotin molecule bound to the C-5 position of the pyrimidine ring through an allyl-

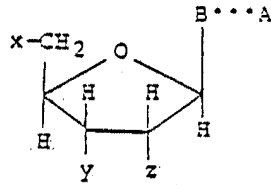
20 amine linker arm. The biotin-labeled nucleotides are efficient substrates for a variety of DNA and RNA polymerases in vitro. Polynucleotides containing low levels of biotin substitution (50 molecules or fewer per kilobase) have denaturation, reassociation and hybridization characteristics similar to those of unsubstituted controls. Biotin-labeled polynucleotides, both single and double stranded, are selectively and quantitatively retained on avidin-Sepharose, even after extensive washing with 8M urea, 6M guanidine hydrochloride or 99% formamide. In

30 addition, biotin-labeled nucleotides can be selectively immunoprecipitated in the presence of anti-biotin antibody and Staphylococcus aurea, Protein A. These unique features of biotin-labeled polynucleotides suggest that they are useful affinity probes for the detection and

35 isolation of specific DNA and RNA sequences. It is

indicated in the article that the subject matter of the article is comprised in a pending U.S. patent application.

5 Compounds or nucleotides have also been prepared which can be incorporated into DNA, such as double-stranded DNA, and which are useful for the preparation of non-radioactive chemically-labeled DNA probes. See, for example, co-pending U.S. patent application Serial No. 255,223, filed April 17, 1981, in which the subject
10 matter of the above-identified article is disclosed, and additionally it is disclosed that compounds having the structure:

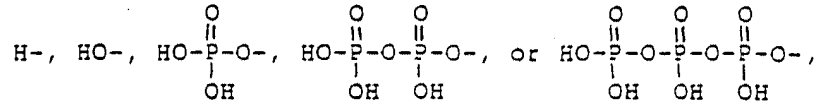


20 wherein B represents a purine, deazapurine, or pyrimidine moiety covalently bonded to the C^{1'}-position of the sugar moiety, provided that when B is purine or 7-deazapurine, it is attached at the N⁹-position of the purine or deazapurine, and when B is pyrimidine, it is attached at the
25 N¹-position;

wherein A represents a moiety consisting of at least three carbon atoms which is capable of forming a detectable complex with a polypeptide when the compound is incorporated into a double-stranded ribonucleic acid; deoxyribonucleic
30 acid duplex, or DNA-RNA hybrid;

wherein the dotted line represents a chemical linkage joining B and A, provided that if B is purine, the linkage is attached to the 9-position of the purine, if B is
35 7-deazapurine, the linkage is attached to the 7-position of the deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine; and

wherein each of x, y and z represents



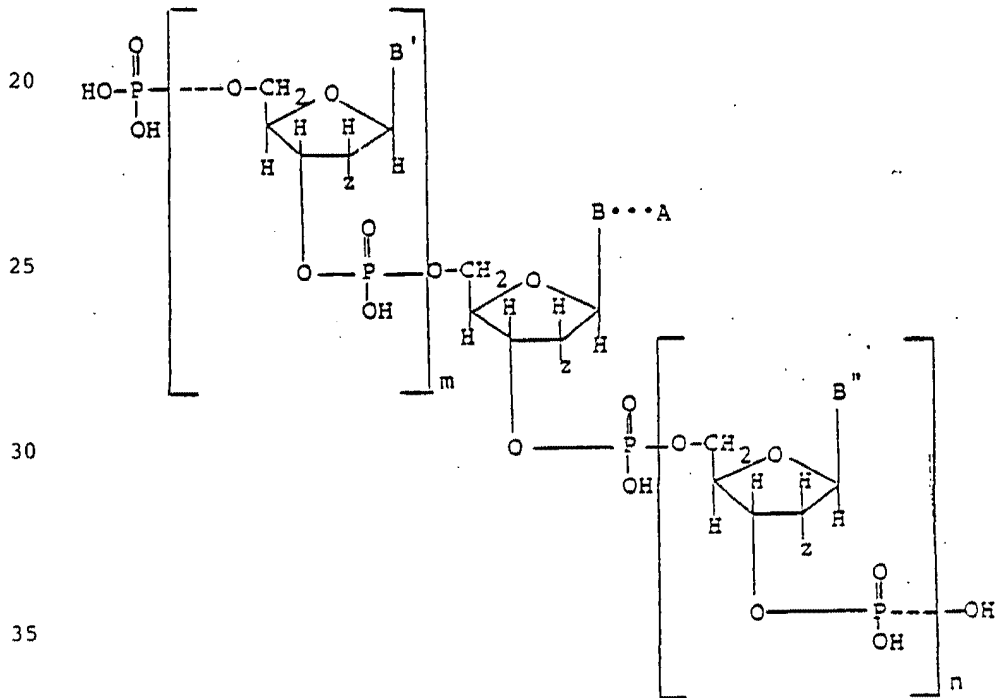
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are widely useful as probes in biomedical research and recombinant DNA technology.

Particularly useful are compounds encompassed within this structure which additionally have one or more of the following characteristics: A is non-aromatic; A is at least C₅; the chemical linkage joining B and A includes an α -olefinic bond; A is biotin or iminobiotin; and B is a pyrimidine or 7-deazapurine.

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U.S. patent application Serial No. 255,223 also discloses compounds having the structure:



wherein each of B, B', and B" represents a purine, 7-deazapurine, or pyrimidine moiety covalently bonded to the C^{1'}-position of the sugar moiety, provided that whenever B, B', or B" is purine or 7-deazapurine, it is attached at the N⁹-position of the purine or 7-deazapurine, and whenever B, B', or B" is pyrimidine, it is attached at the N¹-position;

wherein A represents a moiety consisting of at least three carbon atoms which is capable of forming a detectable complex with a polypeptide when the compound is incorporated into a double-stranded duplex formed with a complementary ribonucleic or deoxyribonucleic acid molecule;

wherein the dotted line represents a chemical linkage joining B and A, provided that if B is purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 8-position of the deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine;

wherein z represents H- or HO-; and

wherein m and n represent integers from 0 up to about 100,000.

These compounds can be prepared by enzymatic polymerization of a mixture of nucleotides which include the modified nucleotides of this invention. Alternatively, nucleotides present in oligo- or polynucleotides may be modified using chemical methods.

The chemically-labeled or modified nucleotides described in the above-referred PNAS article and in U.S. patent application Serial No. 255,223, as indicated hereinabove is the structure:

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