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21 June 2001

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ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: NUCLEIC ACID LABELING COMPOUNDS

(57) Abstract: Nucleic acid labeling compounds containing heterocyclic derivatives are disclosed. The heterocyclic derivative containing compounds are synthesized by condensing a heterocyclic derivative with a cyclic group (e.g. a ribofuranose derivative). The labeling compounds are suitable for enzymatic attachment to a nucleic acid, either terminally or internally, to provide a mechanism of nucleic acid detection.



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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07H19/04 C07H21/00 C07H19/12 C07H19/052 C12Q1/68
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07H C12Q C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 28176 A (AMERSHAM INT PLC ;BROWN DANIEL (GB); HAMILTON ALAN (GB); LOAKES DA) 7 August 1997 (1997-08-07) page 1 -page 4 claims	1,25,43, 49
X	WO 97 27317 A (CHEE MARK ;LAI CHAOQIANG (US); LEE DANNY (US); AFFYMETRIX INC (US)) 31 July 1997 (1997-07-31) page 36 -page 40 examples 11,15 claims 1,42,46,47 figure 23A	1,5,9, 17,21
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
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23 February 2001

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POCHET, SYLVIE ET AL: "Ambiguous base pairing of 1-(2-deoxy-.beta.-D-ribofuranosyl) imidazole-4-carboxamide during PCR" NUCLEOSIDES NUCLEOTIDES (1997), 16(7-9), 1749-1752 , XP000982354	1
A	page 1749	25,31, 43,49
X	LE BEC, CHRISTINE ET AL: "Derivatives of imidazole-4-carboxamide as substrates for various DNA polymerases" NUCLEOSIDES NUCLEOTIDES (1997), 16(7-9), 1301-1302, XP002135339	1
A	the whole document	25,31, 43,49
A	JOHNSON, W. TRAVIS ET AL: "The preparation and stability of oligodeoxyribonucleotides containing the deoxyadenosine mimic 1-(2'-deoxy-.beta.-D-ribofuranosyl)imidazole-4- carboxamide" NUCLEIC ACIDS RES. (1997), 25(3), 559-567, XP002135338	1,25,31, 43,49
A	page 559 -page 560 page 566, "Conclusion" figures 1-4,7	
A	AOYAGI M ET AL: "NUCLEOSIDES AND NUCLEOTIDES. 115. SYNTHESIS OF 3-ALKYL-3-DEAZAINOSINS VIA PALLADIUM-CATALYZED INTRAMOLECULAR CYCLIZATION: A NEW CONFORMATIONAL LOCK WITH THE ALKYL GROUP AT THE 3-POSITION OF THE 3-DEAZAINOSINE IN ANTI-CONFORMATION" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 34, no. 1, 1 January 1993 (1993-01-01), pages 103-106, XP000653639 ISSN: 0040-4039	1,25,31, 43,49
	page 103, paragraph 1 page 104, scheme	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAMILTON, HARRIET W. ET AL: "C4-Substituted 1-.beta.-D-ribofuranosylpyrazolo'3,4-d!pyr imidines as adenosine agonist analogs" J. MED. CHEM. (1983), 26(11), 1601-6, XP002161218 RN 86954-46-3 and 86954-47-4 chart I scheme III table I ---	5
X	RIDEOUT, JANET L. ET AL: "Pyrazolo'3,4-d!pyrimidine ribonucleosides as anticoccidials. 2. Synthesis and activity of some nucleosides of 4-(alkylamino)-1H- pyrazolo'3,4-d!pyrimidines" J. MED. CHEM. (1982), 25(9), 1040-4, XP002161219 RN 82436-59-7, 82436-60-0, 82436-61-1 and 82436-68-8 table I scheme I ---	5
X	AVILA, JOSE LUIS ET AL: "Biological action of pyrazolopyrimidine derivatives against Trypanosoma cruzi. Studies in vitro and in vivo" COMP. BIOCHEM. PHYSIOL., C: COMP. PHARMACOL. TOXICOL. (1987), 86C(1), 49-54, XP000982480 page 50, table 1, compounds 26 (RN 102353-70-8), 28 (102353-71-9), 29 (102353-72-0) and 30 (102353-73-1) ---	5
X	SEELA M. AND ZULAUF M.: "Synthesis of 7-alkynylated 8-aza-7-deaza-2'-deoxyadenosines via the Pd-catalysed cross-coupling reaction" J. CHEM. SOC., PERKIN TRANS. 1, no. 19, 1998, pages 3233-3239, XP002161221 page 3233 scheme 1 ---	9
X	ROSEMEYER H. ET AL: "Stereoelctronic effects of modified purines on the sugar conformation of nucleosides and fluorescence properties" NUCLEOSIDES & NUCLEOTIDES, vol. 16, no. 5-6, 1997, pages 821-828, XP002161222 page 822, formula scheme 2, compound 34 page 827, paragraph 5 - paragraph 8 ---	9
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PETRIE C R ET AL: "A NOVEL BIOTINYLATED ADENYLATE ANALOGUE DERIVED FROM PYRAZOLU3,4-DPYRIMIDINE FOR LABELING DNA PROBES" BIOCONJUGATE CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY, WASHINGTON, vol. 2, no. 6, 1 November 1991 (1991-11-01), pages 441-446, XP000572789 ISSN: 1043-1802 cited in the application page 441, left-hand column -right-hand column, paragraph 3 page 442, scheme I	9-11, 27, 35, 45, 53
P, X	SEELA F. AND ZULAUF M.: "Synthesis of oligonucleoides containing pyrazolo[3,4-d]-pyrimidines: the influence of 7-substituted 8-aza-7-deazaadenines on the duplex structure and stability" J. CHEM. SOC., PERKIN TRANS. 1, no. 4, 9 February 1999 (1999-02-09), pages 479-488, XP002161220 page 479	9
X	CHEMICAL ABSTRACTS, vol. 113, no. 26, 24 December 1990 (1990-12-24) Columbus, Ohio, US; abstract no. 243960, GALUSHKO, S. V. ET AL: "Reversed-phase HPLC of N4- and O'-derivatives of 6-azacytidine" XP002161231 abstract & ZH. ANAL. KHIM. (1990), 45(5), 984-9, RN 31698-11-0 and 130816-26-1	13
X	CHEMICAL ABSTRACTS, vol. 102, no. 25, 24 June 1985 (1985-06-24) Columbus, Ohio, US; abstract no. 216761, LAZURKEVICH, Z. V. ET AL: "Growth activity of 6-substituted azauracils" XP002161232 abstract & FIZIOL. BIOKHIM. KUL'T. RAST. (1985), 17(1), 48-54 , RN 96360-27-9	13

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INTERNATIONAL SEARCH REPORT

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PCT/US 99/12390

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 74, no. 21, 24 May 1971 (1971-05-24) Columbus, Ohio, US; abstract no. 112367, CHERNETSKII, V. P. ET AL: "Anomalous nucleosides and related compounds. XIV. Derivatives of 6-azacytidine" XP002161233 abstract & KHIM. GETEROTSIKL. SOEDIN. (1970), (7), 986, RN 31698-11-0	13
X	--- GALUSHKO, S. V. ET AL: "Relationship between retention parameters in reversed-phase high-performance liquid chromatography and antitumor activity of some pyrimidine bases and nucleosides" J. CHROMATOGR. (1991), 547(1-2), 161-6, XP000979356 page 162; figure 1 (e.g. compound V)	13
A	--- HOLY, ANTONIN ET AL: "Oligonucleotidic compounds. XVII. Synthesis of oligonucleotides containing 6-azauridine and 6-azacytidine" COLLECT. CZECH. CHEM. COMMUN. (1967), 32(8), 2980-97 , XP002161223 RN 17120-68-2 and 17120-69-3 page 2981 schemes 2 and 3 compounds in page 2986	13,28,37
A	--- US 3 891 623 A (VORBRUGGEN HELMUT ET AL) 24 June 1975 (1975-06-24) RN 31698-10-9 example 10	13
X	--- BOBEK, MILAN ET AL: "Nucleic acid components and their analogs. XCVII. Synthesis of 5-hydroxymethyl-6-aza-2'-deoxyuridine and 5-hydroxymethyl-6-aza-2'- deoxycytidine" COLLECT. CZECH. CHEM. COMMUN. (1967), 32(10), 3581-6, XP002161224 page 3581, page 3582. page 3582, compounds I (RN 20258-34-8) and II (RN 20317-01-5) --- -/--	17,21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 594 339 A (LOPEZ CARLOS ET AL) 10 June 1986 (1986-06-10) claim 1 claim 2, column 19 lines 47-48, column 20 lines 1-2 and 13-14 ---	17
A	PRYSTAS, MIROSLAV ET AL: "Nucleic acids components and their analogs. CXXI. Glycosylation of 6-azathymine by the silylation process" COLLECT. CZECH. CHEM. COMMUN. (1969), 34(3), 1104-7 , XP002161225 page 1105, compound VII (RN 23701-72-6) ---	17
X	BRODY, RICHARD S. ET AL: "The purification of orotidine-5'-phosphate decarboxylase from yeast by affinity chromatography" J. BIOL. CHEM. (1979), 254(10), 4238-44 , XP002161226 page 4238 page 4239, scheme 1 ---	21
X	MITCHELL, WILLIAM L. ET AL: "Synthesis and antiviral properties of 5-(2-substituted vinyl)-6-aza-2'-deoxyuridines" J. MED. CHEM. (1986), 29(5), 809-16 , XP002161227 page 809 -page 810, paragraph 1 compounds disclosed in pages 810 and 811 ---	21
X	WO 97 39120 A (PEYMAN ANUSCH ;UHLMANN EUGEN (DE); COSSUM PAUL A (US); RANDO ROBER) 23 October 1997 (1997-10-23) figures 1,2 ---	21
X	BASNAK, I. ET AL: "Some 6-aza-5-substituted-2'-deoxyuridines show potent and selective inhibition of herpes simplex virus type 1 thymidine kinase" NUCLEOSIDES NUCLEOTIDES (1998), 17(1-3), 187-206, XP002161228 scheme 1 ---	21
X	DEPELLEY, JEAN ET AL: "New non-aromatic triazinic nucleosides: synthesis and antiretroviral evaluation of beta.-ribosylamine nucleoside analogs" NUCLEOSIDES NUCLEOTIDES (1996), 15(5), 995-1008 , XP002161229 scheme 3 ---	21
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NISHIDA, MASAKAZU ET AL: "Facile perfluoroalkylation of uracils and uridines at the C-5 position" J. FLUORINE CHEM. (1993), 63(1-2), 43-52, XP002161230 page 46, compounds 10a-c and 11a-c ---	21
X	US 3 352 849 A (SHEN T.-Y. ET AL.) 14 November 1967 (1967-11-14) column 1 -column 2, paragraph 1 claim 1 examples 8,9 ---	21
A	WO 92 02258 A (ISIS PHARMACEUTICALS INC) 20 February 1992 (1992-02-20) page 1, line 4 - line 12 examples 1,2 ---	21
A	LANGER P R ET AL: "ENZYMATIC SYNTHESIS OF BIOTIN-LABELED POLYNUCLEOTIDES: NOVEL NUCLEIC ACID AFFINITY PROBES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA,US,NEW YORK, NY, vol. 78, no. 11, November 1981 (1981-11), pages 6633-6637, XP000904705 cited in the application page 6633 -----	1-60

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4, 25, 31, 32, 43, 49, 50

Nucleic acid labeling compounds of formula I (found in claim 1), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

2. Claims: 5-8, 26, 33, 34, 44, 51, 52

Nucleic acid labeling compounds of formula II (found in claim 5), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

3. Claims: 9-12, 27, 35, 36, 45, 53, 54

Nucleic acid labeling compounds of formula III (found in claim 9), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

4. Claims: 13-16, 28, 37, 38, 46, 55, 56

Nucleic acid labeling compounds of formula IV (found in claim 13), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

5. Claims: 17-20, 29, 39, 40, 47, 57, 58

Nucleic acid labeling compounds of formula V (found in claim 17), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

6. Claims: 21-24, 30, 41, 42, 48, 59, 60

Nucleic acid labeling compounds of formula VI (found in claim 21), nucleic acid derivatives, hybridization products, methods of synthesis and detection.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9728176 A	07-08-1997	AU 712310 B AU 1610397 A CA 2243679 A EP 0885235 A GB 2309969 A,B JP 2000504009 T	04-11-1999 22-08-1997 07-08-1997 23-12-1998 13-08-1997 04-04-2000
WO 9727317 A	31-07-1997	AU 2253397 A EP 0880598 A	20-08-1997 02-12-1998
US 3891623 A	24-06-1975	DE 2122991 A BE 783026 A CH 579585 A CS 171723 B FR 2135249 A GB 1395764 A NL 7206058 A	16-11-1972 06-11-1972 15-09-1976 29-10-1976 15-12-1972 29-05-1975 07-11-1972
US 4594339 A	10-06-1986	NONE	
WO 9739120 A	23-10-1997	AU 2733697 A BR 9708701 A CA 2251945 A EP 0910634 A JP 2000509259 T	07-11-1997 04-01-2000 23-10-1997 28-04-1999 25-07-2000
US 3352849 A	14-11-1967	CH 481135 A DE 1620048 A FR 1496355 A GB 1118269 A NL 6614804 A	15-11-1969 19-03-1970 22-12-1967 25-04-1967
WO 9202258 A	20-02-1992	AT 154246 T AU 641565 B AU 8720591 A BR 9106702 A CA 2088258 A DE 69126530 D DE 69126530 T EP 0544824 A JP 8000074 B JP 6501389 T US 5614617 A	15-06-1997 23-09-1993 02-03-1992 08-06-1993 28-01-1992 17-07-1997 05-02-1998 09-06-1993 10-01-1996 17-02-1994 25-03-1997