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(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.

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PEGYLATION PROCESS

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.

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

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.

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:

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

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

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

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.

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

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

Cyanogen Bromide Method: see USP 4,301,144

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

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.

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