



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 47/48	A1	(11) International Publication Number: WO 98/32466 (43) International Publication Date: 30 July 1998 (30.07.98)
(21) International Application Number: PCT/GB98/00253 (22) International Filing Date: 28 January 1998 (28.01.98) (30) Priority Data: 9701800.6 29 January 1997 (29.01.97) GB 9701804.8 29 January 1997 (29.01.97) GB 9704653.6 6 March 1997 (06.03.97) GB 9708055.0 22 April 1997 (22.04.97) GB (71) Applicant (for all designated States except US): POLYMASC PHARMACEUTICALS PLC [GB/GB]; Fleet Road, London NW3 2EZ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): FRANCIS, Gillian, Eliz- abeth [GB/GB]; Summer Cottage, Cane End, Reading, Berkshire RG4 9GH (GB). FISHER, Derek [GB/GB]; 34 Corinium Gate, St. Albans, Hertfordshire AL3 4HY (GB). MALIK, Farooq [GB/GB]; 67 Gracefield Gardens, Streatham, London SW16 2TS (GB). (74) Agent: NACHSHEN, Neil, Jacob; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, 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). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: PEGYLATION PROCESS (57) Abstract The present invention relates to the attachment of a polyethylene glycol (PEG) moiety to a target substrate. Processes for such attachment will be hereinafter referred to as "PEGylation" of the substrate. In particular, the present invention relates to a process for direct covalent PEGylation of a substrate, comprising the reaction of a halogenated PEG with the substrate wherein the halogen of the halogenated PEG acts as a leaving group in the PEGylation reaction.		

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.

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

PEGYLATION PROCESS

5

The present invention relates to the attachment of a polyethylene glycol (PEG) moiety to a target substrate. Processes for such attachment will be hereinafter referred to as "PEGylation" of the substrate. In particular, the present invention relates to a process for direct covalent PEGylation of a substrate, comprising the reaction of a halogenated PEG with the substrate wherein the halogen of the halogenated PEG acts as a leaving group in the PEGylation reaction.

15

Covalent attachment of PEG to molecules such as proteins or structures such as liposomes is well known to improve their pharmacological and physiological properties.

20

EP-A-354855 describes a liposome which comprises a PEG-bound phospholipid wherein the PEG moiety is bonded to a phospholipid present in the liposome membrane. This is claimed to provide a reduction in the absorption of proteins to the liposome *in vivo* and hence an increase in its *in vivo* stability.

25

EP-A-154316 describes a method for chemically modifying lymphokines by attachment of a PEG moiety wherein the PEG is bonded to at least one primary amino group of the lymphokine. This is claimed to result in the delayed clearance of lymphokines when used as drugs and to decrease their antigenicity.

There are many methods for achieving covalent coupling of PEG to substrates. All such methods require the activation of the PEG by attachment of a group usually referred to as an "activating moiety" or by converting a terminal moiety of the PEG into an activating moiety. This is followed by a second step where the PEG couples to the target molecule, usually via a residual portion of the activating moiety which may be referred to as the "coupling moiety".

Examples of known techniques include:

10

Succinimidyl Active Ester Methods: see e.g. US Patent 4,412,989; WO 86/04145; WO 87/00056; EP-A-0 247 860, C. Monfardini, O. Shiavon, P. Caliceti, M. Morpurgo, J. M. Harris, and F. M. Veronese, "A branched monomethoxypoly(ethylene glycol) for protein modification," Bioconjugate Chem., 6,62-69 (1995), Zalipsky, S. et al. (1991) in "Polymeric Drugs and Drug Delivery Systems" (R. L. Dunn & R. M. Ottenbrite, eds.) ACS, Washington, DC, Chapter 10, Zalipsky, S. et al. (1992) Biotechnol. Appl. Biochem. 15:100, Chiu, H.-C. et al. (1993) Bioconjugate Chem. 4:290, Sirokman, G. & Fasman, G. (1993) Protein Sci. 2:1161, Veronese, F. M. et al (1989) J. Controlled Release 10:145, Abuchowski, A. et al (1984) Cancer Biochem. Biophys. 7:175, Joppich, M. & Luisi, P.L. (1979) Macromol. Chem. 180:1381, Klibanov, A. L. et al (1990) FEBS Letters 268:235, Sartore, L. et al (1991) Appl. Biochem. Biotech. 31:213

20

25

Carbonyldiimidazole Method: see e.g. EP-A-0 154 432.

Phenylchloroformate Methods: see e.g. WO 89/06546 and WO 90/15628.

5 **PEG-Succinate Mixed Anhydride Methods:** see e.g. Ahlstedt et al (1983) Int. Arch. Allergy Appl. Immunol., 71,228-232; Richter and Akerblom (1983) Int. Arch. Allergy Appl. Immunol, 70, 124-131;

Organic Sulphonyl Halide Methods: see e.g. US Patent 4,415,665.

10 **PEG-Maleimide and Related Methods:** see e.g. Goodson & Katre (1990) Biotechnology, 8, 343-346.

Phenylglyoxal Method: see e.g. EP-A-0 340 741

15 **Succinimide Carbonate Method:** see e.g. WO 90/13540; WO 91/07190

Cyanogen Bromide Method: see USP 4,301,144

20 **Poly-PEG Maleic Acid Anhydride Method:** Yoshimoto et al (1987) Biochem. and Biophys. Res. Commun. 148, 876-882.

25 **Cyanuric chloride method:** Abuchowski, A. van Es, T., Palczuk, N.C., & David, F.F. (1977). Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J. Biol. Chem., 252, 3578-3581.

PEG acetaldehyde methods: Royer, G.P. US 4,002,531 EP-A-0154316. Harris, J. M., Yoshinaga, K. Paley, M.S., & Herati, M. R.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.