



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C07H 19/06, 19/16, 21/02, A61K 31/70, C12N 9/00</p>	A3	<p>(11) International Publication Number: WO 97/26270 (43) International Publication Date: 24 July 1997 (24.07.97)</p>
<p>(21) International Application Number: PCT/US96/20527 (22) International Filing Date: 23 December 1996 (23.12.96)</p> <p>(30) Priority Data: 08/585,682 16 January 1996 (16.01.96) US 08/600,429 13 February 1996 (13.02.96) US 08/632,882 16 April 1996 (16.04.96) US</p> <p>(71) Applicant: RIBOZYME PHARMACEUTICALS, INC. [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).</p> <p>(72) Inventors: WINCOTT, Francine; 7920 N. 95th Street, Boulder, CO 80501 (US). USMAN, Nassim; 2954 Kalmia #37, Boulder, CO 80304 (US). BEIGELMAN, Leonid; 5530 Colt Drive, Longmont, CO 80503 (US). HAE-BERLI, Peter; 3260 47th Street #A108, Boulder, CO 80301 (US). MATULIC-ADAMIC, Jasenka; 760 South 42nd Street, Boulder, CO 80303 (US). KARPEISKY, Alexander; 5121 Williams Fork Trail #209, Boulder, CO 80301 (US). SWEDLER, David; 956 St. Andrews Lane, Louisville, CO 80027 (US). JARVIS, Thale; 3720 Smuggler Place, Boulder, CO 80301 (US). DIRENZO, Anthony; 1197 Ravenwood Road, Boulder, CO 80303 (US).</p>	<p>(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon L.L.P., First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p>(88) Date of publication of the international search report: 20 November 1997 (20.11.97)</p>	
<p>(54) Title: SYNTHESIS OF METHOXY NUCLEOSIDES AND ENZYMATIC NUCLEIC ACID MOLECULES</p>		
<p>(57) Abstract</p>		
<p>This invention relates to chemical synthesis of 2'-O-methyl, 3'-O-methyl and 5'-O-methyl nucleosides, incorporation of novel chemical modifications in enzymatic nucleic acid molecules and improved methods for the synthesis of enzymatic nucleic acid molecules.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LR	Liberia	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/20527

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07H19/06 C07H19/16 C07H21/02 A61K31/70 C12N9/00

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 6 C07H

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEOSIDES & NUCLEOTIDES., vol. 14, no. 3-5, 1995, INC US, pages 241-425, XP002033737 BEIGELMAN L. ET AL: "Alternative routes to the synthesis of 2'-O-Me nucleosides" see the whole document ---	1,4-10, 13
A	BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, vol. 51, no. 6, 1978, TOKYO JP, pages 1903-1904, XP002033738 KIMURA J AND MITSUNOBU O: "Studies on Nucleosides and Nucleotides. VI. A Facile Cleavage of the Anhydro Bond of 2',3'-O-Isopropylidene-02,5'-cyclouridine with the Use of Proton Acid or Lewis Acid" see the whole document --- -/--	3-5,12

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
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

4

Date of the actual completion of the international search 26 June 1997	Date of mailing of the international search report 06-10-1997
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer DAY, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/20527

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 95, no. 4, 31 May 1995 & JP 07 017994 A (TOKYO YATSUKA UNIV), 20 January 1995, see abstract ---	1-13
A	WO 95 35102 A (NEXSTAR PHARMACEUTICALS) 28 December 1995 see claims 35-40 ---	1,4-10, 13
A	JOURNAL OF ORGANIC CHEMISTRY, vol. 39, no. 13, 1974, EASTON US, pages 1891-1899, XP002033777 ROBINS M.J. ET AL: "Nucleic Acid Related Compounds. 12. The Facile and High-Yield Stannous Chloride Catalyzed Monomethylation of the Cis-Glycol System of Nucleosides by Diazomethane" see the whole document ---	1,2, 4-11,13
A	BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, vol. 59, no. 9, 1 September 1986, pages 2947-2949, XP000571563 YAMAUCHI K ET AL: "SELECTIVE 2'-O-METHYLATION OF PYRIMIDINE-RIBONUCLEOSIDES BY TRIMETHYLSULFONIUM HYDROXIDE IN THE PRESENCE OF MG ²⁺ AND CA ²⁺ IONS" see the whole document -----	1,2, 4-11,13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 20527

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 ANNEX

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.:

1-13

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/ISA210

1. Claims 1-13

Process for the synthesis of 2',3' or 5'-O-methyl nucleosides from 2,2',2,3' or 2,5'-anhydro-1-(beta-D-arabinofuranosyl) bases respectively, using Lewis acid and methanol as the methylating agent

2. Claim 14

Process for the synthesis of 2'-O-methyl adenosine from N⁴-acetyl-5',3'-di-O-acetyl-2'-O-methyl cytidine

3. Claims 15-18,24 and 25

Process for the synthesis of 2'-O-methyl nucleosides from, using sodium hydride, DMF, and methyl iodide as the methylating agent

4. Claims 19-23

Process for the synthesis of 2'-O-methyl nucleosides from, using metal acetylacetonate, DMF, and trimethylsulphonium hydroxide as the methylating agent

5. Claims 26-40

Method for the synthesis of enzymatic nucleic acids

6. Claims 41-90

5' and / or 3'- capped enzymatic nucleic acid molecules, method of production of the same and cell comprising the same

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/20527

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9535102 A	28-12-95	AU 2649695 A	15-01-96
		CA 2192950 A	28-12-95
		EP 0767657 A	16-04-97
