

**MIXTURES OF DRUG-OLIGOMER
CONJUGATES COMPRISING
POLYALKYLENE GLYCOL, USES THEREOF,
AND METHODS OF MAKING SAME**

FIELD OF THE INVENTION

The present invention relates to drug-oligomer conjugates.

BACKGROUND OF THE INVENTION

Pharmaceutically active molecules such as proteins and polypeptides have been conjugated with polydispersed mixtures of polyethylene glycol or polydispersed mixtures of polyethylene glycol containing polymers to provide polydispersed mixtures of drug-oligomer conjugates. For example, U.S. Pat. No. 4,179,337 to Davis et al. proposes conjugating polypeptides such as insulin with various polyethylene glycols such as MPEG-1900 and MPEG-5000 supplied by Union Carbide.

U.S. Pat. No. 5,567,422 to Greenwald proposes the conjugation of biologically active nucleophiles with polyethylene glycols such as m-PEG-OH (Union Carbide), which has a number average molecular weight of 5,000 Daltons.

U.S. Pat. No. 5,405,877 to Greenwald et al. proposes reacting bovine hemoglobin with thiazolidine thione activated PEG, which was prepared using m-PEG carboxylic acid having a number average molecular weight of 5,000 Daltons.

U.S. Pat. No. 5,359,030 to Ekwuribe proposes conjugating polypeptides such as insulin with polyethylene glycol modified glycolipid polymers and polyethylene glycol modified fatty acid polymers. In this patent, the number average molecular weight of polymer resulting from each combination is preferred to be in the range of from about 500 to about 10,000 Daltons.

PEG is typically produced by base-catalyzed ring-opening polymerization of ethylene oxide. The reaction is initiated by adding ethylene oxide to ethylene glycol, with potassium hydroxide as catalyst. This process results in a polydispersed mixture of polyethylene glycol polymers having a number average molecular weight within a given range of molecular weights. For example, PEG products offered by Sigma-Aldrich of Milwaukee, Wis. are provided in polydispersed mixtures such as PEG 400 (M_n , 380–420); PEG 1,000 (M_n , 950–1,050); PEG 1,500 (M_n , 1,400–1,600); and PEG 2,000 (M_n , 1,900–2,200).

It is desirable to provide non-polydispersed mixtures of drug-oligomer conjugates that comprises polyalkylene glycol.

SUMMARY OF THE INVENTION

A mixture of drug-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may exhibit higher in vivo activity than a polydispersed mixture of similar conjugates, where the polydispersed mixture has the same number average molecular weight as the mixture according to the present invention. This heightened activity may result in lower dosage requirements. Moreover, a mixture of drug-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may be more effective at surviving an in vitro model of intestinal digestion than polydispersed mixtures of similar conjugates. Furthermore, a mixture of drug-oligomer conjugates comprising polyalkylene glycol

according to embodiments of the present invention may result in less inter-subject variability than polydispersed mixtures of similar conjugates.

According to embodiments of the present invention, a substantially monodispersed mixture of conjugates each comprising a drug coupled to an oligomer that comprises a polyalkylene glycol moiety is provided. The mixture preferably is a monodispersed mixture and, more preferably, is a purely monodispersed mixture. The polyalkylene glycol moiety preferably has at least 2, 3 or 4 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety preferably has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety is preferably a polyethylene glycol moiety or polypropylene glycol moiety. The oligomer preferably further comprises a lipophilic moiety. The conjugate is preferably amphiphilically balanced such that the conjugate is aqueously soluble and able to penetrate biological membranes. The oligomer may comprise a first polyalkylene glycol moiety covalently coupled to the drug by a non-hydrolyzable bond and a second polyalkylene glycol moiety covalently coupled to the first polyalkylene glycol moiety by a hydrolyzable bond.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an in vivo activity that is greater than the in vivo activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to still other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an in vitro activity that is greater than the in vitro activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to yet other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to still other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a molecular weight distribution with a standard deviation of less than about 22 Daltons.

According to yet other embodiments of the present invention, a mixture of conjugates is provided where each