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(54) Title: DISCRETE-LENGTH POLYETHYLENE GLYCOLS			
(57) Abstract The present invention provides discrete-length polyethyl- ene glycol and polyethylene glycol containing compounds and methods for their preparation.			
L Step 1 ↓ H-(-P-L-P)-H Step 2 ↓ H-(-P-L-P)L(P-L-P)-H Step 3 ↓ H(-P-L-P-L-P-L-P)L(P-L-P-L-P-L-P)-H			

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DISCRETE-LENGTH POLYETHYLENE GLYCOLS

Field of the Invention

The present invention is generally directed to polyethylene glycols and, more specifically, to discrete-length polyethylene glycols.

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Background of the Invention

Polyethylene glycols are a family of polymers produced from the condensation of ethylene glycol, and have the general formula $\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$ where n , the number of ethylene glycol groups, is greater than or equal to 4. Generally, the designation of a polyethylene glycol (PEG) includes a number that corresponds to its average molecular weight. For example, polyethylene glycol 1500 refers to a mixture of polyethylene glycols having an average value of n between 29 and 36 and a molecular weight range of 1300 to 1600 grams/mole.

The properties of polyethylene glycols vary with the polymer's molecular weight. Polyethylene glycols have been used in plasticizers, softeners and humectants, ointments, polishes, paper coating, mold lubricants, bases for cosmetics and pharmaceuticals, solvents, binders, metal and rubber processing, permissible additives to foods and animal feed, and laboratory reagents, among others.

Polyethylene glycols are generally linear or branched, neutral polyether molecules that are soluble in water and organic solvents. In addition to the uses noted above, polyethylene glycols have proven to be valuable in many biotechnical and biomedical applications. Polyethylene glycols have been advantageously employed in

these applications for their ability to impart water solubilization and surface protective properties, and also because these polymers are only weakly immunogenic.

Polyethylene glycols have also been covalently coupled to proteins to alter their properties in ways that extend their potential uses. Due to *in vivo* instability, the efficacy of a number of therapeutic proteins is severely limited. While many approaches to stabilization of such proteins have been made, the covalent modification of proteins with hydrophilic polymers such as dextrans and polyethylene glycols has been most successful. Typically, polyethylene glycol-protein conjugates are more stable than the native protein *in vivo* and often, the modified proteins exhibit enhanced resistance to proteolytic degradation. The result is an increase in the therapeutic protein's life in circulation and a reduction in its immunogenicity. In some instances, the therapeutic efficiency of these conjugates is greatly enhanced compared to the native protein.

The improved performance of PEG-modified conjugates has resulted in their development as therapeutic agents. For example, enzyme deficiencies where the native enzyme was ineffective due to its rapid clearance and/or immunological reactions have now been treated with equivalent PEG-enzymes. In fact, PEG-adenosine deaminase has obtained FDA approval. Because PEG-enzymes can act as catalysts in organic solvents, the use of such PEG-enzymes to produce desired stereoisomers offers an advantage to classical organic synthesis which typically produces acemic mixtures of organic products. *See, e.g.,* Degado et al., *Crit. Rev. Ther. Drug Carrier Systems*, Vol. 9, pp. 249-304, 1992.

Examples of polyethylene glycol-modified proteins include PEG-adenosine deaminase (PEG-ADA), which has been used in enzyme replacement therapy for immunodeficiency due to ADA deficiency (M.S. Hershfield, *Clin. Immunol. Immuno. Pathol.*, Vol. 76, S 228-232, 1995); PEG-recombinant human granulocyte colony stimulating factor (PEG-rhG-CSF), which showed an increase in stability and retention of *in vivo* bioactivity and has been suggested as a suitable form of the protein for inclusion in an oral delivery formulation (P.-K.E. Jensen et al., *Pharm. Res.*, Vol. 13, pp. 102-107, 1996); PEG-natural human tumor necrosis factor alpha, which showed a gradual decrease in specific activity with increasing degree of PEG-modification and a drastic increase in plasma half-life upon PEG-modification (Y. Tsutsumi et al., *Br. J. Cancer*, Volume 71, pp. 963-968, 1995); PEG-recombinant human interleukin-2, which retains the *in vitro* and *in vivo* activity of interleukin-2, but exhibits a markedly prolonged circulating half-life (T. Menzel et al., *Cancer Bio.*

Ther., Vol. 8, pp. 199-212, 1993); and PEG-asparaginase, which has shown promise in patients suffering from acute lymphocytic leukemia (N. Burnham, *Am. J. Hosp. Pharm.*, Vol. 52, pp. 210-218, 1994). Polyethylene glycol conjugates of oligonucleotides have also been prepared and show a more than tenfold increase in exonuclease stability (A. Jaschke et al., *Nucleic Acids Research*, Vol. 22, pp. 4810-4817, 1994).

Other PEG-modified proteins include papain (C. Woghiren et al., *Bioconjugate Chemistry*, Vol. 4, pp. 314-318, 1993), asialofetuin (L. Roseng et al., *J. Biol. Chem.*, Vol. 267, pp. 22987-22993, 1992), collagen (C.J. Doillon et al., *Biomaterial Sciences Polymers*, Vol. 6, pp. 715-728, 1994), RGDT peptides (I. Saiki, *Japanese J. Cancer Research*, Vol. 84, pp. 558-565, 1993), serum IgG (R. Cunningham et al., *J. Immunol. Methods*, Vol. 152, pp. 177-190, 1992), alpha 1-proteinase inhibitor (A. Mast et al., *J. Lab. Clin. Med.*, Vol. 116, pp. 58-65, 1990), growth hormone releasing factor (A. Felix, *Int. J. Peptide Protein Research*, Vol. 46, pp. 253-264, 1995), basic fibroblast growth factor (S. Kusstatscher et al., *J. Pharmacol. Exp. Ther.*, Vol. 275, pp. 456-61, 1995), and catalase, uricase, honey bee venom, hemoglobin, and ragweed pollen extract. As indicated by the number of utilities noted above, polyethylene glycol has recently been widely used to develop new therapeutic agents.

Despite the widespread use of polyethylene glycols to modify therapeutic agents, their use has not been without associated disadvantages. The covalent attachment of polyethylene glycol to superoxide dismutase produces a heterogeneous mixture of modified protein species. The heterogeneity of the product derives from, in part, the polydispersity of the polyethylene glycol reagent (J. Snyder et al., *J. Chromatography*, Vol. 599, pp. 141-155, 1992.)

Commercially available polyethylene glycols having molecular weights greater than about 300 grams/mole are available only as mixtures of varying length polymers. The range of PEG polymer lengths results from the polymerization process by which the PEG polymers are prepared. Commercially available PEG polymers include polymers having average molecular weights of 100, 200, 300, 400, 600, 900, 1000, 1500, 2000, 3400, 4600, 8000, and 10000 grams/mole from Aldrich Chemical Co. (Milwaukee, WI); 3350 and 20000 grams/mole from Sigma Chemical Co. (St. Louis, MO); and 1000, 2000, 3000, 5000, 10000, 20000 and 25000 grams/mole from Shearwater Polymers, Inc. (Huntsville, AL). The exact composition of these

mixtures are not generally provided, but are considered of relatively narrow range except where terminal monomethyl ethers are desired.

Inherent problems with the utilization of such polymeric mixtures of PEG molecules exist. One of the most significant problems is that a mixture of compounds is obtained when these compounds are modified or used to modify other compounds. Having a mixture of compounds complicates purification and characterization of the compounds. Further, even though PEG molecules are relatively innocuous in the biological system, different compounds within these mixtures are likely to have different pharmacokinetics, pharmacodynamics, and even varying degrees of toxicity, making such a mixture questionable for pharmaceutical applications.

Yet another consideration in having mixtures is the role of sizes in obtaining the desired biological properties. While small differences might be expected in solubilization within a "narrow" average molecular weight range, properties such as rendering proteins nonimmunogenic may be compromised by having a mixture of sizes on the protein surface. The fact that there are variances in lengths may make the protein more recognizable. While immunogenic proteins modified by PEG compounds often have benefits arising from that process, the fact that a mixture of PEG compounds is used may result in obtaining weakly immunogenic responses.

Accordingly, there remains a need in the art for alternatives to PEG polymers composed of a mixture of lengths and molecular weights to overcome the difficulties associated with the preparation, purification, characterization, and therapeutic administration of such PEG mixtures. The present invention seeks to fulfill these needs and provides further related advantages.

Summary of the Invention

In one aspect of the invention, polyethylene glycol containing compounds of discrete-length are disclosed. In one embodiment, functionalized polyethylene glycol containing compounds are disclosed. In another aspect, the present invention discloses a convergent synthetic method for the preparation of polyethylene containing compounds having discrete-lengths.

Brief Description of the Drawings

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same becomes better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is an illustration of some commercially available PEG compounds;

FIGURE 2 is a schematic representation of the preparation of a representative discrete-length PEG of the present invention;

FIGURE 3 is a schematic representation of the preparation of a representative
5 amine-linked discrete-length PEG-containing compound of the present invention;

FIGURE 4 is a schematic representation of the preparation of a representative amide-linked discrete-length PEG-containing compound of the present invention;

FIGURE 5 is a schematic representation of a synthetic method of the present invention;

10 FIGURE 6 is a schematic representation of the preparation of a representative alkoxy-terminated discrete-length PEG-containing compound of the present invention;

FIGURE 7 is a schematic representation of the preparation of a radiolabel PEG building block useful in the preparation of the discrete-length PEG-containing
15 compounds of the present invention;

FIGURE 8 is a schematic representation of the preparation of a fluorescent label PEG building block useful in the preparation of the discrete-length PEG-containing compounds of the present invention;

FIGURE 9 is a schematic representation of the preparation of a biotin PEG
20 building block useful in the preparation of the discrete-length PEG-containing compounds of the present invention;

FIGURE 10 is the schematic illustration of the preparation of a representative ubiquinone (50) carbamate linked discrete-length PEG containing compound of the present invention; and

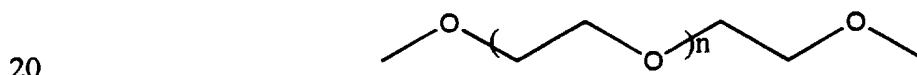
25 FIGURE 11 illustrates representative vitamin B₁₂ biotin compositions covalently linked by representative discrete-length PEG-containing moieties of the present invention.

Detailed Description of the Preferred Embodiment

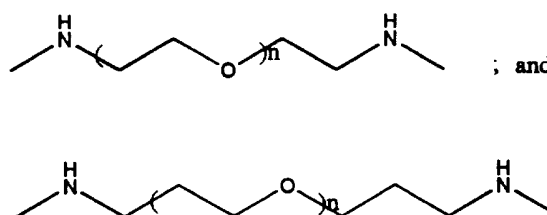
30 The present invention is generally directed to polyethylene glycol (PEG) and polyethylene glycol containing compounds and compositions and methods related to their preparation and use. More specifically, the present invention is directed to polyethylene glycols and polyethylene glycol containing compounds having a discrete-length and molecular weight. As used herein, the phrase "discrete-length" refers to a polyethylene glycol prepared by a nonpolymeric process such that the polyethylene

glycol and have a specific discernible molecular weight. Polyethylene glycols having discernible molecular weights can be contrasted to polymeric processes currently utilized which produce polyethylene glycols having a range of lengths and a range of molecular weights. The present invention provides discrete-length polyethylene glycols, discrete-length polyethylene glycol containing compounds, including polyethylene glycol containing compositions that include the foregoing and methods for the preparation of the same.

One aspect of the present invention provides a polyethylene glycol compound that includes at least one discrete length polyethylene glycol moiety having a molecular weight of at least about 300 grams/mole. Particularly suitable moieties for most uses, generally have a molecular weight in the range of about 1000 to about 50,000 grams/mole, preferably in the range of about 2000 to about 20,000 grams/mole, more preferably in the range of about 2500 to about 7500, specifically about 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, and 6500. The discrete length polyethylene glycol moiety generally includes any moiety having a discrete length and molecular weight and includes the repeated ethylene glycol unit (i.e., $-(OCH_2CH_2)_n-$). The repeating unit may be linked by any number of suitable molecules or cross-linking agents described below in detail and may include one or more of the following radicals.



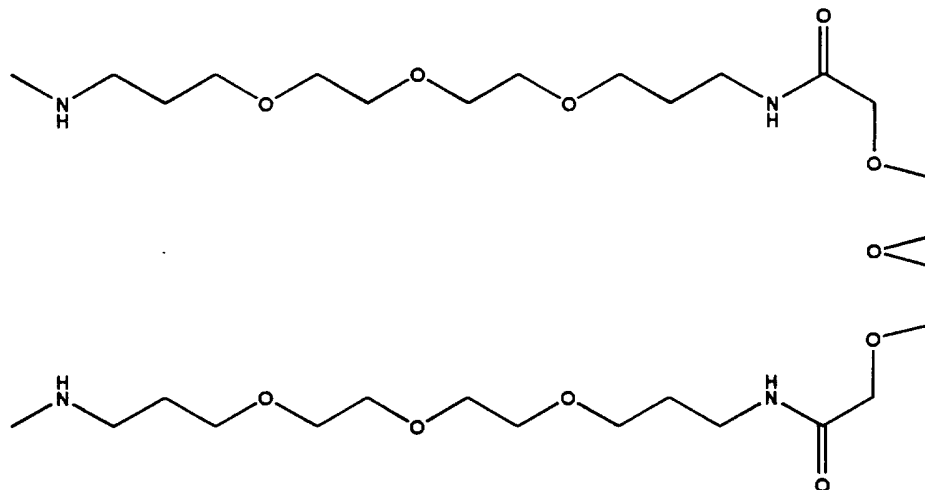
wherein generally, $n = 1-1000$, preferably 45-700, more preferably 50-150, and in a particular preferred embodiment, $n = 100-125$, and



wherein $n = 1-3$.

25 The radicals noted above are polyethylene glycol and polyethylene glycol containing moieties found in representative polyethylene glycol building blocks, such as those shown in FIGURE 1, utilized to produce the compounds of the present invention and further described below.

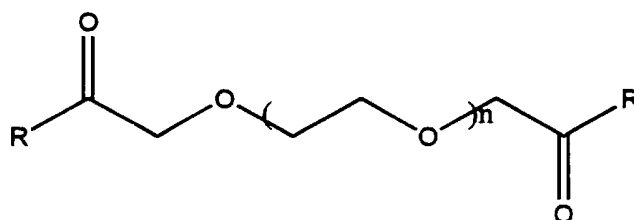
- Suitable polyethylene glycol containing compounds that serve as building blocks (i.e., starting materials and intermediates) for the preparation of the polyethylene glycols and polyethylene glycol containing compounds of the present invention include alkyl oxy oxycarboxy compounds having the general formula
- 5 $R-O-(CH_2CH_2O)_n-CH_2CO_2H$, where $n = 0-2$; oxy dicarboxy compounds having the general formula $HO_2C-CH_2O-(CH_2CH_2O)_n-CH_2CO_2H$ where $n = 0-2$; oxy diamino compounds having the formula $NH_2-(CH_2CH_2O)_n-CH_2CH_2NH_2$ where $n = 1-3$ and $NH_2CH_2-(CH_2CH_2O)_n-CH_2CH_2CH_2NH_2$ where $n = 1-3$; and oxy diol compounds having the formula $HO-(CH_2CH_2O)_n-CH_2CH_2OH$ where $n = 1-4$. In
- 10 one preferred embodiment, the discrete-length polyethylene glycol moiety is a 4,7,10-trioxy-1,13-tridecanediamine radical (the second oxy diamino compound noted above where $n = 3$). In another preferred embodiment, the discrete-length polyethylene glycol moiety is a 3,6,9-trioxyundecanedioic acid radical (the oxy dicarboxy compound noted above where $n = 2$).
- 15 In a preferred embodiment, the polyethylene glycol containing compound of this invention includes both a 4,7,10-trioxy-1,13-tridecanediamine radical and a 3,6,9-trioxyundecanedioic acid radical, and has the following formula:



- In one embodiment, a polyethylene glycol containing compound of this
- 20 invention includes a discrete-length polyethylene glycol moiety having at least six polyethylene glycol units and at least one cross-linking agent. Generally, the moiety will have 6-1000 units, preferably 45-700 units, more preferably 50-150 units, and in a particularly preferred embodiment 100-125 units. As used herein, the term "cross-linking agent" includes a chemical compound having at least two reactive or

activatable functional groups capable of covalent coupling with suitably reactive discrete-length polyethylene glycol derivatives. The cross-linking agents useful in the present invention preferably do not interfere or significantly reduce the moiety's advantageous PEG characteristics and properties.

- 5 The cross-linking agents useful in the present invention include bifunctional, trifunctional, tetrafunctional, or other polyfunctional agents. Preferably, the cross-linking agent is a bifunctional or trifunctional cross-linking agent. Cross-linking agents useful in the present invention include reactive or activatable functional groups that form covalent bonds with the functional groups of the discrete-length
10 polyethylene glycol derivatives. Preferably, the cross-linking agent is a carboxylic acid or a carboxylic acid derivative. Suitable carboxylic acid derivatives include acid halides, acid anhydrides, and activated esters such as, for example, N-hydroxysuccinimide esters, tetrafluorophenyl esters, and p-nitrophenyl esters. In a preferred embodiment, the cross-linking agent is a bifunctional cross-linking agent
15 having the formula:



wherein $n = 0-3$, and R is independently selected from -OH, halide, -OCOR' where R' is an aryl or lower alkyl group, activated ester leaving group such as N-hydroxysuccinimide, p-tetrafluorophenol and p-nitrophenol.

- 20 In addition to the carboxylic acid cross-linking agents, other cross-linking agents are useful in the methods of the present invention. The cross-linking agent can also include a reactive alkyl moiety, such as an alkyl alcohol that has been derivatized to include a leaving group that may be readily displaced by, for example, an alcohol or amine containing compound. For example, diols substituted with leaving groups may
25 be displaced by polyethylene glycol diols to provide ether linked polyethylene glycols, as shown schematically in FIGURE 2. Similarly, suitably substituted diols may be displaced by polyethylene glycol diamines to provide an amine linked polyethylene glycol as shown in the reaction scheme of FIGURE 3. The reaction of a dicarboxylic acid cross-linking agent with a polyethylene glycol diol produces an ester linked

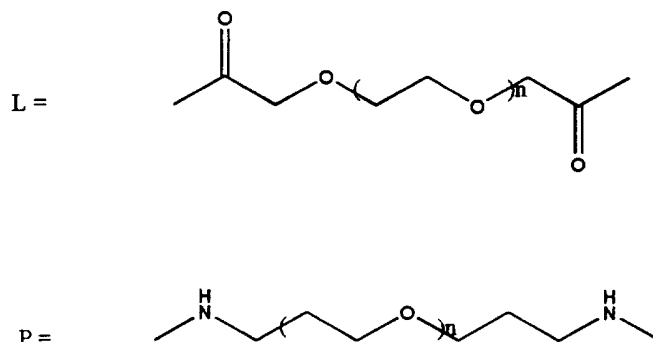
polyethylene glycol, and reaction with a polyethylene glycol diamine produces an amide linked polyethylene glycol as shown in FIGURE 4.

In another aspect, the present invention provides a method for preparing a polyethylene glycol containing compound having a discrete-length polyethylene glycol moiety that has a molecular weight of at least about 300 grams/mole. Generally, the method includes the step of reacting a cross-linking agent with a discrete-length polyethylene glycol derivative having a molecular weight of at least about 300 grams/mole to form a covalent bond between the cross-linking agent and the polyethylene glycol derivative. The general reaction sequence is schematically represented in FIGURES 2-4.

In one embodiment, the method for preparing a polyethylene glycol containing compound of this invention is a convergent synthetic method. By such a method, high molecular weight discrete-length polyethylene glycol containing compounds can be rapidly and efficiently assembled. Referring to FIGURES 2-4, discrete-length polyethylene glycol derivatives are added to each functional group of the cross-linking agent in the method. The method of the present invention further includes the additional step of reacting the cross-linking agent with the discrete-length polyethylene glycol (prepared in a previous step) in a repeated manner (i.e., repeated cycles) to rapidly increase the length and molecular weight of the discrete-length polyethylene glycol. Such a method effectively "dimerizes" the starting discrete-length PEG derivative.

A representative example of three-step reaction sequence that provides discrete-length polyethylene glycol containing compounds having molecular weights of about 600, 1400, and 3000, is shown in FIGURE 5. Referring to FIGURE 5, reaction of a cross-linking agent (i.e., an activated dicarboxylic acid), L, with a diamine, R, produces a discrete-length polyethylene glycol containing compound having a molecular weight of 627 grams/mole. In a second step, the discrete-length polyethylene glycol produced in the first step is reacted with the cross-linking agent. The second step serves to cross-link two discrete-length polyethylene glycol containing compounds produced in the first reaction and provide a discrete-length polyethylene glycol containing compounds having a molecular weight of 1,440 grams/mole. Treatment of the discrete-length polyethylene glycol containing compounds formed by the second step reaction with the same cross-linking agent results in the formation of a discrete-length polyethylene glycol containing compounds having a molecular weight of 3,066 grams/mole. As illustrated by FIGURE 5, the

method of the present invention provides high molecular weight polyethylene glycols having discrete-lengths in a straightforward synthetic manner (e.g., the formation of a discrete-length polyethylene glycol having a molecular weight of about 3,000 grams/mole from relatively low molecular weight starting materials in three synthetic steps). In a preferred embodiment,



wherein for L, $n = 0-2$, and for P, $n = 1-3$.

As can be seen by the representative reaction sequence shown in FIGURE 5, a wide variety of discrete-length polyethylene glycols may be prepared by the method of the present invention by substitution of either one or both of the discrete-length polyethylene glycol building blocks and/or the cross-linking agent. Although FIGURE 5 illustrates a preparation of a polyethylene glycol where both the starting materials are polyethylene glycol-containing compounds, it will be appreciated that the present invention includes discrete-length polyethylene glycols prepared from cross-linking agents that include polyethylene glycol functional groups (i.e., $-(OCH_2CH_2)_n-$) as well as cross-linking agents that do not include polyethylene glycol groups. The use of cross-linking agents other than bifunctional cross-linking agents results in the formation of branched discrete-length polyethylene glycols. Thus, the method of the present invention is versatile and by selection of cross-linking agents provides for the preparation of a wide variety of discrete-length polyethylene glycols. Preferably, the discrete polyethylene glycol containing compounds of the present invention retain the advantageous properties and characteristics of PEG.

The method of the present invention may also be used to prepare a composition that includes a discrete-length polyethylene glycol or PEG containing compound having a molecular weight of at least 300 grams/mole coupled to a second component through a covalent bond. In the method, a polyethylene glycol-containing

compound having a discrete-length ethylene glycol moiety of a molecular weight of at least about 300 grams/mole, is reacted with a second component to form a covalent bond. Thus, in one embodiment, the method of the present invention produces a polyethylene glycol derivatized compound by reacting the compound with a discrete-length polyethylene glycol moiety having a molecular weight of at least 300 grams/mole under conditions suitable to form a covalent bond between the polyethylene glycol moiety and the compound.

The compound, or second component, may include any one of a number of compounds including reporter molecules such as radiolabel compounds and precursors to radiolabel compounds, as shown in FIGURE 7; a fluorescent compound, as shown in FIGURE 8; a protein binding ligand, such as biotin, as shown in FIGURE 9; or a therapeutic agent such as ubiquinone 50, as shown in FIGURE 10. The discrete-length polyethylene glycols of the present invention may also serve as linkers between two components or compounds. For example, as shown in FIGURE 11, discrete-length polyethylene glycols of the present invention can be used to link, for example, vitamin B₁₂ to biotin. The discrete-length polyethylene glycol and PEG containing compounds of this invention can also be coupled to hydrophobic moieties to form biphasic compounds useful in the formation of liposomes and micelles.

Because of the well-known water solubilizing properties of polyethylene glycol, the discrete-length polyethylene glycol and PEG containing compounds and composition of the present invention have high water solubility. Accordingly, the polyethylene glycol compounds and compositions of the present invention are particularly useful in solubilizing components that have low solubility in aqueous environments. Furthermore, because of polyethylene glycol's well-known property of stabilizing therapeutic agents in the bloodstream, the discrete-length polyethylene glycol and PEG containing compounds of the present invention may also be useful in stabilizing biologically active molecules including therapeutic agents. The discrete-length polyethylene glycol and PEG containing compounds of this invention may be coupled to therapeutic agents to increase their water solubility, increase their stability *in vivo*, and to facilitate their administration as water soluble formulations. While the discrete-length polyethylene glycols may be useful in forming compositions with a number of therapeutic agents, preferred therapeutic agents include ubiquinones, particularly ubiquinone 50, and its derivatives, and vitamin B₁₂ and its derivatives.

The discrete-length polyethylene glycol containing compounds and compositions of the present invention includes a therapeutic agent covalently attached to the polyethylene glycol through a covalent bond. Where the covalent bond is cleavable and/or degradation of *in vivo*, the compound or conjugate is a prodrug of the therapeutic agent. Accordingly, the discrete-length polyethylene glycol and PEG containing compounds of the present invention are useful in combination with therapeutic agents as prodrugs and may be effective in the delivery of therapeutic agents such as ubiquinones, vitamin B₁₂, and their derivatives.

In another aspect, the present invention provides a pharmaceutical composition that includes a discrete-length polyethylene glycol containing compound or composition having a discrete-length polyethylene glycol moiety with a molecular weight of at least about 300 grams/mole, preferably 2500, 3000, 3500, 4000, or 5000 grams/mole, coupled to a therapeutic agent, and a pharmaceutically acceptable carrier and/or diluent.

The method of the present invention provides a variety of PEG-containing compounds having discrete-lengths, molecular weights, and functional groups. As noted above and described below, the method of the invention provides (a) ether-linked PEG compounds having bis-alcohol termini; (b) amine-linked PEG compounds having bis-amine termini; (c) amide-linked PEG compounds having bis-amine termini; (d) single terminus substituted PEG compounds having, for example, alkyl, radiolabel, fluorescent, and protein binding (e.g., biotin) termini; and (e) PEG compounds substituted at both termini to provide, for example, prodrugs and other linkers, all of which have discrete lengths.

(a) Ether Linked - PEG Compounds - Bis-Alcohol Termini

In one embodiment, the method of the present invention includes a convergent synthesis that effectively "dimerizes" a discrete-length PEG derivative through a bifunctional cross-linking agent. A general scheme for the preparation of an ether-linked PEG compound having bis-alcohol termini traditional polyethylene glycos (i.e., H-(OCH₂CH₂)_n-OH) is represented in FIGURE 2. Referring to FIGURE 2, the dimerization of a discrete-length PEG diol with a di-activated discrete-length PEG diol is accomplished by using an excess of the first PEG (which can be recycled upon separation from product). The coupling reaction shown employs good leaving groups such as sulfonates (e.g., tosylate, mesylate, triflate). The reaction conditions typically employ a base (e.g., NaH, KOtBu) under conditions generally applied to ether formation. By selecting high molecular starting materials and/or repeating the

reaction sequence (i.e., employing additional reaction cycles, as indicated in FIGURE 5), the method of this invention can rapidly assemble PEG compounds of discrete-length and molecular weight. For a diol having n ethylene glycol units and a cross-linking agent having n' ethylene glycol units, the product polyethylene glycol has
5 $n'' = 2n + n' + 2$ ethylene glycol units.

Referring to FIGURE 2, an excess of commercially available penta(ethylene glycol) [$n = 4$] may be reacted with commercially available pentaethylene glycol di-*p*-toluenesulfonate [$n' = 4$] to yield a pentadecane(ethylene glycol) [$n'' = 14$]. The same reaction can be conducted, in a second cycle, with the newly generated
10 pentadecane(ethylene glycol) [$n'' = 14$] to provide a compound of 34 ethylene glycol units (i.e., $-(CH_2CH_2-O)_{34}-$). When larger derivatives are desired, the PEG diol may be having, for example, pentadecane(ethylene glycol) [$n = 14$] and the PEG ditosylate may be the corresponding di-*p*-tosylate. The product of such a coupling is a PEG compound having 44 ethylene glycol units. While these compounds are provided by
15 way of example, any size of discrete-length PEG [$1 < n < 500$] (commercially available or prepared from a discrete-length compound) may be used to prepare PEG compounds having greater discrete-lengths and molecular weights.

(b) Amine Linked PEG Compounds - Bis-Amine Termini

Substitution of a PEG diamine for the PEG diol in the reaction sequence
20 described above provides an amine-linked PEG, a PEG-like compound that has a -NH-group in place of the -O-group present in traditional polyethylene glycols at the cross-link positions. A general scheme for the preparation of an amine-linked PEG compound having bis-amine termini is represented in FIGURE 3. For a diamine having n ethylene glycol units and a cross-linking agent having n' ethylene glycol units,
25 the product amine linked polyethylene glycol has $n'' = 2n + n'$ ethylene glycol units.

Referring to FIGURE 3, an excess of commercially available 4,7,10-trioxa-1,13-tridecanediamine [$n = 3$] is reacted with commercially available pentaethylene glycol *p*-toluenesulfonate [$n' = 4$] to yield a compound containing 10 ethylene glycol units, two secondary amines and two primary amines. Although the number of
30 ethylene glycol units is decreased over the corresponding simple PEG molecule, the amines will impart water solubility as they will be protonated at neutral pH. The product from this reaction (having a 43-atom length, and molecular weight of 629) can be further reacted to provide a dimer having a molecular weight of about 1500 grams/mole. Further PEG units may be added to these compounds by traditional
35 chemical means to produce even larger PEG compounds.

(c) Amide Linked PEG Molecules - Bis-Amine Termini

Substitution of the diactivated diol cross-linking agent in the reaction sequence described above with a diactivated dicarboxylic acid derivative provides an amide linked PEG, a PEG-like compound that has an $\text{-NHCOCH}_2\text{-}$ group in place of the
5 -O- group present in traditional polyethylene glycols at the cross-link positions. A generalized scheme for the preparation of an amide-linked PEG compound having bis-amine termini is represented in FIGURE 4. For a diamine having n ethylene glycol units and a cross-linking agent having n' ethylene glycol units, the product amide-linked polyethylene glycol has $n'' = 2n + n'$ ethylene glycol units.

10 Referring to FIGURE 4, an excess of commercially available 4,7,10-trioxa-1,13-tridecanediamine [$n = 3$] may be reacted with readily prepared di-tetrafluorophenyl ester of 3,6,9-trioxaundecane dioic acid [$n' = 2$] to yield a PEG containing molecule having the equivalent of 9 PEG units. While the number of PEG units is decreased by introduction of amide bonds, the amide link still has an increased
15 water solubility relative to saturated methylene units. The large advantage of using the amide forming reactions is their relative ease of reaction and the high yields obtained. Such linkage may also be useful for those PEG containing compounds in which cleavable linkages are desirable.

(d) Single Terminus Substituted PEG Compounds

20 The PEG containing compounds and methods of this invention may also be used to prepare PEG containing molecules for attachment to molecules or surfaces where they can impart desired effects including water solubilization or detoxification. In some instances, both ends of the PEG compound may be modified. One end of the PEG compound may be simply "capped" to render it non-reactive in the reaction used
25 to attach the molecule to another for imparting PEG properties. Alternatively, one end of the PEG compound may be capped with a reporter functional molecule such as a fluorescent dye or radiolabeling moiety. Other terminus substitution may provide derivative having moieties that: 1) target tumors through variety of receptor modalities; 2) bind with biologically active proteins; 3) cause the molecule to be
30 excluded from cells; 4) cause it to enter cells; 5) cause it to alkylate other moieties or itself; 6) cause it to target infections; and 7) cause it to otherwise be handled in a manner dissimilar to the PEG derivative without the modification. A generalized scheme for coupling of one end of a discrete-length PEG derivative with a second compound is provided below.

Alkyl Terminated PEG Derivatives. Referring to FIGURE 6, the tetrafluorophenyl (TFP) ester of commercially available 2-[2-(2-methoxyethoxy)ethoxy]acetic acid is reacted with an excess of the diaminodiamide nonaoxa product of the specific reaction shown in FIGURE 4 to provide a monomethyl terminated PEG containing compound (51 atoms long, MW = 787).

Radiolabeled Group (Aryl Iodide) PEG Derivatives. A method for introducing an iodinated phenyl group into a PEG compound is shown in FIGURE 7. The TFP ester of *p*-iodobenzoate is used as the reagent in the second step of the scheme. Replacement of the reagent with the TFP ester of *p*-tri-*n*-butylstannylbenzoate provides a compound which can be used to introduce a radioiodine (by substitution of the stannyl group with radioiodine) into a PEG compound. The radioiodination can take place as the last step of any derivatization with the arylstannyl-PEG. The TFP ester may then be reacted with a diamino-PEG containing compounds in a manner as described above for the TFP ester of the methyl terminated PEG in FIGURE 6.

Fluorescent Group (Dansyl) PEG Derivatives. A method for introducing a fluorescent group (i.e., dansyl) is shown in FIGURE 8. Dansyl chloride is reacted with the diglycolic anhydride adduct of commercially available 4,7,10-trioxa-1,13-tridecanediamine. Activation of the carboxylic acid functionality with tetrafluorophenyl ester is accomplished with the TFP-acetate. The TFP ester is reacted with a diamino-PEG containing compound as described above and illustrated in FIGURE 6.

Protein Binding Group (Biotin) PEG Derivatives. A method for introducing a biotin molecule into a PEG derivative is shown in FIGURE 9. In the reaction sequence, the biotin carboxylate is activated by preparing the TFP ester, then reacted with commercially available 4,7,10-trioxa-1,13-tridecanediamine. The resultant amino compound is reacted with diglycolic anhydride, and the resultant carboxylate is activated by formation of the TFP ester. The TFP ester is then reacted with a representative diamino-PEG derivative to yield a PEG containing compound having a biotin at one terminus and an amino group at the other terminus.

(e) Substitution at Both Terminus of the Discrete-length PEG Containing Molecules.

Many examples of PEG containing compounds of interest have both terminus substituted with compounds. Some examples are noted below, but it will be

appreciated that the PEG compounds of the present invention may be useful in other applications as well.

Water Solubilizing Prodrugs. The PEG containing compounds of this invention may be prodrugs. The PEG containing compounds of this invention can be used simply to provide enhanced water solubility to compounds and components to which they are coupled. The compounds may further be designed to release the parent compound once it been administered. In some cases, all that is of interest is to have one non-reactive terminus (e.g., methoxy terminated) and a cleavable linkage (e.g., ether, ester, carbamate, carbonate) at the other terminus. An example of this can be made where a highly water insoluble compound, coenzyme Q₁₀ (ubiquinone 50), can be made water soluble with PEG derivatives. These derivatives are made wherein the PEG containing compounds are attached to the hydroquinone alcohols produced by gentle reduction of native coenzyme Q₁₀. The coupling at a PEG to ubiquinone 50 is shown in FIGURE 10, where an isocyanate (produced from a terminal amino group) containing PEG is attached to the hydroquinone through formation of a cleavable carbamate group. The discrete-length PEG containing compounds of this invention are preferred because of the difficulty in purifying and characterizing the product obtained using a mixture of different sized PEG compounds. Other examples where the terminus away from the coenzyme Q₁₀ molecule contains a hepatic localizing moiety, a cell membrane permanent agent, a charged species, or a number of other biologically relevant molecules may be prepared as part of the water solubilizing discrete-length PEG containing molecules.

Water Solubilizing Linkers Within Compounds. The PEG containing compounds of this invention may be used as water solubilizing linkers between two compounds that have different biological properties. One such example is the use of the commercially available 4,7,10-trioxa-1,13-tridecanediamine as a water solubilizing linker molecule to conjugate vitamin B₁₂ (VB₁₂) to biotin. A similar sized (non-PEG containing) linker, 1,12-diaminododecane had been used previously, and the resulting conjugate was found to have low water solubility. However, the conjugate prepared using the PEG containing diamine was found to be quite water soluble. Importantly, PEG containing linkers of greater and discrete length than those available commercially are of interest. Lengthy linkers are advantages to separate the two proteins that bind with vitamin B₁₂ and biotin. Examples of several different linkers with discrete-lengths being used as water solubilizing spacer (linker) molecules are shown in FIGURE 11. The water solubilization has been found to be particularly

important in the preparation and application of vitamin B₁₂ dimers. The PEG containing linkers have been shown to improve the water solubility dramatically. FIGURE 12 schematically depicts a representative use of the PEG containing compounds as linkers to solubilize compounds having low solubility in aqueous environments.

PEG compounds of this invention have discrete-lengths and molecular weights. These compounds overcome the difficulties of preparing, characterizing and administering mixtures of compounds. Although polymerization is a cost attractive method of making large molecules including PEG compounds, the problems associated with polymer purification ultimately raise the overall costs significantly. Alternatives to polymerization for making long chain compounds include a systematic building of the molecule by a stepwise process (e.g., peptide synthesis) and convergent synthesis, wherein increasingly larger pieces are prepared and combined in a few step process. Generally, a convergent method is preferred for producing PEG containing compounds having discrete-lengths. The convergent synthesis method also provides the advantage of being readily scaled up for production.

Although the PEG containing compounds having discrete-lengths may have physical and biological properties similar to those obtained from PEG mixtures (i.e., nondiscrete-lengths and molecular weights) produced by polymerization processes, the advantages of introducing other functional groups within the discrete-length PEG containing compounds. For example, one advantage of synthesizing discrete PEG containing compounds is that, unlike PEG compounds produced by polymerization, functional groups (e.g., amines) which readily allow derivatization within the polymer chain is possible. Also, it is possible to introduce functional groups within the chain which permit physical or biological degradation of the chain (e.g., peptidase sensitive (amides), esterase sensitive (esters), pH sensitive (carbonates and carbamates), photosensitive groups (aryl nitro compounds), and thermal sensitive groups). The introduction of the cleavable groups within a PEG compound may be used to impart new and valuable control to a variety of processes that these compounds are involved in, including altering the *in vivo* pharmacokinetics of compounds derivatized with them. A convergent synthesis also has an advantage over polymerization methods as derivatization at either one, or both, ends of the PEG containing molecule is more readily accomplished.

Example 1**Synthesis of a Representative Discrete-Length Polyethylene Glycol-Containing Compound**

The preparation of a representative discrete-length polyethylene glycol containing compound of the present invention is described in this example. Briefly, the representative discrete-length PEG containing compound is synthesized by reacting the ditetrafluorophenyl ester (diTFP) of 3,6,9-trioxaundecanedioic acid (a representative cross-linking agent) with 4,7,10-trioxa-1,13-tridecanediamine.

3,6,9-Trioxaundecanedioic acid diTFP ester: 3,6,9-Trioxaundecanedioic acid (45 mmol, 10 g) was dissolved in anhydrous DMF (150 mL). Tetrafluorophenol (115 mmol, 19 g) was added followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (108 mmol, 21 g). The reaction was stirred overnight at room temperature, and the solvent was removed under vacuum. The residue was extracted with ether (300 mL) and the ether was washed with saturated sodium bicarbonate solution (3 x 25 mL), then with water (2 x 25 mL). The ether solution was dried over anhydrous sodium sulfate and then the ether was removed under vacuum. The resultant product was dried under vacuum at 50-55°C to yield 15.8 g (76%) of product as an oil.

^1H NMR (CDCl_3 , δ): 3.6-3.8 (m, 8H), 4.46 (s, 4H), 6.9 (m, 2H).

9-Oxadiamine: 4,7,10-Trioxa-1,13-tridecanediamine (66 g, 300 mmol) (prepared as described above) was added in a dry flask and a solution of 3,6,9-trioxaundecanedioic acid diTFP ester (15.8 g, 30.5 mmol) in 250 mL of acetonitrile was dripped into it dropwise, at 0°C over a period of 1 h. The reaction was stirred at room temperature for 1 h and then the solvent was distilled. The residue was stirred with ether and the ether layer was discarded. The crude product was loaded over a silica column and the column was eluted with methanol. The fractions containing product were collected, solvent removed, and the product was dried under vacuum to yield 19 g of g-oxadiamine, a representative discrete-length polyethylene glycol-containing compound of this invention.

^1H NMR ($\text{MeOH}-d_4$, δ) 4.0 (s, 3H), 3.5-3.7 (m, 35H), 3.3 (t, 4H), 2.8 (t, 6H) 1.7-1.8 (m, 10H).

Example 2

Synthesis of a Discrete-Length Polyethylene Glycol-Containing Compound Coupled to Vitamin B₁₂

The preparation of a vitamin B₁₂ derivative covalently coupled to a discrete-length polyethylene glycol-containing compound is described. Briefly, the example describes a procedure for the reaction of a cyanocobalamin monocarboxylic acid with the discrete-length polyethylene glycol containing compound prepared as described above in Example 1.

Cyanocobalamin monocarboxylic acid and N-hydroxysuccinimide are dissolved in water and sodium cyanide is added. The discrete-length polyethylene glycol prepared as described in Example 1 is then added and the pH adjusted to 6 with 1 N HCl. N-ethyl-N'-dimethylamino-propyl-carbodiimide-hydrochloride (EDC) is added and the pH of the solution readjusted to 5.5. The reaction mixture is then stirred overnight in the dark at room temperature. In 5 intervals of 6 to 14 h, N-hydroxysuccinimide and EDC are added to the solution, readjusting the pH value to 5.5 each time. After a total reaction time of 4 days (HPLC monitored) the solution is evaporated to dryness, the residue digested with acetone and the solvent decanted. The solid residue is dissolved in 50 mL of H₂O and applied to an Amberlite XAD-2 column. The column is eluted with water, and the desired product is eluted with methanol. The methanol fractions are evaporated to dryness, and the residue dissolved in 25 mL of water and applied to a Dowex Cl⁻ column. The final product is eluted using water, thereby leaving non-converted acid bound to the column, which is later eluted with 0.04 mol/L sodium acetate buffer pH 4.7. The fractions containing the final product are evaporated to dryness, digested with acetone and filtered. The solid product, a discrete-length polyethylene glycol containing compound coupled to vitamin B₁₂, is obtained by recrystallized from aqueous acetone.

Example 3

Synthesis of a Discrete-Length Polyethylene Glycol-Containing Compound Coupled to Ubiquinone (50)

The preparation of ubiquinone (50) covalently coupled to a discrete-length polyethylene glycol-containing compound is described. Briefly, the example describes a procedure for the reaction of ubiquinone (50) with a suitably reactive form of the discrete-length polyethylene glycol prepared as described above in Example 1.

In a first step, ubiquinone (50) is reduced to the corresponding ubiquinol according Cheng and Casida (*J. Labl. Compd. Radiopharm.*, 6:66-75, 1970). Briefly,

a mixture of 1 g of ubiquinone (50), 5 mL of acetic acid and 0.3 g of zinc dust was heated at 50°C for 5 min., quenched with 5 mL of water and extracted in hexane (45 mL), in an argon atmosphere. Hexane extract was washed with water (3 x 5 mL), dried over MgSO₄, concentrated on a rotoevaporator to obtain 1.10 g of colorless viscous material which was dried at high vacuum (0.05 mm) at room temperature for 1 h to yield 0.980 g of reduced ubiquinol (50). This material is used in the following reactions without further purification.

The ubiquinol prepared as described above can be covalently coupled to various functionalized discrete-length polyethylene glycol-containing compounds to produce a variety of ubiquinone linked PEG-containing compounds having, for example, ether linkages, carbamate linkages, carbonate linkages, and ester linkages.

Ether Linked PEG Compounds. The following procedure provides a general methodology for preparing a ubiquinone having a discrete-length PEG-containing moiety coupled directly to a ubiquinol with an ether linking group.

In a three necked round bottom flask equipped with a dropping funnel and a reflux condenser, ubiquinol (reduced CoQ10 prepared as described above) is dissolved in anhydrous tetrahydrofuran (THF)(added via a syringe) under an argon atmosphere. To this solution is added sodium hydride. The reaction mixture is stirred at room temperature for 15 min., then cooled to 0°C using an ice bath. To the cooled solution is added a PEG-tresylate, a reactive form of the discrete-length PEG-containing compound prepared as described in Example 1, PEG-tresylate anhydrous tetrahydrofuran via a dropping funnel over a period of 20 min. After stirring the reaction mixture at 0°C, the ice bath is removed and the reaction mixture stirred for an additional 1 h. The reaction mixture is then brought to the reflux temperature by slowly raising the temperature of oil bath to 100°C. After reflux, heating is discontinued and the reaction mixture was allowed to cool to room temperature. The reaction mixture is quenched with a mixture of brine and THF. The solids vacuum filtered and solvent evaporated on a rotoevaporator. The material obtained is dissolved in chloroform and washed with water. The organics dried over anhydrous magnesium sulfate, filtered, concentrated on a rotoevaporator, and dried under vacuum at room temperature to yield a ubiquinone (50) ether linked discrete-length PEG-containing compound.

Carbamate Linked PEG Compounds. The following procedure provides a general methodology for preparing a ubiquinone having a discrete-length PEG-containing moiety coupled directly to a ubiquinol with a carbamate linking group.

The following synthesis of CoQ10-carbamate-PEG is a two-step process. In the first step, the succinimidyl carbamate of the discrete-length PEG-containing compound prepared as described in Example 1 prepared by dissolving dry dioxane and warming to 50°C to achieve solution. To the cooled solution is added a solution of N,N'-disuccinimidyl carbonate in acetone. A solution of dimethylaminopyridine in acetone is then added and the resulting reaction mixture stirred overnight at room temperature. The crude product is precipitated from the solution by the addition of diethyl ether, filtered, and dried at under vacuum at room temperature to yield the succinimidyl carbamate of the PEG.

In a flask equipped with a dropping funnel and argon balloon was added ubiquinol (reduced CoQ10 prepared as described above) in anhydrous tetrahydrofuran. To this solution is added sodium hydride. The reaction mixture is stirred at room temperature, then cooled to 0°C using an ice bath. To the cooled solution is added 500 mg the succinimidyl carbamate PEG, prepared as described above, in anhydrous tetrahydrofuran via a dropping funnel. After stirring the reaction mixture at 0°C, the reaction mixture is quenched with a mixture of brine and THF, and the solvent evaporated on a rotoevaporator. The crude product is taken up in 50 mL of chloroform and washed with water (2 x 10 mL). The organics are dried over anhydrous magnesium sulfate, filtered, concentrated on a rotoevaporator, and dried under vacuum at room temperature to yield the end product.

The crude product is loaded on a flash silica column and eluted with chloroform as the solvent. CoQ10 and other related impurities are eluted with chloroform and the PEG product is eluted with 50% methanol in chloroform to yield a ubiquinone (50) carbamate linked discrete-length PEG-containing compound.

Carbonate Linked PEG Compounds. The following procedure provides a general methodology for preparing a ubiquinone having a discrete-length PEG-containing moiety directly to a ubiquinol with a carbonate linking group. Treatment of a succinimidyl carbonate of the discrete-length PEG-containing compound prepared as described in Example 1 with reduced coenzyme Q10 in the presence of appropriate base (such as sodium hydride) in appropriate solvent (such as tetrahydrofuran) provides a ubiquinone (50) carbonate linked discrete-length PEG-containing compound.

Ester Linked PEG Compounds. The following procedure provides a general methodology for preparing a ubiquinone prodrug having a solubilizing moiety coupled directly to a ubiquinol with an ester linking group.

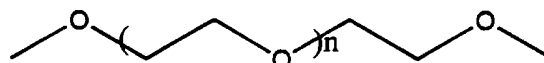
Treatment of reduced coenzyme Q10 with an N-hydroxysuccinimidyl active ester of a succinimide derivative of the discrete-length PEG-containing compound prepared as described in Example 1 in the presence of appropriate base (such as triethylamine or pyridine) in appropriate solvent (such as tetrahydrofuran) provides a
5 ubiquinone (50) ester linked discrete-length PEG-containing compound.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

We claim:

1. A polyethylene glycol-containing compound, comprising at least one discrete-length polyethylene glycol moiety having a molecular weight of at least about 300 grams/mole.

2. The polyethylene glycol-containing compound of Claim 1, wherein the discrete-length polyethylene glycol moiety comprises a radical selected from the group consisting of

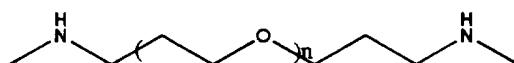


radicals, wherein $n = 7 - 1000$.

3. The polyethylene glycol-containing compound of Claim 1, wherein the discrete-length polyethylene glycol moiety comprises a radical selected from the group consisting of



; and



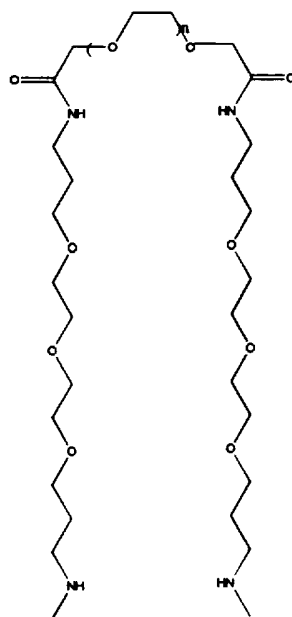
radicals, wherein $n = 1 - 3$.

4. The polyethylene glycol-containing compound of Claim 3, wherein the discrete-length polyethylene glycol moiety comprises a 4,7,10-trioxa-1,13-tridecane diamine radical.

5. The polyethylene glycol-containing compound of Claim 3, wherein the discrete-length polyethylene glycol moiety comprises a 3,6,9-trioxaundecanedioic acid radical.

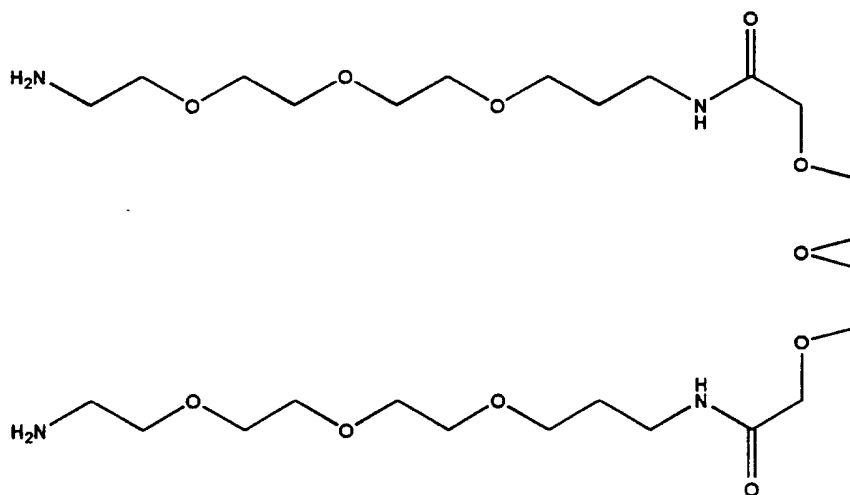
6. The polyethylene glycol-containing compound of Claim 3, wherein the discrete-length polyethylene glycol moiety comprises a radical selected from the group consisting of

-24-



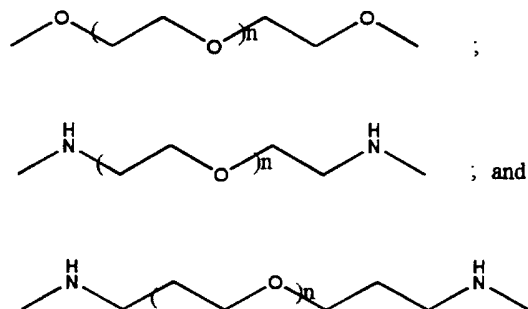
radicals, wherein $n = 0 - 2$.

7. The polyethylene glycol-containing compound



8. A polyethylene glycol-containing compound, comprising a discrete-length polyethylene glycol containing moiety having at least six ethylene glycol units and at least one cross-linking agent.

9. The polyethylene glycol-containing compound of Claim 8, wherein the discrete-length polyethylene glycol containing moiety comprises a radical selected from the group consisting of

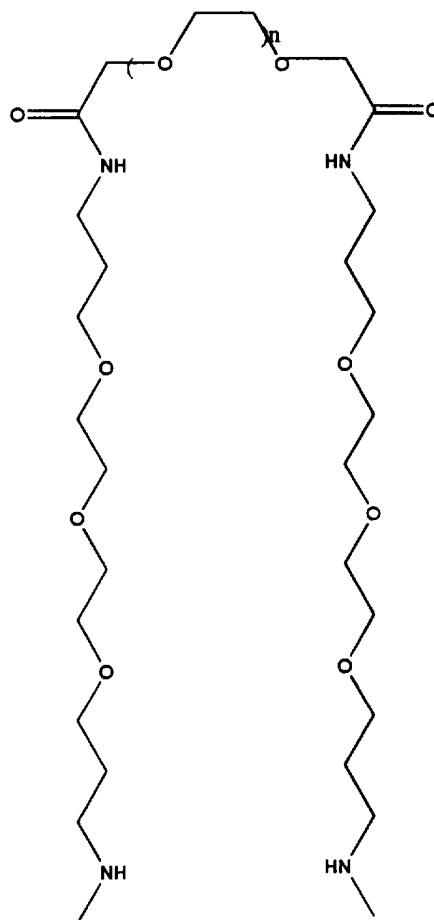


radicals, wherein $n = 1 - 3$.

10. The polyethylene glycol-containing compound of Claim 8, wherein the discrete-length polyethylene glycol containing moiety comprises a 4,7,10-trioxa-1,13-tridecanediamine radical.

11. The polyethylene glycol-containing compound of Claim 8, wherein the discrete-length polyethylene glycol containing moiety comprises a radical selected from the group consisting of

-26-



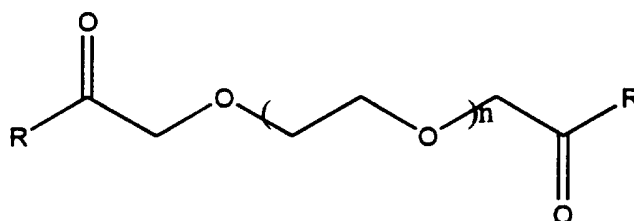
radicals, wherein $n = 0 - 2$.

12. The polyethylene glycol-containing compound of Claim 8, wherein the cross-linking agent is selected from the group consisting of bifunctional and trifunctional cross-linking agents.

13. The polyethylene glycol-containing compound of Claim 8, wherein the cross-linking agent is selected from the group consisting of an alkyl moiety having a displaceable leaving group, a carboxylic acid and a carboxylic acid derivative.

14. The polyethylene glycol-containing compound of Claim 13, wherein the carboxylic acid derivative is selected from the group consisting of an acid halide, an acid anhydride, and activated esters.

15. The polyethylene glycol-containing compound of Claim 12, wherein the bifunctional cross-linking agent is selected from the group consisting of



wherein $n = 0-3$, and R is independently selected from -OH, halide, -OCOR' wherein R' is an aryl or lower alkyl group, and an activated ester leaving group.

16. A method for preparing a discrete-length polyethylene glycol-containing compound, comprising:

reacting at least one cross-linking agent with at least one discrete-length polyethylene glycol derivative to form at least one covalent bond between the cross-linking agent and the discrete-length polyethylene glycol derivative,

to form a discrete-length polyethylene glycol-containing compound having at least six ethylene glycol units.

17. The method of Claim 16, wherein the step comprising reacting the cross-linking agent with the discrete-length polyethylene glycol derivative is repeated a multiple of times to provide a discrete-length polyethylene glycol or discrete-length polyethylene glycol-containing compound having from about 6 to about 1000 ethylene glycol units.

18. The method of Claim 16, further comprising:

reacting the discrete-length polyethylene glycol-containing compound with a second component to form a covalent bond between the polyethylene glycol-containing compound and the second component.

19. A composition, comprising a discrete-length polyethylene glycol moiety having a molecular weight of at least about 300 grams/mole coupled to a second component through a covalent bond.

20. A composition comprising a discrete-length polyethylene glycol containing moiety having at least six ethylene glycol units and at least one cross-linking agent coupled to a second component through a covalent bond.

21. The composition of Claim 19 or 20, wherein the second component has a low solubility in an aqueous environment.

22. The composition of Claim 19 or 20, wherein the second component is a biologically active molecule.

23. The composition of Claim 19 or 20, wherein the second component is a therapeutic agent.

24. The composition of Claim 23, wherein the therapeutic agent is selected from the group consisting of a ubiquinone and derivatives thereof.

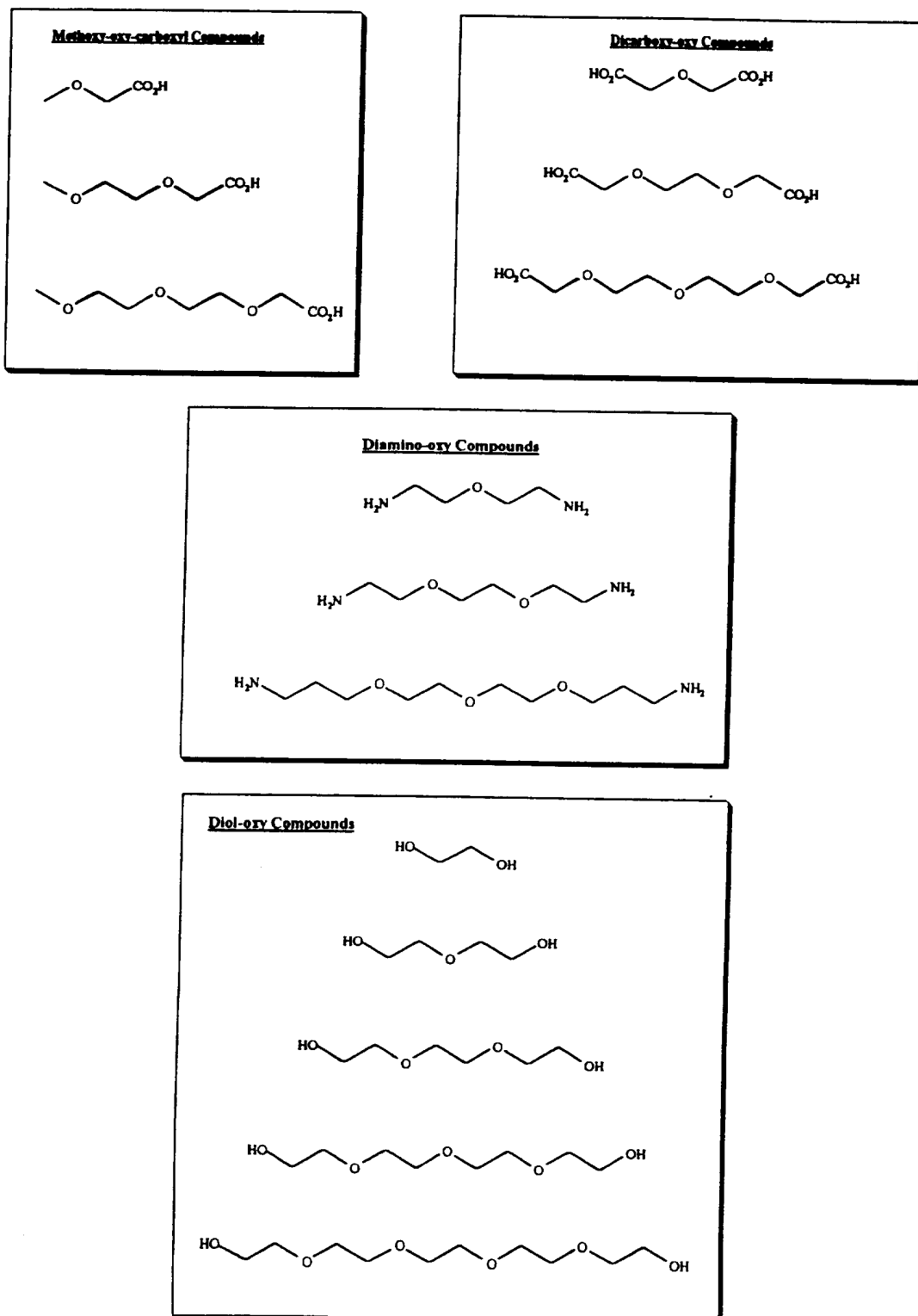
25. The composition of Claim 24, wherein the ubiquinone is ubiquinone 50.

26. The composition of Claim 25, wherein the therapeutic agent is selected from the group consisting of vitamin B₁₂ and derivatives thereof.

27. The composition of Claim 26, wherein the covalent bond is cleavable *in vivo*.

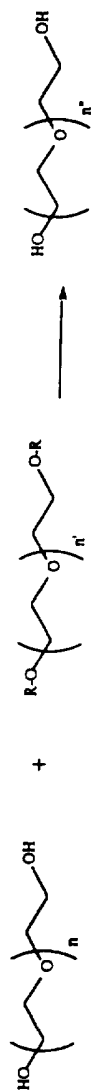
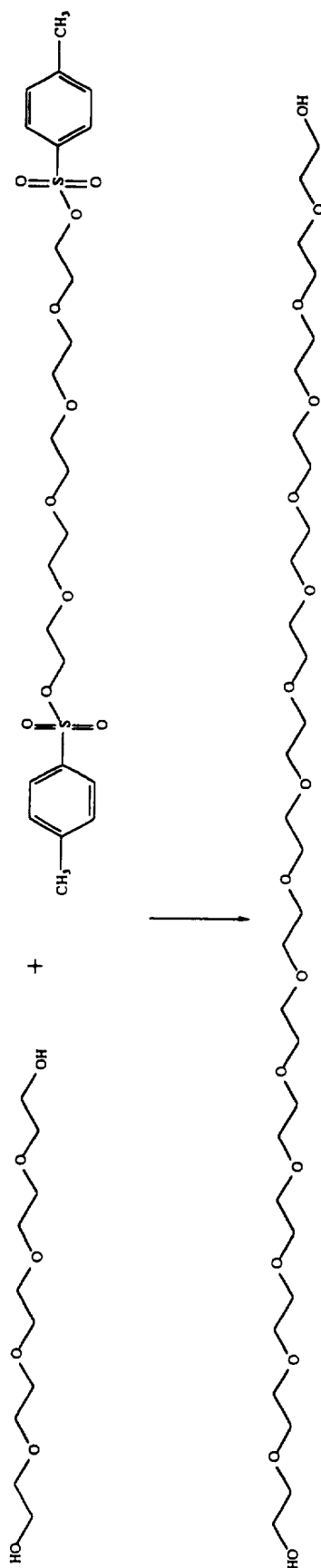
28. A pharmaceutical composition, comprising the composition of Claim 23 and a pharmaceutically acceptable carrier and/or diluent.

Figure 1

Commercially Available PEG Building Blocks

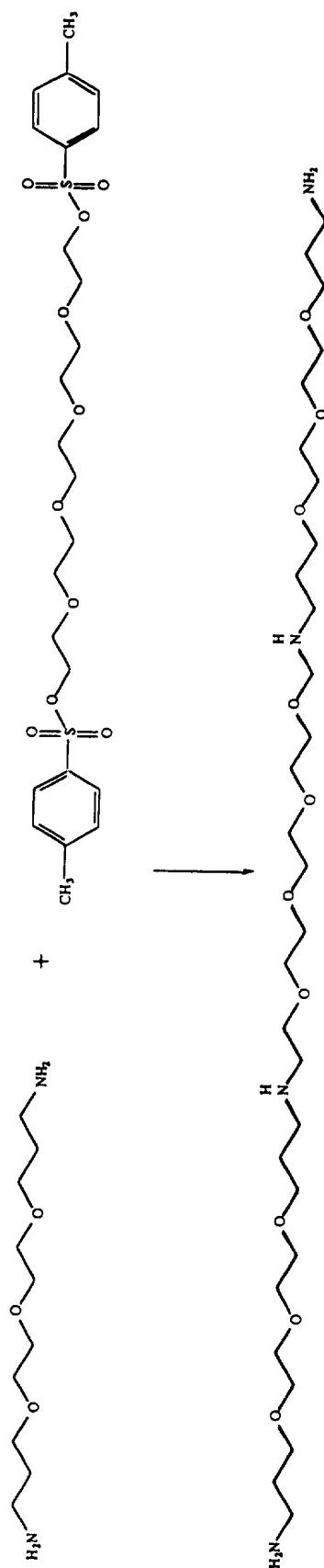
2/11

Figure 2

General Reaction:**Specific Example**

3/11

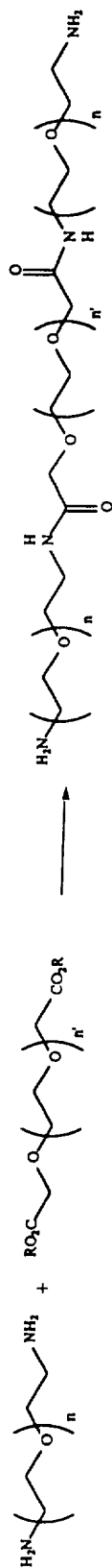
Figure 3

General Reaction:**Specific Example**

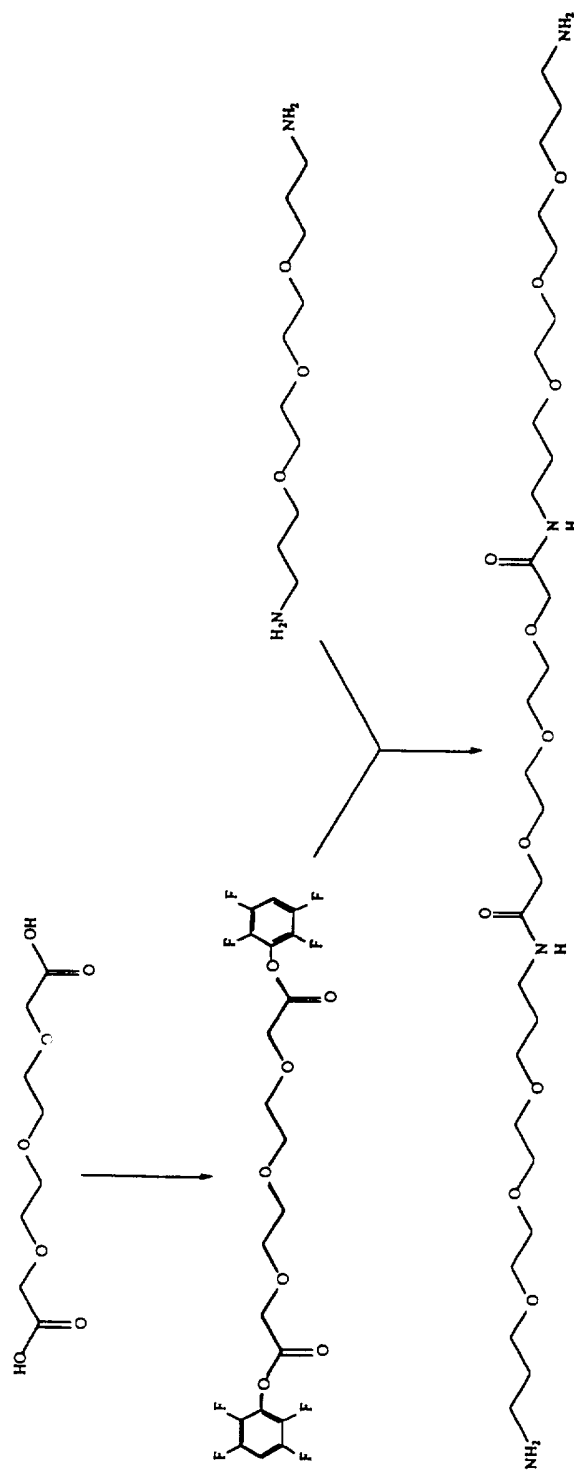
4/11

Figure 4

General Reaction:



Specific Example



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L

Step 1 ↓

$$\text{H}(-\text{P}-\text{L}-\text{P}-)\text{H}$$

Step 2 ↓

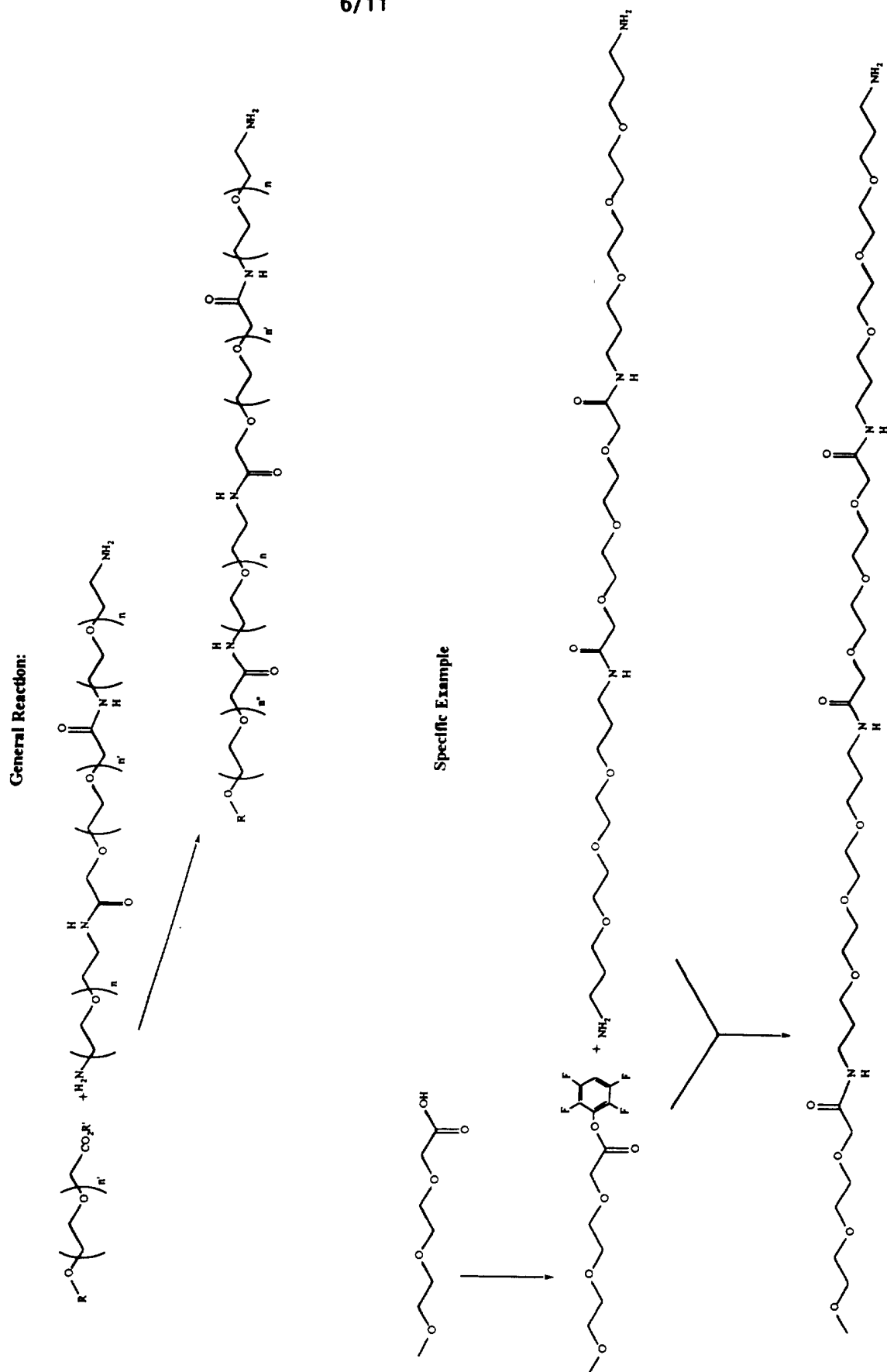
$$\text{H}(-\text{P}-\text{L}-\text{P})\text{L}(\text{P}-\text{L}-\text{P}-)\text{H}$$

Step 3 ↓

$$\text{H}(-\text{P}-\text{L}-\text{P}-\text{L}-\text{P}-\text{L}-\text{P})\text{L}(\text{P}-\text{L}-\text{P}-\text{L}-\text{P}-\text{L}-\text{P}-)\text{H}$$

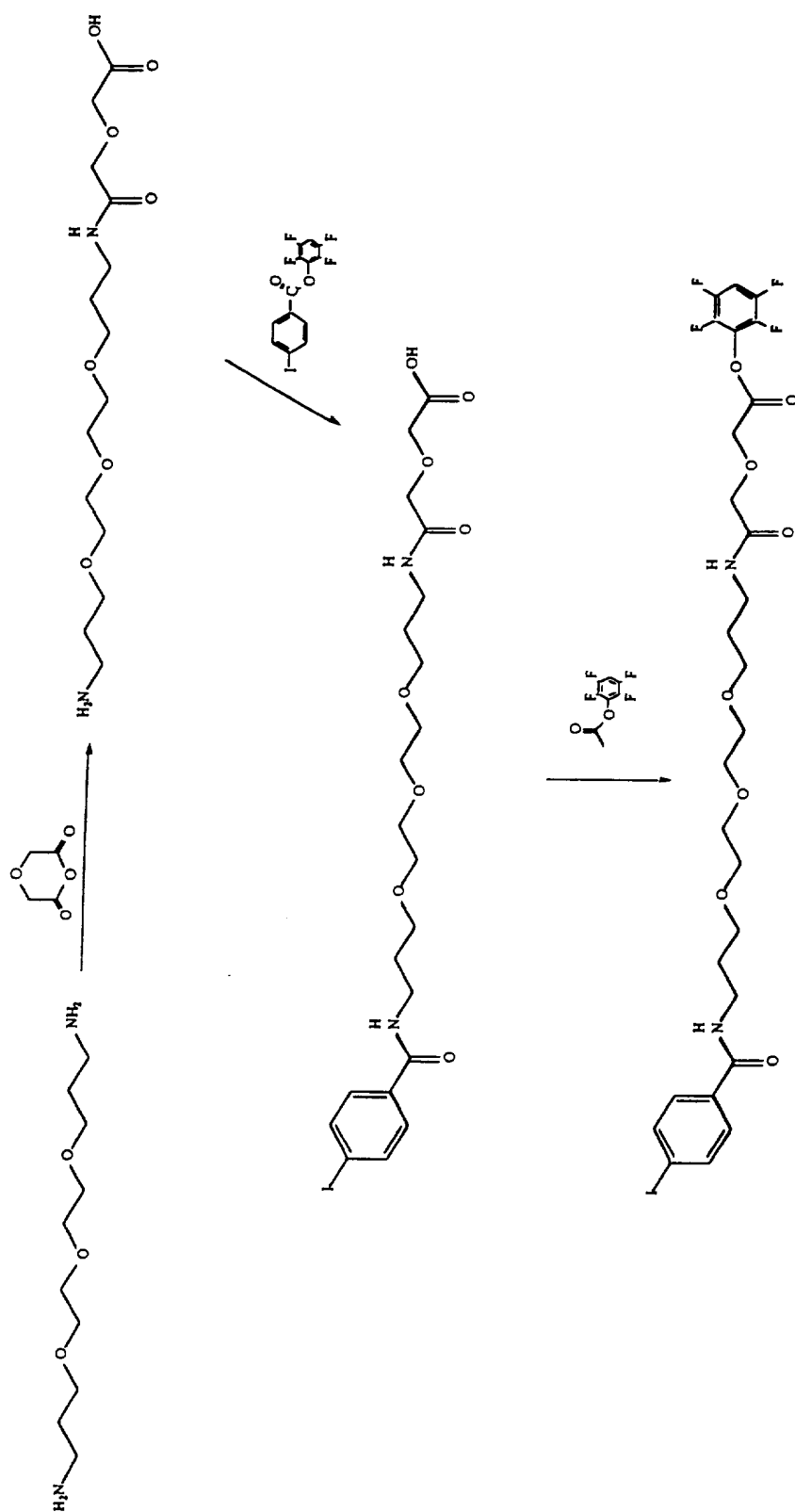
FIGURE 5

Figure 6



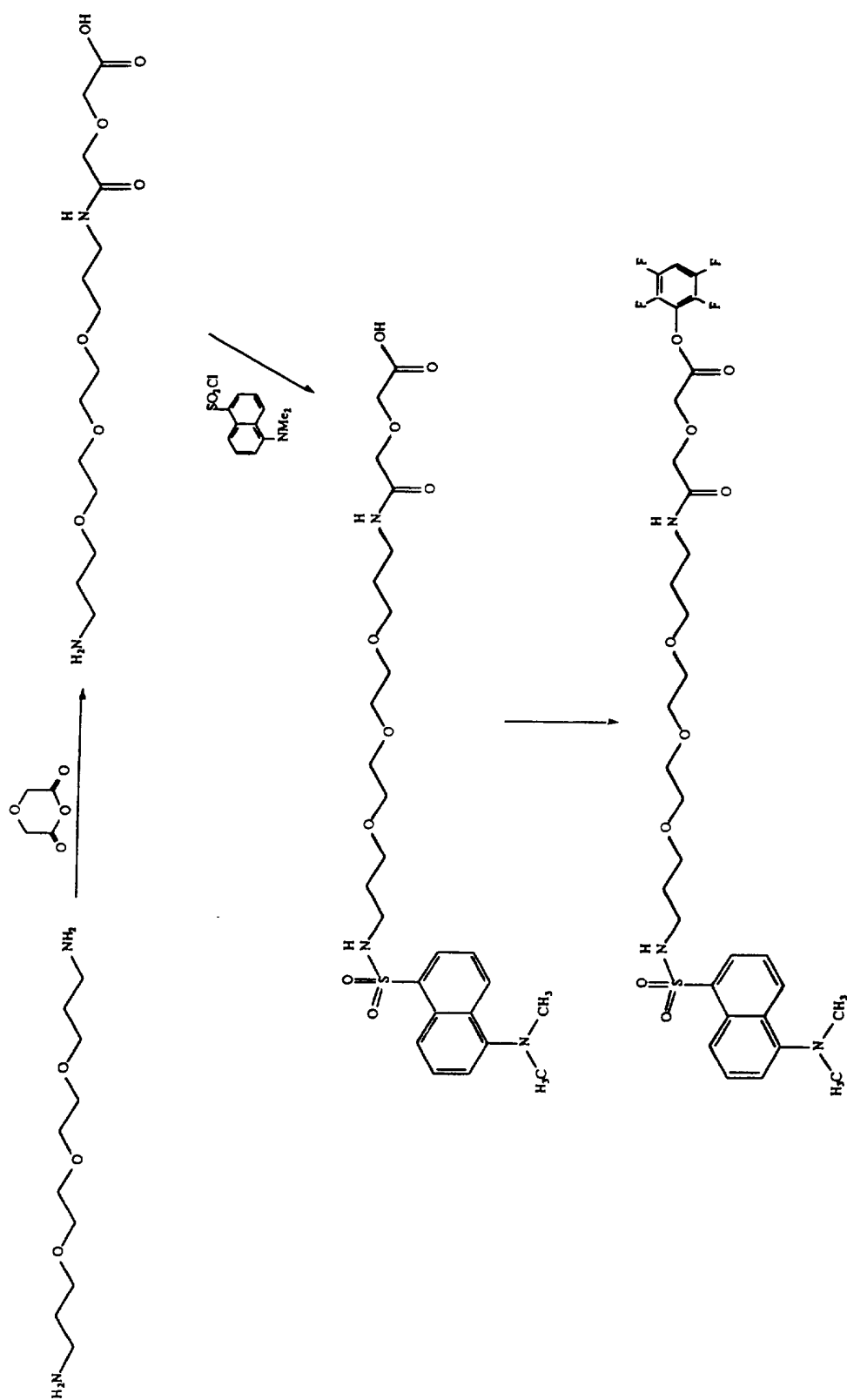
7/11

Figure 7



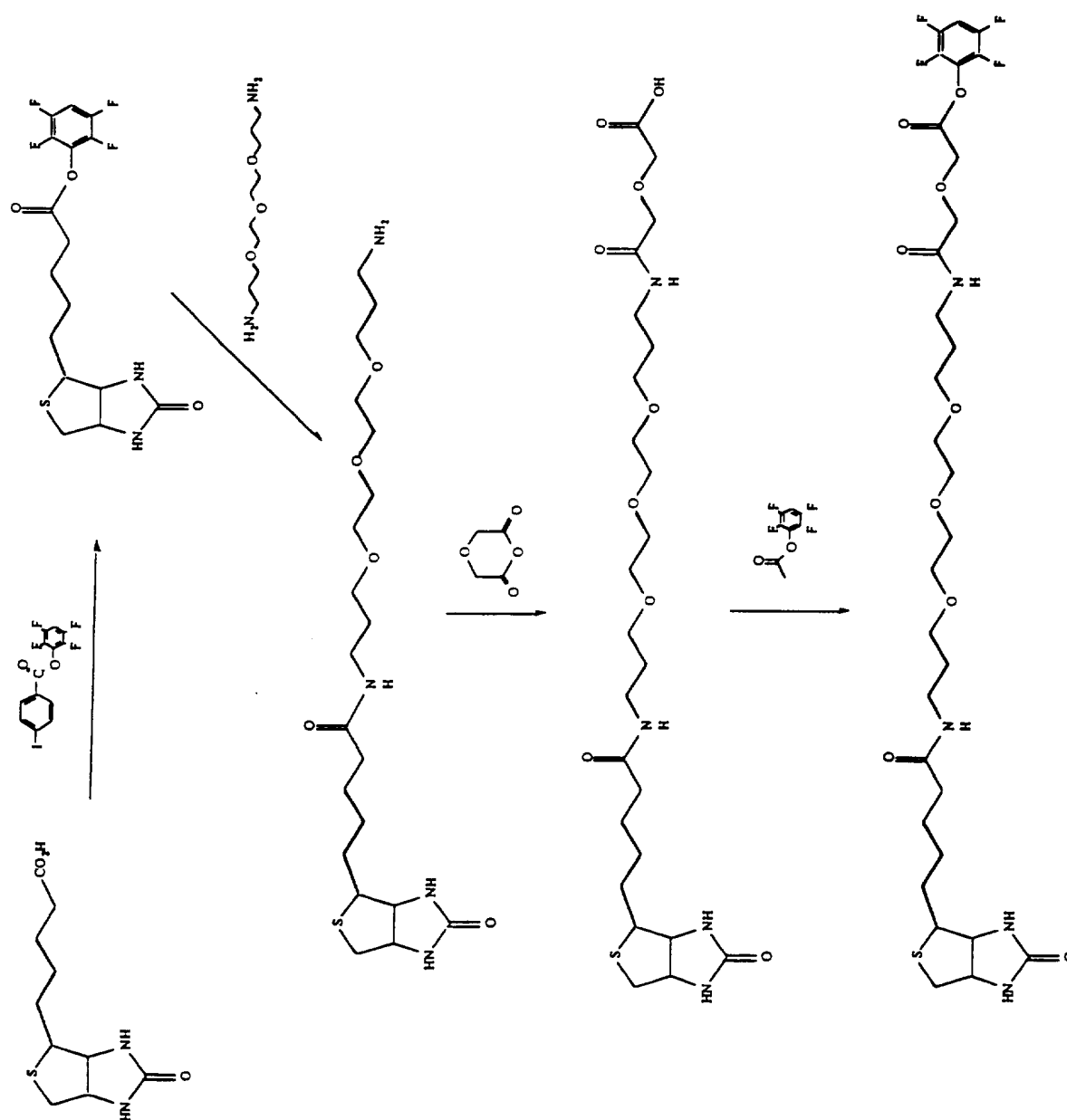
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Figure 8



9/11

Figure 9



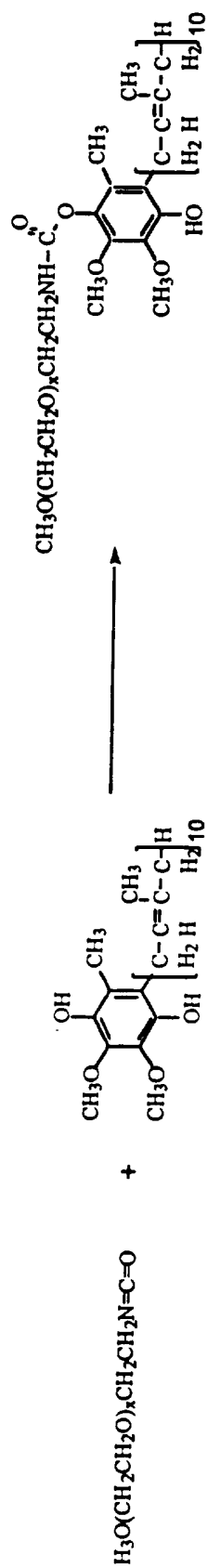
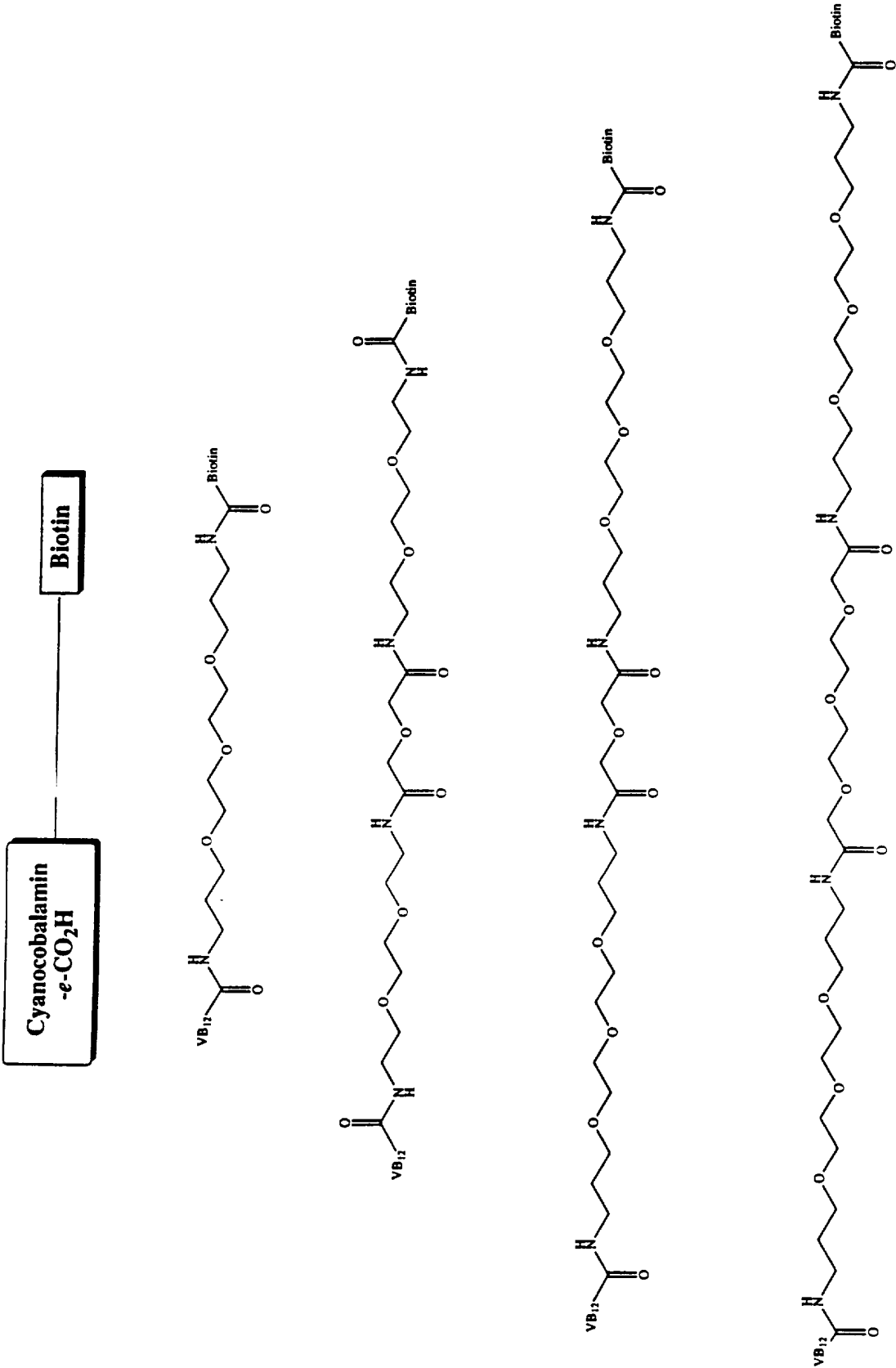


Figure 10

Figure 11



INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/16760

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C08G65/32 C08G65/34 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08G A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAKROMOLEKULARE CHEMIE, vol. 185, no. 8, 1984, HEIDELBERG , DEUTSCHLAND, pages 1559-1563, XP002024820 YEATES S.G. ET AL: "Ethylene glycol oligomers" see page 1559 - page 1560 ---	1,2,8,9, 12-14, 16,17
X	J. CONTROLLED RELEASE , vol. 34, no. 3, June 1995, AMSTERDAM, NL, pages 223-232, XP000505551 BONINA F.P. ET AL: "In vitro and in vivo evaluation of polyoxyethylene indomethacin esters as dermal prodrugs" see page 224; figure 1 --- -/-	1,19-23, 28

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

7 February 1997

Date of mailing of the international search report

21.02.97

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INTERNATIONAL SEARCH REPORT

International Application No

PCI/US 96/16760

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,95 25763 (BRACCO S.P.A) 28 September 1995 see table 4 ---	1-28
A	EP,A,0 335 972 (TERUMO CORP) 11 October 1989 see claims 1,5,16,18-20 ---	1,19-28
A	EP,A,0 301 716 (TEXACO DEVELOPMENT CORP) 1 February 1989 see table 1 -----	1,6,7,11

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PC I/US 96/16760

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		JP-A- 63130069	02-06-88
		DE-A- 3784518	08-04-93
		WO-A- 8803814	02-06-88

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		US-A- 5128441	07-07-92
