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**SOLID PHASE METHOD FOR SYNTHESIS
PEPTIDE-SPACER-LIPID CONJUGATES,
CONJUGATES SYNTHESIZED THEREBY
AND TARGETED LIPOSOMES CONTAINING
THE SAME**

This application is a continuation-in-part application Ser. No. 10/016,569 filed on Dec. 7, 2001, now abandoned.

FIELD OF THE INVENTION

The present invention is related to solid phase synthesis method for preparing peptide-spacer-lipid conjugates and uses of the conjugates.

BACKGROUND OF THE INVENTION

Drug delivery plays a crucial role in the improvement of agents for therapeutic treatment, since many agents have unfavorable drawbacks if they are directly applied to a human body. Therefore, developing a delivery system is necessary for a particular agent to improve its availability such as reduction of side effects, enhancement of efficacy, and convenience in usage. For example, antineoplastic chemotherapies are limited by adverse side effects resulting from their widespread toxicity to normal tissues. Therefore, a delivery system which could prevent drug diffusion and concentrate the drug to the disease site is required.

Liposomes can provide several advantages for use as a drug delivery system for the reasons that they are safe to a biological system, have an excellent spherical bilayer for carrying ether hydrophilic or hydrophobic drugs, and can prevent drugs from degradation and diffusion. Moreover, liposomes can be modified to have additional functions for specific purposes. A successful example is shown as polyethylene-glycerol-grafted (PEG-grafted) liposomes. These modified liposomes can evade the reticuloendothelial system and have prolonged circulation time in blood. Furthermore, cytotoxic cancer drugs encapsulated in the PEG-grafted liposomes provide a remarkable enhancement in anti-tumor activity effect and decrease the side effect of the toxicity to the normal cells. The PEG-grafted liposomes thereby gained commercial application and opened the possibility for further modification of these PEG-grafted liposomes for targeted delivery.

Several types of targeted liposomes have been developed (Maruyama et al., *Biochim Biophys Acta.* 1995, 1234, 74–80; and Allen T M, *Trends Pharmacol Sci.* 1994, 15, 215–220). Commonly used targeted liposomes include (1) targeting ligands linked at the lipid headgroups on the conventional liposomes (Type A); (2) targeting ligands linked at the lipid headgroups on the PEG-grafted liposomes (Type B); and (3) targeting ligands attached at the distal end of the PEG chain on the PEG-grafted liposomes (Type C). To date, studies have shown that targeted liposomes of Type C provide a better liposomal structure for targeted delivery (Maruyama et al., *Biochim Biophys Acta.* 1995, 1234, 74–80). Based on this liposomal structure, several types of molecules, such as antibodies (Ahmad et al., *Cancer Res.* 1993, 53, 1484–8; and Suzuki et al., *Biochim Biophys Acta.*, 1995, 1245, 9–16), proteins (Eavarone et al., *J. Biomed Mater Res.* 2000, 51, 10–4) small synthesis molecules (Gabizon et al., *Bioconjug Chem.* 1999, 10, 289–98) and peptides (Zalipsky et al., *Bioconjug Chem.* 1997, 8, 111–8), have been developed as the targeting ligands for binding the target sites. Among these types of molecules, peptides are considered as highly potential targeting ligands, since a

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peptide can serve as a recognition component in protein-protein interactions such as receptor-ligand interactions. Furthermore, many cellular membrane receptors associated with diseases have been studied.

Peptides, such as RGD-peptides, somatostatin, chemotactic peptides, vasoactive intestinal peptide, and mimetics thereof, are good candidates as the targeting ligands. Many counter receptors of these peptides have been found being overexpressed in various tumor cells. Moreover, peptides and peptide mimetics have several unique advantages over other type of molecules (e.g. antibodies). Generally, these peptides bind to target cells with a ligand-receptor association at high affinity and enter the intercellular compartments through receptor-mediate endocytosis. However, an antibody-based targeted liposome may not utilize the endocytosis pathway into the interior of the cells by the antigen on the cell membrane. Furthermore, peptides have less opportunity to be recognized by the reticuloendothelial system and are, thus, cleared from the blood circulation system. Peptide mimetics can provide a higher binding affinity and a better resistance to the proteases degradation than nature peptides.

Currently, two approaches for preparing peptide-based targeted liposomes have been developed, whereby the peptide ligands can be attached at the distal end of PEGs. The first approach is incorporating end-group functionalized PEG-lipid conjugates into liposomes and then conjugating with peptide ligands (Zalipsky et al., *Bioconjug. Chem.*, 1995, 6, 705–8). However, when the end-group functionalized PEGs are conjugated to peptide ligands, a non-homogeneous conjugation may happen if there is more than one reaction group in the peptide ligands. Furthermore, the unreacted end-groups of functionalized PEG are difficult to define and are completely deactivate after the coupling reaction. The second approach is directly incorporating the peptide-PEG-lipid conjugates into liposomal membranes (Zalipsky et al., *Bioconjug. Chem.*, 1997 8, 111–8). This approach can provide a structurally well-defined targeted liposome component.

Although peptide-PEG-lipid conjugates are the expected molecules for preparing the targeted liposomes, the available conjugates are still very limited and the synthesis is difficult. This is so, because, in the peptide-PEG-lipid conjugates, the chemical property of the side chains in peptides is diverse, the molecular mass of PEG is heterogeneous, and the nature of lipids is amphiphilic. These properties cause difficulty in the synthetic processes of side chain protection, purification, and reaction and is evident in that very few peptide-PEG-lipid conjugates have been synthesized and in that the conjugation of a peptide, a spacer, and a lipid often induce the formation of a clumsy linker and an unusual functional group.

Zalipaky et al. (1997) discloses the method for synthesizing YIGSR-PEG-lipid conjugates (SEQ ID NO: 1 Tyr-Ile-Gly-Ser-Ary). However, this method cannot be used as a general method for synthesizing a broad range of peptide-PEG-lipid conjugates as a nonspecific reaction of bromoacetyl group with strong nucleophilic residues, such as an amino group or other thiol group, in the peptide may occur. Furthermore, in the YIGSR-PEG-lipid conjugate, a thioacetyl (—S—CH₂—CO—) linker was used for conjugating to the peptide and PEG, which is unfavorable in industrialization since an additional modification at the ends of peptide and PEG to a bromoacetyl group and a thiol group, respectively, is required. The urethane linkage between PEG and lipid in the YIGSR-PEG-lipid conjugate is unnatural and