PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **A61K 47/48, C12N 9/64, C07K 5/06, 5/02 C07D 405/00, 403/12, 239/00**	**A3**	**(11) International Publication Number:** **WO 95/13095** **(43) International Publication Date:** 18 May 1995 (18.05.95)

(21) International Application Number: PCT/GB94/02483

(22) International Filing Date: 11 November 1994 (11.11.94)

(30) Priority Data:
9323429.2 12 November 1993 (12.11.93) GB

(71) Applicant *(for all designated States except US)*: THE WELLCOME FOUNDATION LIMITED [GB/GB]; Unicorn House, 160 Euston Road, London NW1 2BP (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SMITH, Gary, Keith [US/US]; 521 Mills Street, Raleigh, NC 27608 (US). BLUMENKOPF, Todd, Andrew [US/US]; 201 Hunter Hill Road, Chapel Hill, NC 27516 (US). CORY, Michael [US/US]; 55 Cedar Street, Chapel Hill, NC 27514 (US).

(74) Agent: STOTT, Michael, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
13 July 1995 (13.07.95)

(54) Title: THERAPY

(57) Abstract

The present invention relates to improvements in targetted enzyme prodrug therapy including antibody-directed enzyme prodrug therapy (ADEPT), it particularly relates to novel enzymes and prodrugs for use in ADEPT.

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.

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

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 94/02483

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48 C12N9/64 C07K5/06 C07K5/02 C07D405/00
C07D403/12 C07D239/00

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 A61K C12N

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.
Y	EP,A,0 423 818 (BRISTOL-MYERS SQUIBB COMPANY) 24 April 1991 see page 7, line 30 - line 31; claims ---	1-3, 12-15, 25-29
X,Y	WO,A,90 07929 (AKZO) 26 July 1990 cited in the application see page 4, paragraph 3; claims 1-11 ---	1-3, 12-15, 25-29
A	PROTEIN ENGINEERING, vol.6, no.4, 1993, ENGLAND GB pages 409 - 415 OLESEN K. ET AL. 'ALTERING SUBSTRATE PREFERENCE OF CARBOXYPEPTIDASE Y BY A NOVEL STRATEGY OF MUTAGENESIS ELIMINATING WILD TYPE BACKGROUND.' see page 409; table 1 --- -/--	1-11, 16-19, 27,29-38

[X] Further documents are listed in the continuation of box C.

[X] 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

7

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 1995	21. 03. 95
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** BERTE, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOCHEMISTRY, vol.31, 1992, EASTON, PA US pages 959 - 963 MARGARET A. PHILLIPS ET AL. 'TRANSITION STATE CHARACTERIZATION: A NEW APPROACH COMBINING INHIBITOR ANANLOGUES AND VARIATION IN ENZYME STRUCTURE.' see abstract	1-19, 29-38
A	WO,A,93 13806 (BAGSHAWE , KENNETH, DAWSON) 22 July 1993 see page 10, line 11 - line 22 see page 19, line 5 - page 22, line 4 see page 24, line 10 - line 17; claims 1,12-17; examples 1,3,5	1-19, 25-38
A	WO,A,89 10140 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 2 November 1989 cited in the application see page 7, line 14 - page 8, line 3 see page 13, line 8 - line 15; examples see page 4, line 11 - line 16; claims 1,17	1-19, 25-38
A	WO,A,88 07378 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 6 October 1988 cited in the application see page 6 see page 7, line 7 - line 9 see page 9, line 1 - line 8; claims	1-19, 25-38
A	EP,A,0 382 411 (ELI LILLY AND COMPANY) 16 August 1990 see page 5, line 41 - line 54; claims 1,2	1-19, 25-38
E	EP,A,0 633 029 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 11 January 1995 see page 3, line 9 - line 16; claims see page 5, line 1 - line 16	1-19, 25-38
A	BIOCHEMISTRY, vol.28, 1989, EASTON, PA US pages 2288 - 2297 ULRIKE KUEFNER ET AL. 'CARBOXYPEPTIDASE-MEDIATED RELEASE OF METHOTREXATE FROM METHOTREXATE ALPHA-PEPTIDES.' cited in the application see page 2296, column 1 see page 2293, column 2	1-7

7

INTERNATIONAL SEARCH REPORT

Inter ɔnal application No.
PCT/GB94/02483

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:
PLEASE SEE ATTACHED SHEET!

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:

PLEASE SEE ATTACHED SHEETS!

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 searches 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.:

1-19,27-38;25-26 partially

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

LACK OF UNITY OF INVENTION

1. Claims: 1-19,27-38, and partially 25-26
a mutant mammalian enzyme; a conjugate; a DNA or RNA molecule encoding such a conjugate or encoding a mutant enzyme; a vector containing such DNA or RNA; a cell line containing the DNA or RNA or vector; a method of targetted chemotherapy using such a conjugate; the use of a prodrug in relation to such a conjugate

2. Claims: 20-24 and partially 25-26
(as far as not comprised in claims 1-19 and 27-38): a compound of general formula II, representing prodrugs; the use of a compound of formula II or a conjugate thereof in medical therapy.

Rule 13.1 PCT deals with the requirement of unity of invention and states the principle that an international application should relate to only one invention or, if there is more than one invention, that the inclusion of those inventions in one international application is only permitted if all inventions are so linked as to form a single general inventive concept.
Rule 13.2 PCT defines the method for determining whether the requirement of unity of invention is satisfied in respect of a group of inventions claimed in an international application.
Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same of corresponding "special technical features."
The expression "special technical features" is defined in Rule 13.2 as meaning those technical features that define a contribution which each of the inventions, considered as a whole, makes over the prior art.

The problem underlying the present application is to provide a prodrug which is stable in vivo and yet activated by a non-immunogenic enzyme (see description, page 3, lines 6-7).
The solution proposed in the present application is to use a mutant mammalian enzyme conjugated to a targetting molecule, capable to catalyse the transformation of the prodrug to its active form, whereas the prodrug is refractory to endogenous catalysis by the wild form of the enzyme.

The claims of the present application relate to:

a. a mutant mammalian enzyme (claims 29-35); a conjugate, according to claims 1-16; a DNA or RNA molecule encoding such a conjugate (claim 17) or encoding a mutant enzyme (claim 36); a vector containing such DNA or RNA (claims 18, 37); a cell line containing the DNA or RNA or vector

International Application No. PCT/GB94/02483

(claim 19,38); a method of targetted chemotherapy using such a conjugate (claim 27); the use of a prodrug in relation to such a conjugate (claim 28); the use of such a conjugate, cell line, vector or DNA or RNA in medical therapy (claims 25-26, partially);

b. a compound of general formula II, representing prodrugs (claims 20-24); the use of a compound of formula II or a conjugate thereof in medical therapy (claims 25-26, partially).

The special technical feature linking the subject matters comprised under a. is the mutant mammalian enzyme.
There is no limitation as to the nature of the conjugate in claims 25-26.
Accordingly, the compounds of formula II may be used in conjunction with any conjugate.
Moreover, the compounds of formula II may be used independently from a conjugate.
Consequently, the prodrugs of formula II cannot be considered as specifically adapted for use in conjunction with the mutant mammalian enzyme.
The subject matter of claims 1-16 (and the other claims referred to under a.), is not restricted to conjugates comprising a prodrug of formula II, but encompasses conjugates of a mutant enzyme and any prodrug.

The special technical feature common to the subject matters comprised under b. is to be seen in the structural features of the (specific) prodrugs of formula II.
These prodrugs may optionally be used as a conjugate thereof (not restricted in any respect), the conjugate only optionally being a conjugate according to claims 1-16.

Accordingly, there is no single general inventive concept linking the prodrugs of formula II, characterised by the structural features of the compounds as a special technical feature, and the subject matters comprised under a., characterised by the special technical feature of the mutant mammalian enzyme.

In the present application no further technical feature(s) can be distinguished that can be regarded as a "special technical feature" involved in the technical relationship among the different inventions.
Consequently, the present application lacks unity of invention, and the different solutions not belonging to a common inventive concept are identified as the different subjects listed in the communication pursuant to Article 17(3)(a) PCT.
Each of the inventions listed is a distinct invention, characterised by its own special technical feature, defining the contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

MEANINGFUL SEARCH NOT POSSIBLE OR INCOMPLETE SEARCH

Incomplete search

In view of the definition of products by means of their biological, chemical and or pharmacological properties, the search has to be restricted for economic reasons.

The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims or examples.
(see guidelines, Part B, Chapter III, paragraph 3.6)

Partially searched claims: 1-5,12-19,27-38

International Application No. PCT/GB94/02483

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Searching these different subjects would have caused major additional searching efforts.
Only the first subject was searched.
The attention of the Applicant is drawn to the possibility that further objections as to lack of unity of invention may be raised for the second subject, should that subject matter show a posteriori lack of unity of invention.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. nal Application No
PCT/GB 94/02483

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0423818	24-04-91	AU-B-	638283	24-06-93
		AU-A-	6470890	26-04-91
		JP-A-	3155781	03-07-91
		US-A-	5270196	14-12-93
WO-A-9007929	26-07-90	AU-B-	648015	14-04-94
		AU-A-	5039790	13-08-90
		CA-A-	2025899	24-07-90
		EP-A-	0454783	06-11-91
		JP-T-	4503068	04-06-92
WO-A-9313806	22-07-93	CA-A-	2124217	22-07-93
		EP-A-	0620742	26-10-94
		GB-A-	2276624	05-10-94
WO-A-8910140	02-11-89	AT-T-	119782	15-04-95
		DE-D-	68921749	20-04-95
		EP-A-	0414741	06-03-91
		EP-A-	0633029	11-01-95
		JP-T-	3503898	29-08-91
WO-A-8807378	06-10-88	DE-D-	3889340	01-06-94
		DE-T-	3889340	01-09-94
		EP-A-	0408546	23-01-91
EP-A-0382411	16-08-90	AT-T-	114974	15-12-94
		AU-A-	3026192	25-02-93
		AU-B-	633867	11-02-93
		AU-A-	4894890	09-08-90
		CA-A-	2009071	02-08-90
		DE-D-	69014666	19-01-95
		DE-T-	69014666	04-05-95
		ES-T-	2064613	01-02-95
		JP-A-	2247164	02-10-90
EP-A-0633029	11-01-95	AT-T-	119782	15-04-95
		DE-D-	68921749	20-04-95
		EP-A-	0414741	06-03-91
		WO-A-	8910140	02-11-89
		JP-T-	3503898	29-08-91