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- [54] **GLYCOSAMINOGLYCAN-SYNTHETIC POLYMER CONJUGATES**
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Related U.S. Application Data

- [63] Continuation-in-part of Ser. No. 907,518, Jul. 2, 1992, Pat. No. 5,324,775, which is a continuation-in-part of Ser. No. 433,441, Nov. 14, 1989, Pat. No. 5,162,430, which is a continuation-in-part of Ser. No. 274,071, Nov. 21, 1988, abandoned.
- [51] **Int. Cl.**⁶ **C08G 63/91**
- [52] **U.S. Cl.** **525/54.2; 525/54.21; 525/54.22; 525/54.23; 525/54.24**
- [58] **Field of Search** **525/54.2, 54.21, 525/54.22, 54.23, 54.24**

References Cited**U.S. PATENT DOCUMENTS**

3,619,371 11/1971 Crook et al. .
3,788,948 1/1974 Kagedal et al. .

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

098110 1/1984 European Pat. Off. .
200574 11/1986 European Pat. Off. .
247860 12/1987 European Pat. Off. .
466300 1/1992 European Pat. Off. .
4-227265 8/1992 Japan .
WO84/01106 3/1984 WIPO .
WO87/04078 7/1987 WIPO .

OTHER PUBLICATIONS

- 07/907,518, Rhee et al., Jul. 2, 1992.
- Abuchowski et al., "Cancer Therapy with Chemically Modified Enzymes. I. Antitumor Properties of Polyethylene Glycol-Asparaginase Conjugates," *Cancer Biochem Biophys* (1984) 7:175-86.
- Balazs, E. A. et al., "Matrix Engineering," *Blood Coagulation and Fibrinolysis* (1991) 2:173-178.
- Balazs, E. A. et al., "Clinical Uses of Hyaluronan," *The Biology of Hyaluronan* (1989) 265-285.
- Bendich, A. et al., "Immunological Effects of Native and Polyethylene Glycol-Modified Asparaginases from *Vibrio Succinogenes* and *Escherichia Coli* in Normal and Tumour-Bearing Mice," *Clin Exp Immunol* (1982) 48: 273-78.
- Chen, R. H. L. et al., "Properties of Two Urate Oxidases Modified by the Covalent Attachment of Poly(ethylene Glycol)," *Biochim Biophys Acta* (1981) 660:293-298.
- Chvapil, M. et al., "Some Chemical and Biological Characteristics of a New Collagen-Polymer Compound Material," *J. Biomed Mater Res* (1969) 3:315-32.
- Davis et al., "Hypouricaemic Effect of Polyethyleneglycol Modified Urate Oxidase," *Lancet* (1981) 2:281-83.
- Doillon, C. J., et al., "Fibroblast Growth on a Porous

- Collagen Sponge Containing Hyaluronic Acid and Fibronectin," *Biomaterials* (1987) 8:195-200.
- Inada et al., "Ester Synthesis Catalyzed by Polyethylene Glycol-Modified Lipase in Benzene," *Biochem & Biophys Res Comm* (1984) 122:845-50.
- Lloyd et al., "Coupling of Acrylic Polymer and Collagen by Use of a Water-Soluble Carbodiimide," *J Polymer Sci. Chem Ed.* (1979) 17:3473-3483.
- Nishida et al., "Hypouricaemic Effect after Oral Administration in Chickens of Polyethylene Glycol-Modified Uricase Entrapped in Liposomes," *J. Pharm Pharmacol* (1984) 36:354-355.
- Pyatak, P. S. et al., "Preparation of a Polyethylene Glycol: Superoxide Dismutase Adduct, and an Examination of its Blood Circulating Life and Anti-Inflammatory Activity," *Res Com Chem Path Pharmacol* (1980) 29:113-27.
- Ramshaw, J. A. M. et al., "Precipitation of Collagens by Polyethylen Glycols," *Anal Biochem* (1984) 141:361-65.
- Savoca, K. V. et al., "Preparation of a Non-immunogenic Arginase by the Covalent Attachment of Polyethylene Glycol," *Biochem Biophys Acta* (1979) 578: 47-53.
- Takahashi et al., "A Chemical Modification to Make Horseradish Peroxidase Soluble and Active in Benzene," *Biochem & Biophys Res Chem* (1984) 121:261-65.
- Viau, A. T. et al. "Safety Evaluation of Free Radical Scavengers PEG-Catalase and PEG-Superoxide Dismutase," *J. Free Rad in Bio & Med* (1986) 2:283-288.
- Viau, A. T. et al. "Toxicologic Studies of a Conjugate of Asparaginase and Polyethylene Glycol in Mice, Rats, and Dogs," *Am J Vet Res* (1986) 47:1398-401.
- Wieder, K. J. et al., "Some Properties of Polyethylene Glycol: Phenylalanine Ammonia-Lyase Adducts," *J Biol Chem* (1979) 254: 12579-87.

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Pharmaceutically acceptable, nonimmunogenic compositions are formed by covalently binding glycosaminoglycans or derivatives thereof, to hydrophilic synthetic polymers via specific types of chemical bonds to provide biocompatible conjugates. Useful glycosaminoglycans include hyaluronic acid, the chondroitin sulfates, keratan sulfate, chitin and heparin, each of which is chemically derivatized to react with a hydrophilic synthetic polymer. The conjugate comprising a glycosaminoglycan covalently bound to a hydrophilic synthetic polymer may be further bound to collagen to form a three component conjugate having different properties. The hydrophilic synthetic polymer may be polyethylene glycol and derivatives thereof having an average molecular weight over a range of from about 100 to about 100,000. The compositions may include other components such as fluid, pharmaceutically acceptable carriers to form injectable formulations, and/or biologically active proteins such as growth factors or cytokines. The conjugates of the invention generally contain large amounts of water when formed. The conjugates can be dehydrated to form a relatively solid implant for use in hard tissue augmentation. The dehydrated, solid implant can further be ground into particles which can be suspended in a non-aqueous fluid and injected into a living being (preferably human) for soft tissue augmentation. Once in place, the solid implants or particles rehydrate and expand in size approximately three- to five-fold.

29 Claims, No Drawings