SYNTHESIS OF HYBRID BLOCK COPOLYMERS AND USES THEREOF

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Kim, et al., “Possibility of wound dressing using poly(L-leucine)/poly(ethylene glycol) poly(L-leucine) triblock copolymer” Biomaterials 21 (2000) 1311-141.

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ABSTRACT

The present invention relates to the field of polymer chemistry and more particularly to multiblock copolymers and methods of preparing the same.

14 Claims, No Drawings
OTHER PUBLICATIONS


Kim, et al., “Possibility of wound dressing using poly(L-leucine)/poly(ethyleneglycol)/poly(L-leucine) triblock copolymer” Biomaterials, 2000, 21, 131-141.


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SYNTHESIS OF HYBRID BLOCK COPOLYMERS AND USES THEREOF

BACKGROUND OF THE INVENTION

Multi-block copolymers comprising a synthetic polymer portion and a poly(amino acid) portion are of great synthetic interest. The poly(amino acid) portion of such polymers is typically prepared by the ring-opening polymerization of an amino acid-N-carboxy-anhydride (NCA). However, methods for preparing the poly(amino acid) block that employ free amines as initiators of the NCA polymerization afford block copolymers with a wide range of polydispersity indices (PDIs) that tend to be quite high. For example, Schléad and coworkers (J. Phys. Chem. B, 2003, 2944-2945) reported a PDI of 7.0 for poly(L-benzyl glutamate) copolymers and a PDI of 1.4 after fractionation. It is believed that, during the reaction, the chain end exists primarily in its unreactive salt form as a dormant species and that the unreactive amine salt is in equilibrium with the reactive amine. The free amine is capable of ring opening the NCA, which adds one repeat unit to the polymer chain. This cycle repeats until all of the monomer is consumed and the final poly(amino acid) is formed. This reported method has limitations in that only a single poly(amino acid) block is incorporated. In addition, this reported method only described the use of a polystyrene macroinitiator. In another publication by Schléad and coworkers (Eur. Phys. J., 2003, 10, 17-23), the author indicates that use of a PEG macroinitiator results in diverse and unpredictable PDIs. The author further indicates that even “the coupling of preformed polymer segments like that of a haloacetylated poly(ethylene oxide) with poly(L-aspartic acid) . . . yields block copolymers that are chemically disperse and are often contaminated with homopolymers.”

The present invention provides methods for the synthesis of block copolymers containing one or more poly(amino acid) blocks and one or more synthetic polymer blocks. The poly(amino acid) portions of these block copolymers are prepared by controlled ring-opening polymerization of cyclic monomers such as N-carboxy anhydrides (NCAs), lactams, and cyclic imides wherein said polymerization is initiated by an amine salt. The amine salt initiators used in this invention are polymers with terminal amine salts (referred to herein as “macroinitiators”). Without wishing to be bound by any particular theory, it is believed that the amine salt reduces or eliminates many side reactions that are commonly observed with traditional polymerization of these reactive monomers. This leads to block copolymers with narrow distributions of block lengths and molecular weights. It has been surprisingly found that the sequential polymerization of monomers provides multi-block copolymers having desirable low polydispersity. The sequential addition of cyclic monomers to a “living” polymer chain end (i.e. a terminal amine salt) affords multi-block copolymers having a variety of poly(amino acid) and synthetic polymer block types. Accordingly, one aspect of the present invention provides a method for preparing a multi-block copolymer comprising two or more different poly(amino acid) blocks and one or more synthetic polymer blocks, wherein said method comprises the step of sequentially polymerizing two or more different cyclic amino acid monomers onto a synthetic polymer having a terminal amine salt wherein said polymerization is initiated by said amine salt.

2. Definitions

Compounds of this invention include those described generally above, and are further illustrated by the embodiments, sub-embodiments, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements. CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in “Organic Chemistry”, Thomas Sorrell, University Science Books, Sausalito: 1999, and “March’s Advanced Organic Chemistry”, 5th Ed., Ed.: Smith, M. B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

As used herein, the term “sequential polymerization”, and variations thereof, refers to the method wherein, after a first monomer (e.g. NCA, lactam, or imide) is incorporated into the polymer, the polymerizes an amino acid “block”, a second monomer (e.g. NCA, lactam, or imide) is added to the reac-
tion to form a second amino acid block, which process may be continued in a similar fashion to introduce additional amino acid blocks into the resulting multi-block copolymers.

As used herein, the term “block copolymer” refers to a polymer comprising at least one synthetic polymer portion and at least one poly(aminoc acid) portion. The term “multi-block copolymer” refers to a polymer comprising at least one synthetic polymer and two or more poly(aminoc acid) portions. These are also referred to as triblock copolymers (having two poly(aminoc acid) portions), tetrablock copolymers (having three poly(aminoc acid) portions), etc. Such multi-block copolymers include those having the format X-W-X, X-W-X', W-X-W', X'-W-X', X'-W-X-W', X'-W-X'-W', or W-X'-X whereby W is a synthetic polymer portion and X, X', or X'' are poly(aminoc acid) chains or “amino acid blocks”. In certain aspects, the synthetic polymer is used as the center block which allows the growth of multiple blocks symmetrically from the center.

As used herein, the term “synthetic polymer” refers to a polymer that is not a poly(aminoc acid). Such synthetic polymers are well known in the art and include polysyrenes, polyalkylene oxides, such as polyethyleneoxide (also referred to as polyethylene glycol or PEG), and derivatives thereof.

As used herein, the term “poly(aminoc acid)” or “amino acid block” refers to a covalently linked amino acid chain wherein each monomer is an amino acid unit. Such amino acid units include natural and unnatural amino acids. In certain embodiments, each amino acid unit is in the L-configuration. Such poly(aminoc acids) include those having suitably protected functional groups. For example, amino acid monomers may have hydroxyl or amino moieties which are optionally protected by a suitable hydroxyl protecting group or a suitable amine protecting group, as appropriate. Such suitable hydroxyl protecting groups and suitable amine protecting groups are described in more detail herein. As used herein, an amino acid block comprises one or more monomers or a set of two or more monomers. In certain embodiments, an amino acid block comprises one or more monomers such that the overall block is hydrophilic.

As used herein, amino acid blocks of the present invention include random amino acid blocks, i.e., blocks comprising a mixture of amino acid residues.

As used herein, the phrase “natural amino acid side-chain group” refers to the side-chain group of any of the 20 amino acids naturally occurring in proteins. Such natural amino acids include the nonpolar, or hydrophobic amino acids, glycine, alanine, valine, leucine isoleucine, methionine, phenylalanine, tryptophan, and proline. Cysteine is sometimes classified as nonpolar or hydrophobic and other times as polar. Natural amino acids also include polar, or hydrophilic amino acids, such as tyrosine, serine, threonine, aspartic acid (also known as aspartate, when charged), glutamic acid (also known as glutamate, when charged), asparagine, and glutamine. Certain polar, or hydrophilic, amino acids have charged side-chains. Such charged amino acids include lysine, arginine, and histidine. One of ordinary skill in the art would recognize that protection of a polar or hydrophilic amino acid side-chain can render that amino acid nonpolar. For example, a suitably protected tyrosine hydroxyl group can render that tyrosine nonpolar and hydrophobic by virtue of protecting the hydroxyl group.

As used herein, the phrase “unnatural amino acid side-chain group” refers to amino acids not included in the list of 20 amino acids naturally occurring in proteins, as described above. Such amino acids include the D-isomer of any of the 20 naturally occurring amino acids. Unnatural amino acids also include homoserine, ornithine, and thyroxine. Other unnatural amino acids side-chains are well known to one of ordinary skill in the art and include unnatural aliphatic side chains. Other unnatural amino acids include modified amino acids, including those that are N-alkylated, cyclized, phosphorylated, acetylated, amidated, labelled, and the like.

As used herein, the phrase “living polymer chain-end” refers to the terminus resulting from a polymerization reaction which enables the polymer to react further with additional monomer or with a polymerization terminator.

As used herein, the term “termination” refers to attaching a terminal group to a polymer chain-end by the reaction of a living polymer with an appropriate compound. Alternatively, the term “termination” may refer to attaching a terminal group to an amine or hydroxyl end, or derivative thereof, of the polymer chain.

As used herein, the term “polymerization terminator” is used interchangeably with the term “polymerization terminating agent” and refers to a compound that reacts with a living polymer chain-end to afford a polymer with a terminal group. Alternatively, the term “polymerization terminator” may refer to a compound that reacts with an amine or hydroxyl end, or derivative thereof, of the polymer chain, to afford a polymer with a terminal group.

As used herein, the term “polymerization initiator” refers to a compound, which reacts with, or whose anion or free base form reacts with, the desired monomer in a manner which results in polymerization of that monomer. In certain embodiments, the polymerization initiator is the compound that reacts with an alkylene oxide to afford a polyalkylene oxide block. In other embodiments, the polymerization initiator is the amine salt described herein.

The term “aliphatic” or “aliphatic group”, as used herein, denotes a hydrocarbon moiety that may be straight-chain (i.e., unbranched), branched, or cyclic (including fused, bridged, and spiro-fused polycyclic) and may be completely saturated or may contain one or more units of unsaturation, but which is not aromatic. Unless otherwise specified, aliphatic groups contain 1-20 carbon atoms. In some embodiments, aliphatic groups contain 1-10 carbon atoms. In other embodiments, aliphatic groups contain 1-8 carbon atoms.

The term “heteroatom” means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon. This includes any oxidized form of nitrogen, sulfur, phosphorus, or silicon, the quaternized form of any basic nitrogen, or a substitutable nitrogen of a heterocyclic ring including: —N— as in 3,4-dihydro-2H-pyrrol, —NH— as in pyrrolidinyl, or —N(R)— as in N-substituted pyrrolidinyl. The term “unsaturated”, as used herein, means that a moiety has one or more units of unsaturation.

The term “aryl” used alone or as part of a larger moiety as in “aralkyl”, “aralkoxy”, or “aryloxoyalkyl”, refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term “aryl” may be used interchangeably with the term “aryl ring”.

As described herein, compounds of the invention may contain “optionally substituted” moieties. In general, the term “substituted”, whether preceded by the term “optionally” or
not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a "optionally substituted" carbon atom of an "optionally substituted" group are independently halogen; -(CH₂)ₙ₂R⁺, -(CH₂)ₙ₋₁OR⁺, -O-(CH₂)ₙ₋₁C(O)OR⁺; -(CH₂)ₙ₋₁CH(O)OR⁺; -(CH₂)ₙ₋₁SR⁺; -(CH₂)ₙ₋₁Ph, which may be substituted with R⁺; -(CH₂)ₙ₋₁O(CH₂)ₘ₁Ph which may be substituted with R⁺; -(CH₂)ₙ₋₁CH₂Ph, which may be substituted with R⁺; -(CH₂)ₙ₋₁NO₂; -(CH₂)ₙ₋₁CN; -(CH₂)ₙ₋₁N=S, -(CH₂)ₙ₋₁NR⁺₂, -(CH₂)ₙ₋₁NNHC(O)R⁺, -(CH₂)ₙ₋₁NNHC(O)OR⁺, -(CH₂)ₙ₋₁NNHS(O)₂R⁺, -(CH₂)ₙ₋₁NR⁺, -(CH₂)ₙ₋₁N=O, -(CH₂)ₙ₋₁S-(CH₂)ₙ₋₁; wherein each R⁺ may be replaced with a suitable substituent, such as a halogen, alkyl, or aryl group further including, but not limited to, esters, carbonates, sulfonates, allyl ethers, ethers, silyl ethers, and the like.

Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: -S-, -(CH₂)ₙ₋₁S-, -(S(O)₂)ₙ₋₁S-, -(S(O)₂)ₙ₋₁S-, -(S(O)₂)ₙ₋₁S-, -(S(O)₂)ₙ₋₁S-, where each S⁻ is independently halogen, -(CH₂)ₙ₋₁S-, where each S⁻ is independently halogen, -(CH₂)ₙ₋₁S-, or -(CH₂)ₙ₋₁S-, which may be substituted with a suitable substituent, such as a halogen, alkyl, or aryl group further including, but not limited to, esters, carbonates, sulfonates, allyl ethers, ethers, silyl ethers, and the like.

Suitable substituents on the aliphatic group of R⁺ include halogen, -(CH₂)ₙ₋₁S-, -(CH₂)ₙ₋₁OR⁺, -(CH₂)ₙ₋₁NR⁺₂, -(CH₂)ₙ₋₁O(OH), -(CH₂)ₙ₋₁SH, -(CH₂)ₙ₋₁N=O, -(CH₂)ₙ₋₁S-, -(CH₂)ₙ₋₁N=O, -(CH₂)ₙ₋₁S-, -(CH₂)ₙ₋₁N=O, -(CH₂)ₙ₋₁S-, or -(CH₂)ₙ₋₁S-, which may be substituted with a suitable substituent, such as a halogen, alkyl, or aryl group further including, but not limited to, esters, carbonates, sulfonates, allyl ethers, ethers, silyl ethers, and the like.
alkyl ethers, arylalkyl ethers, and alkoxyalkyl ethers. Examples of suitable esters include formates, acetates, propionates, pentanoates, crotonates, and benzoates. Specific examples of suitable esters include formate, benzoate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4’-(ethylene|dithio)pentanoate, pyr- 
aldehyde(3-trimethylacetal), crotonate, 4-methoxy-crotonate, benzoate, p-benzybenzoate, 2,4,6-trimethylbenzoate. Examples of suitable carbonates include 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethy, 2-(p- 
henylylsulfonyl)vinyl, allyl, and p-nitrobenzyl carbonate. Examples of suitable silyl ethers include trimethylsilyl, tri- 
ethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, tris(o-
propylsilyl)ether, and other trialkysilyl ethers. Examples of suitable alkyl ethers include methyl, benzy, p-
hydroxymethyl, 3,4-dimethoxybenzyl, triyl, t-butyl, and allyl ether, or derivatives thereof. Alkoxyalkyl ethers include acetals such as methoxymethyl, methyliothio methyl, (2-methoxethoxy) methyl, benzoxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrachloropyran-2-yl ether. Examples of suitable arylalkyl ethers include benzy, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-
hydrobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-pico-
lyl ethers.

Protected amines are well known in the art and include those described in detail in Greene (1999). Suitable mono-
protected amines further include, but are not limited to, anilinlamines, carbamates, allyl amine, amides, and the like. Examples of suitable mono-protected amino moieties include t-
butoxy carbonylaminio (-NHBOC), ethoxycarbonyl-
amino, methoxycarbonylamino, trichloroethoxycarbo-
nylamino, allyloxycarbonylamino (-NHAla), benzoxyst-
carbonylamino (-NICO2), allylamino, benzylaminio (-NHBn), fluorenylmethy carbonyl (-NHFmoc), formamide, acetamido, chloroacetamido, dichloroacetamido, trichloro-
acetamido, phenylacetamido, trithiocetaenamido, benzami-
do, t-butyldiphenylsilyl, and the like. Suitable di-protected amines include amides that are substituted with two substitu-
teus independently selected from those described above as mono-
protected amines, and further include cyclic imides, such as pthalimide, maleimide, succinimide, and the like. Suitable di-protected amines also include pyrroles and the like, 2,2,5,5-tetramethyl-[1,2,5] 

zoazasilolidine and the like, and azide.

Protected aldehydes are well known in the art and include those described in detail in Greene (1999). Suitable protected aldehydes further include, but are not limited to, acyclic acetals, cyclic acetals, hydrazones, imines, and the like. Examples of such groups include dimethyl acetal, diethyl acetal, disopropyl acetal, dibenzyl acetal, bis(2-nitrobenzyl) acetal, 1,3-dioxanes, 1,3-dioxolanes, semicarbazones, and derivatives thereof.

Protected carboxylic acids are well known in the art and include those described in detail in Greene (1999). Suitable protected carboxylic acids further include, but are not limited to, optionally substituted C6-8 aliphatic esters, optionally sub-
stituted ary1 esters, silyl esters, activated esters, amides, hydrazides, and the like. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, ben-
zy1, and phenyl ether, wherein each group is optionally sub-
stituted. Additional suitable protected carboxylic acids include oxazolines and ortho esters.

Protected thiols are well known in the art and include those described in detail in Greene (1999). Suitable protected thiols further include, but are not limited to, disulfides, thioethers, silyl thioethers, thioesters, thiacarbonates, and thiothea-

mols, and the like. Examples of such groups include, but are not limited to, alkyl thioethers, benzyl and substituted benzyl thioethers, triphenylmethyl thioethers, and trichloroethoxy-
benzoyl thioester, to name but a few.

A “crown ether moiety” is the radical of a crown ether. A crown ether is a mononuclear polyether comprising of repeating units of —CH2CH2O—. Examples of crown ethers include 12-crown-4, 15-crown-5, and 18-crown-6.

Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastere- 
meric, and geometric or conformational) forms of the struc-
ture; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E 
conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise 


described, structures depicted herein are also meant to include compounds that differ only in the presence of one or 
mores isolatedly enriched atoms. For example, compounds 
having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a 
carbon by a 13C- or 14C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as 
analytical tools or probes in biological assays.

As used herein, the term “detectable moiety” is used inter-
changeably with the term “label” and relates to any moiety 
capable of being detected (e.g., primary labels and secondary 
labels). A “detectable moiety” or “label” is the radical of a 
detectable compound.

“Primary” labels include radioisotope-containing moieties (e.g., moieties that contain 52P, 33P, 35S, or 34S), mass-tags, and 
fluorescent labels, and are signal-generating reporter groups which can be detected without further modifications.

Other primary labels include those useful for positron emission tomography including molecules containing radio-
isotopes (e.g., 18F) or ligands with bound radioactive metals (e.g., 52Cu). In other embodiments, primary labels are contrast agents for magnetic resonance imaging such as gadolinium,
gadolinium chelates, or iron oxide (e.g. Fe3O4 and Fe2O3) 
particles. Similarly, semiconducting nanoparticles (e.g. cad-
mium selenide, cadmium sulfide, cadmium telluride) are use-
ful as fluorescent labels. Other metal nanoparticles (e.g. col-
loidal gold) also serve as primary labels.

“Secondary” labels include moieties such as biotin, or 
protein antigens, that require the presence of a second com-
ponent to produce a detectable signal. For example, in the case 
of a biotin label, the second compound may include strepta-
vidin-enzyme conjugates. In the case of an antigen label, the 
second compound may include an antibody-enzyme conjuga-
t. Additionally, certain fluorescent groups can act as sec-
ondary labels by transferring energy to another compound or 
group in a process of nonradiative fluorescent resonance energy transfer (FRET), causing the second compound or 
group to then generate the signal that is detected.

Unless otherwise indicated, radioisotope-containing moi-
eytes are optionally substituted hydrocarbon groups that con-
tain at least one radioisotope. Unless otherwise indicated, 
radioisotope-containing moieties contain from 1-40 carbon 
atoms and one radioisotope. In certain embodiments, radio-
isotope-containing moieties contain from 1-20 carbon atoms 
and one radioisotope.

The terms “fluorescent label”, “fluorescent group”, “flu-
orescent compound”, “fluorescent dye”, and “fluorophore”, as 
used herein, refer to compounds or moieties that absorb light
energy at a defined excitation wavelength and emit light energy at a different wavelength. Examples of fluorescent compounds include, but are not limited to: Alexa Fluor dyes (Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660 and Alexa Fluor 680), AMCA, AMCA-S, BODIPY dyes (BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665), Carboxy-rhodamine 6G, carboxy-X-rhodamine (ROX), Cascade Blue, Cascade Yellow, Coumarin 343, Cyamine dyes (Cy3, Cy5, Cy3.5, Cy5.5), Dapsyl, Dapoxyl, Diallylaminocoumarin, 4',5'-Dichloro-2',7'-dimethoxy-fluorescein, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IR Dyes (IRD40, IRD 700, IRD 800), JOE, Lissamine rhodamine B, Marina Blue, Methoxyxocoumarin, Naphthofluorescein, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, PyMPO, Pyrene, Rhodamine B, Rhodamine 6G, Rhodamine Green, Rhodamine Red, Rhodol Green, 2',4',5',7'-Tetramethylrhodamine (TMR), Carboxytetramethylrhodamine (TAMRA), Texas Red, Texas Red-X. The term “mass-tag” as used herein refers to any moiety that is capable of being uniquely detected by virtue of its mass using mass spectrometry (MS) detection techniques. Examples of mass-tags include electrophore release tags such as N-[3-4'-[p-Methoxytetrafluorobenzoyl]oxyphenyl]-3-methyglycerolyl]isonicotic Acid, 4'-[2,3,5,6-Tetrafluorouracil][methyl acetonaphenyl], and their derivates. The synthesis and utility of these mass-tags is described in U.S. Pat. Nos. 4,650,750, 4,709,016, 5,360,819, 5,516,931, 5,602,273, 5,604,104, 5,610,020, and 5,650,270. Other examples of mass-tags include, but are not limited to, nucleotides, dideoxynucleotides, oligonucleotides of varying length and base composition, oligopeptides, oligosaccharides, and other synthetic polymers of varying length and monomer composition. A large variety of organic molecules, both neutral and charged (biomolecules or synthetic compounds) of an appropriate mass range (100-2000 Daltons) may also be used as mass-tags.

The term “substate”, as used herein refers to any material or macromolecular complex to which a functionalized endgroup of a block copolymer can be attached. Examples of commonly used substrates include, but are not limited to, glass surfaces, silica surfaces, plastic surfaces, metal surfaces, surfaces containing a metallic or chemical coating, membranes (e.g., nylon, polysulfone, silica), micro-beads (e.g., latex, polystyrene, or other polymer), porous polymer matrices (e.g., polysacrylamide gel, polysaccharide, polymethacrylate), macromolecular complexes (e.g., protein, polysaccharide).

3. Description of Exemplary Embodiments

As described generically above, one aspect of the present invention provides a method for preparing a multi-block copolymer comprising one or more poly(amicid acid) blocks and one or more synthetic polymer blocks, wherein said method comprises the steps of sequentially polymerizing one or more cyclic amino acid monomers onto a synthetic polymer having a terminal amine salt wherein said polymerization is initiated by said amine salt. In certain embodiments, said polymerization occurs by ring-opening polymerization of the cyclic amino acid monomers. In other embodiments, the cyclic amino acid monomer is an amino acid NCA, lactam, or imide.

As described generally above, the synthetic polymers used in methods of the present invention have a terminal amine salt for initiating the polymerization of a cyclic amino acid monomer. Such salts include the acid addition salts of an amino group formed with an inorganic acid such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid or perchloric acid. It is also contemplated that such amine salts include the acid addition salts of an amino group formed with an organic acid such as acetic acid, oxalic acid, malonic acid, tartaric acid, citric acid, succinic acid, malonic acid, and the like, or by using other methods used in the art such as ion exchange.

As described generally above, the synthetic polymers used in methods of the present invention have a terminal amine salt. In certain embodiments, the synthetic polymer is poly (ethylene glycol) (PEG) having a terminal amine salt (“PEG macrorinitiator”) which initiates the polymerization of NCAs to provide PEG-poly(amiacid acid) multi-block copolymers. Such synthetic polymers having a terminal amine salt may be prepared from synthetic polymers having a terminal amine. Such synthetic polymers having a terminal amine group are known in the art and include PEG-aminates. PEG-aminates may be obtained by the deprotection of a suitably protected PEG-amine. Preparation of such suitably protected PEG-aminates, and methods of deprotecting the same, is described in detail in U.S. patent application Ser. No. 11/256,735, filed Oct. 24, 2005 the entirety of which is hereby incorporated herein by reference.

As described in U.S. Ser. No. 11/256,735, suitably protected PEG-aminates may be formed by terminating the living polymer chain end of a PEG with a terminating agent that contains a suitably protected amine. The suitably protected amine may then be deprotected to generate a PEG that is terminated with a free amine that may subsequently be converted into the corresponding PEG-amine salt macroinitiator. In certain embodiments, the PEG-amine salt macroinitiator of the present invention is prepared directly from a suitably protected PEG-amine by deprotecting said protected amine with an acid. Accordingly, in other embodiments, the terminating agent has suitably protected amino group wherein the protecting group is acid-labile.

Alternatively, suitable synthetic polymers having a terminal amine salt may be prepared from synthetic polymers that contain terminal functional groups that may be converted to amine salts by known synthetic routes. In certain embodiments, the conversion of the terminal functional groups to the amine salts is conducted in a single synthetic step. In other embodiments, the conversion of the terminal functional groups to the amine salts is achieved by way of a multi-step sequence. Functional group transformations that afford amines, amine salts, or protected amines are well known in the art and include those described in Larock, R. C., “Comprehensive Organic Transformations:” John Wiley & Sons, New York, 1999.

Alternatively, and as described in detail in U.S. Ser. No. 11/256,735, suitably protected PEG-aminates may be formed by initiating the polymerization of ethylene oxide with a compound that contains a suitably protected amine moiety. The PEG formed therefrom may be terminated by any manner known in the art, including those described in U.S. Ser. No. 11/256,735. The method of termination may incorporate a additional suitably protected amine functional group, or a precursor thereto, such that each terminal of the PEG formed therefrom may be subsequently converted to an amine salt that may be employed in the polymerization of the cyclic monomers described herein. In certain embodiments, only one terminal of such a PEG is converted to an amine salt that is then employed in the formation of one or more poly(amicid acid) blocks. Following such polymerizations, the amine salt
terminus may be converted to an unreactive form, and then the other terminus may be converted to an amine salt for use in the introduction of additional poly(amino acid) blocks.

In another embodiment, both termini of a PEG are converted to amine salts that are then employed in bidirectional polymerization to introduce poly(amino acid) blocks concomitantly at each end. Such bidirectional polymerization is depicted in Scheme 2, below.

One of ordinary skill in the art would recognize that the embodiments described above and herein that employ PEG as the synthetic polymer block can be readily applied to other synthetic polymers. Therefore, this invention contemplates multiblock copolymers of the permutations described herein that employ synthetic polymers other than PEG. In certain embodiments, the synthetic polymer block is polypropylene oxide (PPO), PEG-PPO-PEG block copolymers (Pluronic®), polyesters, polyamides, poly(ethylene imine), polyphosphazenes, polyacrylates, or polymethacrylates.

In certain embodiments, the synthetic polymer is poly(ethylene glycol) (PEG) having one or two terminal amine salt(s) ("PEG macroinitiator") to initiate the polymerization of NCAs to provide a PEG-poly(amo acid) multi-block copolymer as illustrated in Schemes 1 and 2, below.

Scheme 1 above depicts a polymerization method of the present invention. A macroinitiator of formula I is treated with a first amino acid NCA to form a compound of formula I-a having a first amino acid block. The second amino acid NCA is added to the living polymer of formula I-a to form a compound of formula II having two differing amino acid blocks. Each of the $R^1$, $A$, $n$, $Q$, $R^2$, $R'$, $m$, and $m'$ groups depicted in Scheme 1 are as defined and described in classes and subclasses, singly and in combination, herein.

Scheme 2
Scheme 2 above depicts the synthesis of a PAA-b-PAA-b-PEG-b-PAA-b-PAA pentablock copolymer according to the present invention, wherein each of \( R^1, R^2, m, n, A, \) and \( Q \) are as defined herein and in classes and subclasses, singly and in combination.

Another aspect of the present invention provides a method of preparing a multi-block copolymer comprising two or more different poly(amine acid) blocks and a PEG synthetic polymer block, wherein said method comprises the steps of:

(a) providing a compound of formula 1:

\[
\begin{align*}
\text{R}^1 & \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{N}^+ \quad \text{A} \\
\text{A-HN} & \quad \text{H} \quad \text{R}^1 \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{N}^+ \quad \text{A}
\end{align*}
\]

wherein:

- \( n \) is 10-2500;
- \( \text{R}^1 \) is \(-Z(\text{CH}_2\text{CH}_2\text{Y})_p(\text{CH}_2)_t\text{R}^3\), wherein:
  - \( Z \) is \(-\text{O}\text{--}, -\text{S}--\text{C}--\text{C}--\text{, or} -\text{CH}_2--\);
  - each \( Y \) is independently \(-\text{O}--\text{ or} -\text{S}--\);
- \( p \) is 0-10;
- \( t \) is 0-10; and
- \( \text{R}^3 \) is \(-\text{N}--\text{,} -\text{CN, a mono-}

(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula 1;

(c) optionally polymerizing a second cyclic amino acid monomer onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer; and

(d) optionally polymerizing additional cyclic amino acid monomers onto the living polymer end.

In certain embodiments, the cyclic amino acid monomers include N-carboxy anhydrides (NCAs), lactams, and cyclic imides. According to one embodiment, the cyclic amino acid monomer is an NCA. NCAs are well known in the art and are typically prepared by the carboxylation of amino acids by a modification of the Fuchs-Farthing method (Kricheldorf, \textit{Aminoacid-N-Carboxy-Anhydrides and Related Heterocycles: Syntheses, Properties, Peptide Synthesis, Polymerization, 1987}). Although reaction conditions vary among different amino acids, most, if not all, natural and unnatural, 2-substituted amino acids can be converted to N-carboxy anhydrides using phosgene gas or triphosgene (for ease of handling). It will be appreciated that, although \( \alpha \)-amino acids are described below, one of ordinary skill in the art would recognize that NCAs may be prepared from \( \beta \)- and \( \gamma \)-amino acids as well. In addition, NCAs can be prepared from dimers or trimers of amino acids. Using an amino acid having an \( R^1 \) side-chain, as defined herein, as an example, Scheme 3 below depicts the typical formation of an NCA using phosgene.
NCAs exhibit reactivity that is well-suited for ring-opening polymerization (ROP). Primary, secondary, and tertiary amines as well as alcohols, water, and acid are known to initiate the ring opening of the NCA. Thus, solvents and all starting materials, including initiators and the NCA monomers, are substantially free from impurities and moisture.

Because a wide variety of functionalities can initiate the polymerizations of NCAs, amino acids containing alcohol, amine, and carboxylic acid functionality are typically protected before polymerization. Such protected hydroxyl groups, protected amine groups, and protected carboxylic acids are well known in the art and include those described above and in Greene (1999).

Examples of suitable hydroxyl protecting groups include, but are not limited to, esters, allyl ethers, ethers, silyl ethers, alkyloxalkyl ethers, and alkoxyalkyl ethers. Examples of such esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzylic formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylene)pentanoate, 5-oxoalbino (trimethylacetyl), crotonate, 4-methoxy- crotonate, benzoate, p-benzybenzoate, 2,4,6-trimethylbenzoate, carboxylates such as methyl, 2-fluorophenylmethyl, ethyl, 2,2,3-trichloroethl, 2-(trimethylsilyl)ethyl, 2-(phenylthio)ethyl, vinyl, allyl, and p-nitrobenzyl. Examples of such silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkysilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, triethyl, t-butyldimethylsilyl, and allyloxycarbonyl ethers or derivatives. Alkoxalkyl ethers include acetics such as methoxymethyl, methoxymethyl- ethyl, 2-(methoxymethylyl)methyl, benzoxymethyl, beta- (trimethylsilyl)ethoxymethyl, and triethanolamino ethers. Examples of arylalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl.

Suitable amino protecting groups include, but are not limited to, anilines, carbamates, cyclic imides, alkyamines, amidines, and the like. Examples of such groups include t-butyloxycarbonyl (BOC), ethoxycarbonyl, methoxycarbonyl, chloroethoxycarbonyl, chloroethylcarbonyl, (Alcoc), benzylcarbonyl (CBZ), allyl, phthalimidine, benzyl (Bn), fluoroethoxycarbonyl (Flmoc), formyl, acetyl, chloroacetetyl, dichloroacetetyl, phenacyl, triethoxycarbonyl, benzoyl, and the like. In certain embodiments, the amino protecting group is phthalimidino. In other embodiments, the amino protecting group is mono- or di-benzyl or mono- or di-allyl. In still other embodiments, the amino protecting group is a tert-butyloxycarbonyl (BOC) group.

Suitable carboxylate protecting groups include, but are not limited to, substituted C₆H₄ aliphatic esters, optionally substituted aryl esters, silyl esters, activated esters, amides, hydrdazides, and the like. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert- butyl, benzyl, and phenyl wherein each group is optionally substituted.

Both D and L NCA enantiomers can be synthesized and any combination of the two stereoisomers can undergo ring-opening polycondensation. Advanced Chemtech (http://www.advancechemtech.com) and Bachem (www.bachem.com) are commercial and widely-referenced sources for both protected and unprotected amino acids. It will be appreciated that amino acid dimers and trimers can form cyclic anhydrides and are capable of ROP in accordance with the present invention.

In certain embodiments, the cyclic amino acid monomer is a carboxylate-protected aspartic acid NCA, a hydroxyl-protected tyrosine NCA, or an amino-protected lysine NCA. In other embodiments, the cyclic amino acid monomer is a t-butyl protected aspartic acid NCA, a benzyl-protected tyrosine NCA, or a BOC-protected lysine NCA.

In certain embodiments, the R³ moieties of the R¹ group of formula I is —Nᵢ —R₃.

In other embodiments, the R³ moieties of the R¹ group of formula I is —CN.

In still other embodiments, the R³ moieties of the R¹ group of formula I is a mono-protected amine or a di-protected amine.

In certain embodiments, the R³ moieties of the R¹ group of formula I is an optionally substituted aliphatic group. Examples include t-butyl, 5-norbornene-2-yl, octane-5-yl, acetylenyl, trimethylsilylacetylenyl, trisopropylsilylacetylenyl, and t-butylidemethylsilylacetylenyl. In some embodiments, said R³ moiety is an optionally substituted alkyl group.

In other embodiments, said R³ moiety is an optionally substituted alkynyl or alkenyl group. When said R³ moiety is a substituted aliphatic group, suitable substituents on R³ include CN, Nᵢ, trimethylsilyl, triisopropylsilyl, t-butyldimethylsilyl, N-methyl propiolamido, N-methyl-4-acetylenylaminino, N-methyl-4-acetylenylbenzoamido, bis-(4-ethynyl- benzyl)-amino, dipropargylamino, di-hex-5-ynyl-amino, di-pent-4-ynyl-amino, di-but-3-ynyl-amino, propargyloxy, hex-5-ynoxyloxy, pent-4-ynoxyloxy, di-but-3-ynoxyloxy. N-methyl-propargylamino, N-methyl-hex-5-ynyl-amino, N-methyl-pent-4-ynyl-amino, N-methyl-but-3-ynyl-amino, 2-hex-5-ynylsulfanyl, 2-pent-4-ynylsulfanyl, 2-but-3-ynylsulfanyl, and 2-propargylsulfanyl. In certain embodiments, the R³ group is 2-(N-methyl-(N-ethynylcarboxy)-amino)ethoxy, 4-ethylbenzoxylxy, or 2-(4-ethylphenox)ethoxy.

In certain embodiments, the R³ moieties of the R¹ group of formula I is an optionally substituted aryl group. Examples include optionally substituted phenyl and optionally substituted pyridyl. When said R³ moiety is a substituted aryl group, suitable substituents on R³ include CN, Nᵢ, NO₂, —CH₃, —CH₂NH₂, —CH₂CO₂H, —CH₂Br, I, F, bis-(4-ethynylbenzyl)- amino, di-propargylamino, di-hex-5-ynyl-amino, di-pent-4-ynyl-amino, di-but-3-ynyl-amino, propargyloxy, hex-5-ynoxyloxy, pent-4-ynoxyloxy, di-but-3-ynoxyloxy, 2-hex-5-ynoxyloxy-ethylsulfanyl, 2-pent-4-ynoxyloxy-ethylsulfanyl, 2-but-3-ynoxyloxy-ethylsulfanyl, 2-propargyloxy-ethylsulfanyl, bis-benzyl-oxyl, and [1,3]dioxolan-2-yl, and [1,3]dioxan-2-yl.

In other embodiments, the R³ moiety is an aryl group substituted with a suitably protected amino group. According to another aspect, the R³ moiety is phenyl substituted with a suitably protected amino group.

In other embodiments, the R³ moieties of the R¹ group of formula I is a protected hydroxyl group. In certain embodiments the protected hydroxy of the R³ moiety is an ester, carbonate, sulfonate, allyl ether, ether, silyl ether, alkyl ether,
aryalkyl ether, or alkoxyalkyl ether. In certain embodiments, the ester is a formate, acetate, propionate, pentanoate, crotonate, or benzoate. Exemplary esters include formate, benzyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxycetate, ρ-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylene)pentanoate, pivaloate(trimethylacetate), crotonate, 4-methoxy-crotonate, benzoate, p-benzenzoate, 2,4,6-trimethylbenzoate. Exemplary carbonates include 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(p-phenylsulfonyloxy)ethyl, vinyl, allyl, and p-nitrobenzyl carbonate. Examples of suitable silyl ethers include triethylsilyl, triethylsilylethyl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl, triisopropylsilyl ether, and other trialkylsilyl ethers. Exemplary alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, triyl, t-butyl, and allyl ether, or derivatives thereof. Exemplary alkoxalkyl ethers include acetal derivatives such as methoxymethyl, methylthiomyethyl, (2-methoxethoxy)methyl, benzoxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyran-2-yl ether. Exemplary arylalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-lallobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picoly ethers.

In certain embodiments, the R³ moiety of the R³ group of formula I is a mono-protected or di-protected amino group. In certain embodiments R³ is a mono-protected amine. In certain embodiments R³ is a mono-protected amine selected from aralkylamines, carbamates, allyl amines, or amides. Exemplary mono-protected amino moieties include t-butylcarboxybenzylamino, ethoxycarbonylaminomethy, methylcarboxylamino, triethoxycarbonylaminomethyl, allyloxybenzylamino, benzyloxycarbonylamino, alanyl, benzylaminomethy, fluorenlymethoxycarbonylmethy, formamidomethyl, acetamido, chloroacetamido, dierloroacetamido, trichloroacetamido, phenylacetamido, trichloroacetamido, benzamido, and t-butyldiphenylsilylamino. In other embodiments R³ is a di-protected amine. Exemplary di-protected amines include dibenzylamine, di-allylamino, phthalimide, maleimide, succinimide, pyrrole, 2,2,5,5-tetramethyl-1,2,5-tetrasidediolide, and azide. In certain embodiments, the R³ moiety is phthalimido. In other embodiments, the R³ moiety is mono- or di-benzylamino or mono- or di-allylamino. In certain embodiments, the R³ group is 2-dibenzaminoethoxy.

In other embodiments, the R³ moiety of the R³ group of formula I is a protected aldehyde group. In certain embodiments the protected aldehyde moiety of R³ is an acyclic acetal, a cyclic acetal, a hydrazone, or an imine. Exemplary R³ groups include dimethyl acetal, diethyl acetal, diisopropyl acetal, dibenzyl acetal, bis(2-nitrobenzyl) acetal, 1,3-dioxane, 1,3-dioxolane, and semicarbazone. In certain embodiments, R³ is an acyclic acetal or a cyclic acetal. In other embodiments, R³ is a dibenzyl acetal.

In yet other embodiments, the R³ moiety of the R³ group of formula I is a protected carboxylic acid group. In certain embodiments, the protected carboxylic acid moiety of R³ is an optionally substituted ester selected from C₁₋₅ aliphatic or aryl, or a silyl ester, an activated ester, an amide, or a hydrazide. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobuty, benzyl, and phenyl ester. In other embodiments, the protected carboxylic acid moiety of R³ is an oxazoline or an ortho ester. Examples of such protected carboxylic acid moieties include oxazolin-2-yl and 2-methoxy-[1,3]dioxin-2-yl. In certain embodiments, the R³ group is oxazolin-2-ylmethyloxy or 2-oxazolin-2-yl-1-propoxy.

According to another embodiments, the R³ moiety of the R³ group of formula I is a protected thiol group. In certain embodiments, the protected thiol of R³ is a disulfide, thiethyl, silyl thiethio, thieto, thieto, thieto, or a thieto-carbamate. Examples of such protected thios include trisopropylsilyl thiethio, t-butyldimethylsilyl thiethio, t-butyldihexyl thiethio, benzyl thiethio, p-methylbenzyl thiethio, trisopropylthiophenyl methyl thiethio, and p-methylphenylthienyl methyl thiethio. In other embodiments, R³ is optionally substituted thiethio selected from alkyly, benzyl, or trishellthio, or trichloroethylcarbonyl thiethio. In certain embodiments, R³ is -S—S—pyridin-2-yl, —S—SBn, —S—SCH₃, or —S—S(p-ethylphenyl). In other embodiments, R³ is —S—S—pyridin-2-yl. In still other embodiments, the R³ group is 2-triphenylmethylsulfanil-ethoxy.

In certain embodiments, the R³ moiety of the R³ group of formula I is a crown ether. Examples of such crown ethers include 12-crown-4, 15-crown-5, and 18-crown-6.

In still other embodiments, the R³ moiety of the R³ group of formula I is a detectable moiety. According to one aspect of the invention, the R³ moiety of the R³ group of formula I is a fluorescence moiety. Such fluorescence moieties are well known in the art and can include coumarins, quinolones, benzoisouquino- lone, hostasol, and Rhodamine dyes, to name but a few. Exemplary fluorescence moieties of the R³ group of R³ include anthracen-9-yl, pyren-4-yl, 9H-carbazol-9-yl, the carbonylcate of rhodamine B, and the carboxylate of coumarin 343.

In certain embodiments, the R³ moiety of the R³ group of formula I is suitable for Crick chemistry. Crick reactions tend to involve high-energy ("spring-loaded") reagents with well-defined reaction coordinates, giving rise to selective bond-forming events of wide scope. Examples include the nucleophilic trapping of strained-ring electrophiles (epoxide, aziridines, aziridinium ions, episulfonium ions), certain forms of carbonyl reactivity (aldehydes and hydrazines or hydroxylamines, for example), and several types of cycloaddition reactions. The azide-alkyne 1,3-dipolar cycloaddition is one such reaction. Crick chemistry is known in the art and one of ordinary skill in the art would recognize that certain R³ moieties of the present invention are suitable for Crick chemistry.

Compounds of formula I having R³ moieties suitable for Crick chemistry are useful for conjugating said compounds to biological systems or macromolecules such as proteins, viruses, and cells, to name but a few. The Click reaction is known to proceed quickly and selectively under physiological conditions. In contrast, most conjugation reactions are carried out using the primary amine functionality on proteins (e.g. lysine or protein end-group). Because most proteins contain a multitude of lysines and arginines, such conjugation occurs uncontrollably at multiple sites on the protein. This is particularly problematic when lysines or arginines are located around the active site of an enzyme or other biomolecule. Thus, another embodiment of the present invention provides a method of conjugating the R³ group of a compound of formula I to a macromolecule via Crick chemistry. Yet another embodiment of the present invention provides a macromolecule conjugated to a compound of formula I via the R³ group.

As defined generally above, Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C₁₋₁₂ alkylamine chain, wherein 0-6 methylene units of Q are independently replaced by -C=O, -O-, -NH-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO₂-, -SO₂-, -NO₂-, -SO₂NH-, -NHCO(OH)-, -C(O)NΗ₂-, -OC(O)NH₂-, or -NHCO(OH)-, wherein -C=O is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, par-
ially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, Q is a valence bond. In other embodiments, Q is a bivalent, saturated C₁₋₁₂ alkylene chain, wherein 0–6 methylene units of Q are independently replaced by -C₉₋₋₉₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~-~
wherein:

n is 10-2500;
m is 1 to 1000;
m' is 0 to 1000;
R' and R'' are each independently a natural or unnatural amino acid side-chain group, wherein R' and R'' are different from each other;

R' is \( \text{Z}(\text{CH}_2 \text{CH}_2 \text{Y})_m \text{CH}_2 \text{R}' \), wherein:

Z is \( \text{O} \), \( \text{S} \), \( \text{C} \), or \( \text{CH}_2 \); each Y is independently \( \text{O} \) or \( \text{S} \); p is 0-10;
t is 0-10; and

R'' is \( \text{N}, \text{CN} \), a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( \text{C}_{1-12} \) alkylene chain, wherein 0-6 methylene units of Q are independently replaced by \( \text{C}_n \), \( \text{O} \), \( \text{N} \), \( \text{S} \), \( \text{C} \), \( \text{O} \), \( \text{SO} \), \( \text{SO}_2 \), \( \text{NH}_2 \text{SO} \), \( \text{NH}_2 \text{NH} \), \( \text{NH}_2 \text{O} \), \( \text{C} \), \( \text{OC} \), \( \text{NH} \), \( \text{OC} \text{NH} \), \( \text{NC} \text{H} \text{O} \), or \( \text{N} \text{HC} \text{H} \text{O} \), wherein:

\( \text{C}_n \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

A is a suitable acid anion,

wherein said method comprises the steps of:

(a) providing a compound of formula I:

\[
\begin{align*}
\text{R'} & \quad \text{O} \\
\text{O} & \quad \text{Q} \\
\text{Q} & \quad \text{NH}_2 \text{A}
\end{align*}
\]

wherein:

n is 10-2500;
R' is \( \text{Z}(\text{CH}_2 \text{CH}_2 \text{Y})_m \text{CH}_2 \text{R}' \), wherein:

Z is \( \text{O} \), \( \text{S} \), \( \text{C} \), or \( \text{CH}_2 \); each Y is independently \( \text{O} \) or \( \text{S} \); p is 0-10;
t is 0-10; and

R'' is \( \text{N}, \text{CN} \), a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( \text{C}_{1-12} \) alkylene chain, wherein 0-6 methylene units of Q are independently replaced by \( \text{C}_n \), \( \text{O} \), \( \text{N} \), \( \text{S} \), \( \text{C} \), \( \text{O} \), \( \text{SO} \), \( \text{SO}_2 \), \( \text{NH}_2 \text{SO} \), \( \text{NH}_2 \text{NH} \), \( \text{NH}_2 \text{O} \), \( \text{C} \), \( \text{OC} \), \( \text{NH} \), \( \text{OC} \text{NH} \), \( \text{NC} \text{H} \text{O} \), or \( \text{N} \text{HC} \text{H} \text{O} \), wherein:

\( \text{C}_n \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

A is a suitable acid anion,

(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula I, wherein said first cyclic amino acid monomer comprises R' and;

(c) optionally polymerizing a second cyclic amino acid monomer, comprising R'', onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer. Each of the classes and subclasses as described for the R' (including R'' and other variables contained therein) and Q groups of formula I also apply singly and in combination to the R' and Q groups of formula II.

In certain embodiments, m' is 0. In other embodiments, m' is 1-1000. According to other embodiments, m and m' are independently 10 to 100 repeat units. In still other embodiments, m is 1-20 repeat units and m' is 10-50 repeat units.

In certain embodiments, one of R' and R'' is a hydrophilic, or crosslinkable, amino acid side-chain group, or suitably protected form thereof, and the other of R' and R'' is a hydrophobic, or ionisable amino acid side-chain group, or suitably protected form thereof. In other embodiments, R' is a hydrophilic or crosslinkable amino acid side-chain group and R'' is a hydrophobic, or ionisable amino acid side-chain group. Such hydrophilic, or crosslinkable, amino acid side-chain groups include tyrosine, serine, cysteine, threonine, aspartic acid (also known as aspartate, when charged), glutamic acid (also known as glutamate, when charged), asparagine, and glutamine. Such hydrophilic amino acid side-chain groups include a suitably protected tyrosine side-chain, a suitably protected serine side-chain, a suitably protected threonine side-chain, phenylalanine, alanine, valine, leucine, tryptophan, proline, benzyl and alkyl glutamates, or benzyl and alkyl aspartates or mixtures thereof. Such ionizable amino acid side-chain groups includes lysine side-chain, arginine side-chain, or a suitably protected lysine or arginine side-chain, an aspartic acid side chain, glutamic acid side-chain, or a suitably protected aspartic acid or glutamic acid side-chain. One of ordinary skill in the art would recognize that protection of a polar or hydrophilic amino acid side-chain can render that amino acid nonpolar. For example, a suitably protected tyrosine hydroxyl group can render that tyrosine nonpolar and hydrophobic by virtue of protecting the hydroxyl group. Suitable protecting groups for the hydroxy, amino, and thiol, and carboxylate functional groups of R' and R'' are as described herein.

In other embodiments, R' comprises a mixture of hydrophobic and hydrophilic amino acid side-chain groups such that the overall poly(amino acid) block comprising R' is hydrophobic. Such mixtures of amino acid side-chain groups
include phenylalanine/tyrosine, phenalanine/serine, leucine/tyrosine, and the like. According to another embodiment, R' is a hydrophobic amino acid side-chain group selected from phenylalanine, alanine, or leucine, and one or more of tyrosine, serine, or threonine.

In other embodiments, one or both of R' and R" comprise functional groups capable of forming cross-links. According to another embodiment, R' comprises a functional group capable of forming cross-links. It will be appreciated that a variety of functional groups are capable of such cross-linking, including, but not limited to, carboxylate, hydroxyl, thiol, and amino groups. Examples of NCA's having functional groups capable of forming cross-links, or protected forms thereof, include protected glutamic and aspartic acids, such as:

protected serines capable of glutaraldehyde crosslinking via the corresponding hydroxyl, such as:

and aldehyde and protected aldehyde capable of glutaraldehyde crosslinking, such as:

protected cysteines capable of forming disulfide crosslinking via the corresponding thiol, such as:

As discussed above, the preparation of poly(amo acid) containing polymers synthesized by the initiation of NCAs using free amine macrorinitiators affords block copolymers with a wide range of PDIs. In certain embodiments, the sequential polymerization methods of the present invention result in the preparation of multi-block copolymers of the present invention having a PDI about equal to or lower than that of the starting synthetic polymer.

Other nonlimiting examples of amino acid monomers suitable for the methods of the present invention include protected glutamic and aspartic acids, such as:
protected lysine, such as:

protected arginine, such as:

protected histidine, such as:

Another aspect of the present invention provides a compound of formula II:

wherein:
\( n \) is 10-2500;
\( m \) is 1 to 1000;
\( m' \) is 0 to 1000;
\( R^\prime \) and \( R^\prime \) are each independently a natural or unnatural amino acid side-chain group, wherein \( R^\prime \) and \( R^\prime \) are different from each other;
\( R^2 \) is \(-Z(CH_2)_pY(CH_2)_qR^3\), wherein:
\( Z \) is \(-O-\), \(-S-,\) \(-C=C-,\) or \(-CH_3-\);
each \( Y \) is independently \(-O-\) or \(-S-\);
\( p \) is 0-10;
\( t \) is 0-10; and
\( R^2 \) is \(-N_3,\) \(-CN,\) a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring hav-
ing 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety;
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by \(-\text{Cy}, -\text{O}, -\text{NH}, -\text{S}, -\text{OC (O)\_}, -\text{C(O)\_}, -\text{NH\_}, \text{-SO\_}, -\text{SO\_}, -\text{NH\_}\text{SO\_}, -\text{SO\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}, -\text{C(O)\_}\text{NH\_}, -\text{OC(O)\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}\text{O-
}
\text{O\_}, -\text{S}, -\text{N}^+\text{C=}, \text{or} -\text{CH}_2\text{-}; each Y is independently \text{O or S};
\text{p is 0-10; and}
\text{R}^3 = \text{N}_{3y} - \text{CN}, \text{a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety; Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by \(-\text{Cy}, -\text{O}, -\text{NH}, -\text{S}, -\text{OC (O)\_}, -\text{C(O)\_}, -\text{NH\_}\text{SO\_}, -\text{SO\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}, -\text{C(O)\_}\text{NH\_}, -\text{OC(O)\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}\text{O-
}
\text{O\_}, -\text{S}, -\text{N}^+\text{C=}, \text{or} -\text{CH}_2\text{-}; each Y is independently \text{O or S};
\text{p is 0-10; and}
\text{R}^3 = \text{N}_{3y} - \text{CN}, \text{a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety; Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by \(-\text{Cy}, -\text{O}, -\text{NH}, -\text{S}, -\text{OC (O)\_}, -\text{C(O)\_}, -\text{NH\_}\text{SO\_}, -\text{SO\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}, -\text{C(O)\_}\text{NH\_}, -\text{OC(O)\_}\text{NH\_}, -\text{NH\_}\text{C(O)\_}\text{O-

A is a suitable acid anion.

In certain embodiments, the m' group of formula II is 1-1000. In certain embodiments, the m' group of formula II is 0. In other embodiments, m' is 1-1000. According to other embodiments, m and m' are independently 10 to 100 repeat units. In still other embodiments, m is 1-20 repeat units and m' is 10-50 repeat units.

In certain embodiments, the R^3 moiety of the R^1 group of formula II is \text{N}_{3y}.

In other embodiments, the R^3 moiety of the R^1 group of formula II is \text{CN}.

In certain embodiments, the R^3 moiety of the R^2 group of formula II is an optionally substituted aliphatic group. Examples include t-butyl, 5-norbornene-2-yl, octane-5-yl, acetylenyl, trimethylsilylacetylenyl, triisopropylsilylacetyle-
In some embodiments, said R² moiety is an optionally substituted alkyl group. In other embodiments, said R² moiety is an optionally substituted aryl group. When said R² moiety is a substituted aryl group, suitable substituents on R² include CN, N₂, trimethylsilyl, trisopropylsilyl, t-butylidinemethylenesilylethylsilyl, N-methyl propionylamido, N-methyl-4-acytelyenylaminino, N-methyl-4-acytelyenylbenzamido, bis(4-ethylbenzyl)-amino, dipropargylamino, di-hex-5-ynyl-amino, di-pent-4-ynyl-amino, di-but-3-ynyl-amino, propargyloxy, hex-5-ynoxy, pent-4-ynoxy, di-but-3-ynoxy, N-methylpropargylamino, N-methyl-hex-5-ynyl-amino, N-methylpent-4-ynyl-amino, N-methyl-but-3-ynyl-amino, 2-hex-5-ynylidisulfanyl, 2-pent-4-ynylidisulfanyl, 2-but-3-ynylidisulfanyl, and 2-propargylidisulfanyl. In certain embodiments, the R² group is 2-(N-ethyl-N-(ethynylcarbonyl)amino)ethoxy, 4-ethynylbenzoxo, or 2-(4-ethynylphenoxy)ethoxy.

In certain embodiments, the R³ moiety of the R¹ group of formula II is an optionally substituted aryl group. Examples include optionally substituted phenyl and optionally substituted pyridyl. When said R³ moiety is an optionally substituted aryl group, suitable substituents on R³ include CN, N₂, —CH₃, —CH₂N₂ —CH—CH₂ —C—CH Br, I, F, bis(4-ethylbenzyl)-amino, dipropargylamino, di-hex-5-ynyl-amino, di-pent-4-ynyl-amino, di-but-3-ynyl-amino, propargyloxy, hex-5-ynoxy, pent-4-ynoxy, di-but-3-ynoxy, 2-hex-5-ynoxy-ethylidisulfanyl, 2-pent-4-ynoxy-ethylidisulfanyl, 2-but-3-ynoxy-ethylidisulfanyl, 2-propargyloxy-ethylidisulfanyl, bis-benzoxo-methyl, [1,3]dioxolan-2-yl, and [1,3]dioxan-2-yl.

In other embodiments, the R³ moiety is an aryl group substituted with a suitably protected amino group. According to another aspect, the R³ moiety is phenyl substituted with a suitably protected amino group.

In other embodiments, the R³ moiety of the R¹ group of formula II is a protected hydroxyl group. In certain embodiments, the protected hydroxyl of the R³ moiety is an ester, carbonate, sulfonate, allyl ether, ether, silyl ether, alky ether, aryalkyl ether, or alkoxalkyl ether. In certain embodiments, the ester is a formate, acetate, propionate, pentonate, crotonate, or benzoate. Exemplary esters include formate, benzoic formate, chloroacetate, trifluoroacetate, methoxycate, triphenylmethyleneacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanato, 4-(4-ethylenedithio)pentanato, pivaloate(trimethyacetate), crotonate, 4-methoxy-crotonate, benzoate, p-benzenesulfo, 2,4,6-trimethylbenzoate. Exemplary carbanates include 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethyilsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, alky, and p-nitrobenzyl carbonate. Examples of suitable silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, trisopropylsilyl ether, and other trialkylsilyl ethers. Exemplary alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, and allyl ether, or derivatives thereof. Exemplary alkoxalkyl ethers include ethers such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzoxylmethyl, beta-(trimethyilsilyl)ethoxymethyl, and tetrahydropropyn-2-yl ether. Exemplary aryalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl ethers.

In certain embodiments, the R³ moiety of the R¹ group of formula II is a mono-protected or di-protected amino group. In certain embodiments R³ is a mono-protected amine. In certain embodiments R³ is a mono-protected amine selected from aralkylamines, carbamates, allyl amines, or amides.

Exemplary mono-protected amino moieties include t-butyloxycarbonylamino, ethoxycarbonylamino, methoxycarbonylamino, trichloroethoxy-carbonylamino, allyloxy carbonylamino, benzoxo carbonylamino, alkyloxycarbonylamino, benzyl carbonylamino, benzamino, benzylamine, fluoroaminomethylcarbonyl, formamido, acetamido, chloroacetamido, dichloroacetamido, trichloroacetamido, phenylocetamido, trifluoroacetamido, benzamido, and t-butylidiphenylsilylamino. In other embodiments R³ is a di-protected amine. Exemplary di-protected amines include di-benzylamine, di-allylamine, phthalimide, maleimide, succinimide, pyrrole, 2,2,5,5-tetramethyl[1,2,5]azadisilololide, and azide. In certain embodiments, the R³ moiety is phthalimido. In other embodiments, the R³ moiety is mono- or di-benzylamino or mono- or di-allylamine. In certain embodiments, the R³ group is 2-dibenzylaminooxytox. In other embodiments, the R³ moiety of the R¹ group of formula II is a protected aldehyde group. In certain embodiments the protected aldehyde moiety of R³ is an acyclic acetal, a cyclic acetal, a hydrazone, or an imine. Exemplary R³ groups include dimethyl acetal, diethyl acetal, disopropyl acetal, dibenzyl acetal, bis(2-nitrobenzoyl)acetal, 1,3-dioxane, 1,3-dioxolane, and semicarbazone. In certain embodiments, R³ is an acyclic acetal or a cyclic acetal. In other embodiments, R³ is a dibenzyl acetal.

In yet other embodiments, the R³ moiety of the R¹ group of formula II is a protected carboxylic acid group. In certain embodiments, the protected carboxylic acid moiety of R³ is an optionally substituted ester selected from C₁₋₄ aliphatic or aryl, or a silyl ester, an activated ester, an amide, or a hydrazide. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, and phenyl ester. In other embodiments, the protected carboxylic acid moiety of R³ is an oxazoline or an ortho ester. Examples of such protected carboxylic acid moieties include oxazolin-2-yl and 2-methoxy-[1,3]dioxinin-2-yl. In certain embodiments, the R³ group is oxazolin-2-ylmethoxy or 2-oxazolin-2-yl-1-propoxy.

According to another aspect, the R³ moiety of the R¹ group of formula II is a protected thiol group. In certain embodiments, the protected thiol of R³ is a disulfide, thioether, silyl thioether, thioester, thiocarbonate, or a thio carbamate. Examples of such protected thiols include trisopropylsilyl thioether, t-butyldimethylsilyl thioether, t-buty1 thioether, benzyl thioether, p-methylenzyl thioether, triphenylmethylene thioether, and p-methoxycarbonyldimethylthioether. In other embodiments, R³ is an optionally substituted thioether selected from alkyl, benzyl, or trisopropylmethyl, or trichloroethoxy carbonyl thioether. In certain embodiments, R³ is S-[S-pyridin-2-yl]-S-S⁻<sub>SBn</sub>, S-S⁻<sub>SC₁₋₄</sub>, or S-S⁻(p-ethynylbenzyl). In other embodiments, R³ is S-[S-pyridin-2-yl]. In still other embodiments, the R³ group is 2-triphenylmethylene sulfanyl ethoxy.

In certain embodiments, the R³ moiety of the R¹ group of formula II is a crown ether. Examples of such crown ethers include 12-crown-4, 15-crown-5, and 18-crown-6.

In still other embodiments, the R³ moiety of the R¹ group of formula II is a detectable moiety. According to one aspect of the invention, the R³ moiety of the R¹ group of formula II is a fluorescent moiety. Such fluorescent moieties are well known in the art and include coumarins, quinolones, benzouisoquinolones, hostasol, and Rhodamine dyes, to name but a few. Exemplary fluorescent moieties of the R¹ group of R³ include anthracen-9-yl, pyren-4-yl, 9-H-carbazol-9-yl, the carbonylate of rhodamine B, and the carbonylate of coumarin 343.

As defined generally above, the Q group of formula II is a valence bond or a bivalent, saturated or unsaturated, straight or branched C₁₋₁₂ alkylene chain, wherein 0-6 methylene linkages are present.
units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)-, -SO-, -SO2-, -NH2SO-, -SO3NH-, -NHC(O)-, -CO(NH)-, or -NH(CO)O-, wherein -Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, Q is a valence bond. In other embodiments, Q is a bivalent, saturated C1-12 alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)-, or -SO-, wherein -Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one aspect of the present invention, -Cy- is an optionally substituted bivalent aryl group. According to another aspect of the present invention, -Cy- is an optionally substituted bivalent alkylene chain. In other embodiments, -Cy- is an optionally substituted 5-8 membered bivalent, saturated carbocyclic ring. In still other embodiments, -Cy- is an optionally substituted 5-8 membered bivalent, saturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Exemplary -Cy- groups include bivalent rings selected from phenyl, pyridyl, pyrimidinyl, cyclohexyl, cyclopentyl, or cyclopropyl.

In certain embodiments, the R3 moiety of the R1 group of formula II is a group suitable for Click chemistry. Click reactions tend to involve high-energy (“spring-loaded”) reagents with well-defined reaction coordinates, giving rise to selective bond-forming events of wide scope. Examples include the nucleophilic trapping of strained-ring electrophiles (epoxide, aziridines, aziridinium ions, episolium ions), certain forms of carbonyl reactivity (aldehydes and hydrazines or hydroxylamines, for example), and several types of cycloaddition reactions. The azide-alkyne 1,3-dipolar cycloaddition is one such reaction. Click chemistry is known in the art and one of ordinary skill in the art would recognize that certain R3 moieties of the present invention are suitable for Click chemistry.

Compounds of formula II having R3 moieties suitable for Click chemistry are useful for conjugating said compounds to biological systems or macromolecules such as proteins, viruses, and cells, to name but a few. The Click reaction is known to proceed quickly and selectively under physiological conditions. In contrast, most conjugation reactions are carried out using the primary amine functionality on proteins (e.g. lysine or protein end-group). Because most proteins contain a multitude of lysines and arginines, such conjugation occurs uncontrollably at multiple sites on the protein. This is particularly problematic when lysines or arginines are located around the active site of an enzyme or other biomolecule. Thus, another embodiment of the present invention provides a method of conjugating the R3 groups of a compound of formula II to a macromolecule via Click chemistry. Yet another embodiment of the present invention provides a macromolecule conjugated to a compound of formula II via the R3 group.

After conjugation to a biomolecule, drug, cell, or the like, the other end-group functionality, corresponding to free amine or salt thereof, group of formula II, can be used to attach targeting groups for cell specific delivery including, but not limited to, detectable moieties, such as fluorescent dyes, covalent attachment to surfaces, and incorporation into hydrogels.

According to one embodiment, the R3 moiety of the R1 group of formula II is an azide-containing group. According to another embodiment, the R3 moiety of the R1 group of formula II is an alkyne-containing group. In certain embodiments, the R3 moiety of the R1 group of formula II has a terminal alkylene moiety. In other embodiments, R3 moiety of the R1 group of formula II is an alkylene moiety having an electron withdrawing group. Accordingly, in such embodiments, the R3 moiety of the R1 group of formula II is

wherein E is an electron withdrawing group and y is 0-6. Such electron withdrawing groups are known to one of ordinary skill in the art. In certain embodiments, E is an ester. In other embodiments, the R3 moiety of the R1 group of formula II is

wherein E is an electron withdrawing group, such as —C(O)O— group and y is 0-6.

Another aspect of the present invention provides a method for preparing a compound of formula II:

wherein:

- n is 10-2500;
- m is 1 to 1000;
- m' is 0 to 1000;
- R2 and R4 are each independently a natural or unnatural amino acid side-chain group, wherein R6 and R7 are different from each other;
- R3 is —Z(CH2)nCH3Y(CH2)mR5, wherein:
  Z is —O—, —S—, —C=C—, or —CH2—;
  each Y is independently —O— or —S—;
  p is 0-10;
  q is 0-10; and
- R1 is —N3, —CN, a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 mem-
bered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety; and

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkylene chain, wherein 0-6 methylene units of \( Q \) are independently replaced by -\( \text{Cy} - \), -\( \text{O} - \), -\( \text{NH} - \), -\( \text{S} - \), -\( \text{OC} (\text{O}) - \), -\( \text{C} (\text{O}) - \), -\( \text{SO} - \), -\( \text{SO}_{2} - \), -\( \text{NHSO}_{2} - \), -\( \text{SO}_{2} \text{NH} - \), -\( \text{NHIC} (\text{O}) - \), -\( \text{C} (\text{O}) \text{NH} - \), -\( \text{OC} (\text{O}) \text{NH} - \), or -\( \text{NHIC} (\text{O}) \text{O} - \), wherein:

-\( \text{Cy} - \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

wherein said method comprises the steps of:

(a) providing a compound of formula I:

R
\[ \text{I} \]

wherein:

n is 10-2500;

R' is \(-\text{Z(CH}_{2}\text{CH}_{2}Y)_{m}\text{(CH}_{2}\text{)}R^3\), wherein:

X is \(-\text{O} - \), \(-\text{S} - \), \(-\text{C} = \text{C} - \), or \(-\text{CH}_{2} - \);

each Y is independently \(-\text{O} - \) or \(-\text{S} - \);

p is 0-10;

t is 0-10; and

R' is \(-\text{N}_{2} - \), \(-\text{CN} - \), a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30 membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkylene chain, wherein 0-6 methylene units of \( Q \) are independently replaced by -\( \text{Cy} - \), -\( \text{O} - \), -\( \text{NH} - \), -\( \text{S} - \), -\( \text{OC} (\text{O}) - \), -\( \text{C} (\text{O}) - \), -\( \text{SO} - \), -\( \text{SO}_{2} - \), -\( \text{NHSO}_{2} - \), -\( \text{SO}_{2} \text{NH} - \), -\( \text{NHIC} (\text{O}) - \), -\( \text{C} (\text{O}) \text{NH} - \), -\( \text{OC} (\text{O}) \text{NH} - \), or -\( \text{NHIC} (\text{O}) \text{O} - \), wherein:

-\( \text{Cy} - \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

A is a suitable acid anion;

(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula I, wherein said first cyclic amino acid monomer comprises R';

(c) optionally polymerizing a second cyclic amino acid monomer, comprising R'', onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer, to form a compound of formula II:

\[ \text{II} \]

and (d) treating said compound of formula II with a base to form a compound of formula II':

\[ \text{II'} \]

One of ordinary skill in the art would appreciate that a variety of bases are suitable for forming the free-base compound of formula II' from the salt form of formula II. Such bases are well known in the art. In certain embodiments, the base utilized at step (d) is pyridine, or a derivative thereof, such as dimethylaminopyridine ("DMAP"), lutidine or collidine. In other embodiments, the base utilized at step (d) is dimethylaninopyridine ("DMAP"). In still other embodiments, inorganic bases are utilized and include ammonia, potassium hydroxide, sodium hydroxide, sodium carbonate, sodium bicarbonate, potassium carbonate, or potassium bicarbonate.

According to yet another embodiment, the present invention provides a compound of formula II':

\[ \text{II'} \]

wherein:

n is 10-2500;

m is 1 to 1000;

m' is 0 to 1000;

R' and R'' are each independently a natural or unnatural amino acid side-chain group, wherein R' and R'' are different from each other;

R' is \(-\text{Z(CH}_{2}\text{CH}_{2}Y)_{m}\text{(CH}_{2}\text{)}R^3\), wherein:

Z is \(-\text{O} - \), \(-\text{S} - \), \(-\text{C} = \text{C} - \), or \(-\text{CH}_{2} - \);

each Y is independently \(-\text{O} - \) or \(-\text{S} - \);

p is 0-10;

t is 0-10; and

R' is \(-\text{N}_{2} - \), \(-\text{CN} - \), a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30 membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety; and

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkylene chain,
wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -OC (O)-, -C(O)O-, -C(O)-, -SO-, -SO2-, -NI=SO2-, -SO2NH-, -NIHC(O)-, -C(O) NH-, -OC(O)NH-, or -NIHC(O)O-, wherein:

-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Each of the embodiments relating to the R', Q, n, m, m', R' and R'' groups of formula I1 apply to the R', n, m, m', R' and R'' groups of formula II both singly and in combination.

It will be appreciated to those ordinarily skilled in the art that a compound of formula II1 may be further derivatized by treatment of that compound with a suitable terminating agent. Thus, another embodiment provides a method for preparing a compound of formula III:

wherein:

n is 10-2500;

m is 1 to 1000;
m' is 0 to 1000;
R' and R'' are each independently a natural or unnatural amino acid side-chain group, wherein R' and R'' are different from each other;
R' = \(\text{Z(CH}_2\text{CH}_2\text{Y})_p\text{CH}_2\text{R}^\text{3}\), wherein:
Z is -O-, -S-, -C=O, or -CH\(_2\)N-;
each Y is independently -O- or -S-;
p is 0-10;

R' = -N\(_3\), -CN, a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety;
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C\(_{1-12}\) alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -OC (O)-, -C(O)O-, -C(O)-, -SO-, -SO2-, -NI=SO2-, -SO2NH-, -NIHC(O)-, -C(O) NH-, -OC(O)NH-, or -NIHC(O)O-, wherein:
-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula I, wherein said first cyclic amino acid monomer comprises R';
(c) optionally polymerizing a second cyclic amino acid monomer, comprising R', onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer, to form a compound of formula II:

(d) treating said compound of formula II with a base to form a compound of formula II':

and

e) treating said compound of formula II' with a suitable terminating agent to form the compound of formula III.

In certain embodiments, the m' group of formula III is 1-1000. In certain embodiments, the m' group of formula III is 0. In other embodiments, m' is 1-1000. According to other embodiments, m and m' are independently 10 to 100 repeat units. In still other embodiments, m is 1-20 repeat units and m' is 10-50 repeat units.

As described generally above, R' = Z(CH2)n CH2Y; (CH2)n CH2Y, wherein Z is O, S, or C=C--; each Y is independently O or S; and p is 0-10; y is 0-10; and R3 is CH2 or CN, a mono-protected amine, a protected amine, a protected alcohol, a protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, a 9-30-membered crown ether, an optionally substituted aliphatic group, an optionally substituted 5-8-membered saturated or partially unsaturated, or aryl ring having 9-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 9-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety.

In certain embodiments, the R' moiety of the R' group of formula III is —R'.'

In other embodiments, the R' moiety of the R' group of formula III is —CN.

In certain embodiments, the R' moiety of the R' group of formula III is an optionally substituted aliphatic group. Examples include t-butyl, 5-norbornene-2-yl, octane-5-yl, acetylenyl, trimethylsilylacetylenyl, triisopropylsilylacetylenyl, and t-butylmethylsilylacetylenyl. In some embodiments, said R' moiety is an optionally substituted alkyl group. In other embodiments, said R' moiety is an optionally substituted alkynyl or alkynyl group. When said R' moiety is a substituted aliphatic group, suitable substituents on R' include CN, N, trimethylsilyl, triisopropylsilyl, t-butylidemethylsilyl, and t-butylidemethylsilyl.

In certain embodiments, the R' group is 2-(N-methyl-N-ethylcarboxylo)ethoxy, 4-ethoxybenzyl, or 2-(4-ethoxyphenoxy)ethoxy. In certain embodiments, R' is other than —OMe.

In certain embodiments, the R' moiety of the R' group of formula III is an optionally substituted aryl group. Examples include optionally substituted phenyl and optionally substituted pyridyl. When said R' moiety is a substituted aryl group, suitable substituents on R' include CN, N, NO2, —CH3, —CH2N, —CH=CH2, —C==CH, Br, I, F, bisis-(4-ethylbenzyl)-amino, dipropargylamino, di-hex-5-ynyl-amino, di-pent-4-ynyl-amino, di-but-3-ynyl-amino, propargyloxy, hex-5-ynoxyloxy, pent-4-ynoxyloxy, di-but-3-ynoxyloxy, N-methyl-propargylamino, N-methyl-hex-5-ynyl-amino, N-methyl-pent-4-ynyl-amino, N-methyl-but-3-ynyl-amino, 2-hex-5-ynylsulfanyl, 2-pent-4-ynylsulfanyl, 2-but-3-ynylsulfanyl, and 2-propargylsulfanyl. In certain embodiments, the R' group is 2-(N-methyl-N-(ethoxycarbonylo))amino)ethoxy, 4-ethoxybenzyl, or 2-(4-ethoxyphenoxy)ethoxy. In certain embodiments, R' is other than —OMe.

In certain embodiments, the R' moiety of the R' group of formula III is an optionally substituted hydroxyl group. In certain embodiments, the protected hydroxyl of the R' moiety is an ester, carbonate, sulfonate, allyl ether, ether, silyl ether, alkyl ether, aryalkyl ether, or alkoxalkyl ether. In certain embodiments, the ester is a formate, acetate, propionate, pantoate, crotonate, or benzoate. Exemplary esters include formate, benzyl formate, chloroacetaete, trifluoroacetate, methoxycetate, trimethylsilylacetate, a-chloroformylacetate, 3-phenylpropionate, 4-oxopentanate, 4,4-(ethylenedioxy)pentanolate, pivaloate(trimethylacetate), crotonate, 4-methoxycrotonate, benzoate, p-benzylenbenzoate, 2,4,6-trimethylbenzoate. Exemplary carbonates include 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(p-hexyloxy)silyl)ethyl, vinyl, allyl, and p-nitrobenzyl carbonate.

Examples of useful silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyle ethers. Examples of useful alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, tert-butyl, and allyl ether, or derivatives thereof. Exemplary alkoxalkyl ethers include acetylacetone such as methoxymethyl, methylethimethylethoxy, (2-methoxethoxy)methoxy, benzoxymethyl, beta-(trimethylsilyl)ethoxyethoxy, and tetrahydropryan-2-yl ether. Examples of aryalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, and 2- and 4-picolyl ethers.

In certain embodiments, the R' moiety of the R' group of formula III is a mono-protected or di-protected amino. In certain embodiments R' is a mono-protected amino. In certain embodiments R' is a mono-protected amino selected from aralkylamines, carbamates, allyl amines, or amidines. Exemplary mono-protected amino moieties include t-butyloxy carbamylaminoo, ethoxyloxy carbamylaminoo, methoxycarbonylaminoo, trichloroethoxy carbamylaminoo, allyloxy carbamylaminoo, benzoyloxy carbamylaminoo, allylaminoo, benzalaminoo, fluorenylmethyl carbonyl, formamido, acetamido, chloroacetamido, dichloroacetamido, trichloroacetamido, phenacylamido, trifluoroacetamido, benzamido, and t-butylidemethylsilylamido. In certain embodiments R' is a protected amine. Exemplary protected amines include dialkylamine, di-allylamine, dialkylamine, maleimide, succinimide, pyrrole, 2,2,5,5-tetramethyl[1,2,5]azadisilidide, and azide. In certain embodiments, the R' moiety is phthal-
imido. In other embodiments, the R³ moiety is mono- or di-benzylamino or mono- or di-allylaminio. In certain embodiments, the R² group is 2-dibenzy laminoethoxy.

In other embodiments, the R³ moiety of the R¹ group of formula III is a protected aldehyde group. In certain embodiments the protected aldehyde moiety of R³ is an acyclic acetal, a cyclic acetal, a hydrzone, or an imine. Exemplary R² groups include dimethyl acetal, diethyl acetal, diisopropyl acetal, dibenzyl acetal, bis(2-nitrobenzyl)acetal, 1,3-dioxane, 1,3-dioxolane, and semicarbazone. In certain embodiments, R³ is an acyclic acetal or a cyclic acetal. In other embodiments, R³ is a dibenzyl acetal.

In yet other embodiments, the R³ moiety of the R¹ group of formula III is a protected carboxylic acido moiety. In certain embodiments, the protected carboxylic acid moiety of R³ is an optionally substituted ester selected from C₁₋₆ aliphatic or aryl, or a silyl ester, an activated ester, an amide, or a hydrazide. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, and phenyl ester. In other embodiments, the protected carboxylic acid moiety of R³ is an oxazoline or an ortho ester. Examples of such protected carboxylic acid moieties include oxazolin-2-yl and 2-methoxy-[1,3]dioxin-2-yl. In certain embodiments, the R² group is oxazolin-2-ylmethoxy or 2-oxazolin-2-yl-1-propoxy.

According to another embodiments, the R³ moiety of the R¹ group of formula III is an acetylated thiol group. In certain embodiments, the protected thiol of R³ is a disulfide, thioether, silyl thioether, thioester, thio carbonate, or a thiocarbamate. Examples of such protected thiols include trisopropylsilyl thioether, t-butyl dimethylsilyl thioether, t-butyl thioether, benzyl thioether, p-methylbenzyl thioether, triphenylmethyl thioether, and p-methoxyphenylphenylmethyl thioether. In other embodiments, R³ is an optionally substituted thioether selected from allyl, benzyl, or triphenylmethyl, or trichloroethoxycarbonyl thioester. In certain embodiments, R³ is

\[ -S-S-pyridin-2-yl \quad -S-SBz \quad -S-SCH₂ \quad or \quad -S-S(ephenthylbenzyl). \]

In other embodiments, R³ is \[ -S-S-pyridin-2-yl. \]

In still other embodiments, the R² group of formula III is a crown ether. Examples of such crown ethers include 12-crown-4, 15-crown-5, and 18-crown-6.

In still other embodiments, the R³ moiety of the R¹ group of either of formula III is a detectable moiety. According to one aspect of the invention, the R³ moiety of the R¹ group of formula III is a fluoroscent moiety. Such fluorescent moieties are well known in the art and include coumarins, quinolones, benzisoxo quinolones, hostasol, and Rhodamine dyes, to name but a few. Exemplary fluorescent moieties of the R³ group of R include anthracen-9-yl, pyren-4-yl, 9H-carbazol-9-yl, the carboxylate of rhodamine B, and the carboxylate of coumarin 343.

In certain embodiments, the R³ moiety of the R¹ group of formula III is a group suitable for Click chemistry. Click reactions tend to involve high-energy ("spring-loaded") reagents with well-defined reaction coordinates, that give rise to selective bond-forming events of wide scope. Examples include nucleophilic trapping of strained-ring electrophiles (epoxide, aziridines, aziridinium ions, episulfonium ions), certain carbonyl reactivity (e.g., the reaction between aldehydes and hydrzones or hydroxylamines), and several cycloaddition reactions. The azide-alkyne 1,3-dipolar cycloaddition is one such reaction. Click chemistry is known in the art and one of ordinary skill in the art would recognize that certain R³ moieties of the present invention are suitable for Click chemistry.

Compounds of formula III having R³ moieties suitable for Click chemistry are useful for conjugating said compounds to biological systems or macromolecules such as proteins, viruses, and cells, to name but a few. The Click reaction is known to proceed quickly and selectively under physiological conditions. In contrast, most conjugation reactions are carried out using the primary amine functionality on proteins (e.g. lysine or protein end-group). Because most proteins contain a multitude of lysines and arginines, such conjugation occurs uncontrollably at multiple sites on the protein. This is particularly problematic when lysines or arginines are located around the active site of an enzyme or other biomolecule. Thus, another embodiment of the present invention provides a method of conjugating the R³ groups of a compound of formula III to a macromolecule via Click chemistry. Yet another embodiment of the present invention provides a macromolecule conjugated to a compound of formula III via the R¹ group.

After conjugation to a biomolecule, drug, cell, substrate, or the like, the other end-group functionality, corresponding to the R² group of formula III, can be used to attach targeting groups for cell-specific delivery including, but not limited to, detectable moieties, such as fluorescent dyes, covalent attachment to surfaces, and incorporation into hydrogels.

According to one embodiment, the R³ moiety of the R¹ group of either of formula III is an azide-containing group. According to another embodiment, the R³ moiety of the R¹ group of either of formula III is an alkyn-containing group.

In certain embodiments, the R³ moiety of the R¹ group of formula III has a terminal alkyn moiety. In other embodiments, the R³ moiety of the R¹ group of formula III is an alkyn moiety having an electron withdrawing group. Accordingly, in such embodiments, the R³ moiety of the R¹ group of formula III is

\[ \text{wherein } E \text{ is an electron withdrawing group and } y = 0-6. \]

Such electron withdrawing groups are known to one of ordinary skill in the art. In certain embodiments, E is an ester. In other embodiments, the R³ moiety of the R¹ group of formula III is

\[ \text{wherein } E \text{ is an electron withdrawing group, such as } -C(O)-O- \text{ and } y = 0-6. \]

Exemplary R¹ groups of compounds of the present invention are set forth in Table 1, below.

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<tr>
<th>Table 1</th>
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<td>a</td>
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Representative R¹ Groups
TABLE 1-continued

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**TABLE 1-continued**
In certain embodiments, the R^1 group of any of formulae I, II, II', and III is selected from any of those R^1 groups depicted in Table 1, supra. In other embodiments, the R^1 group of any of formulae I, II, II', and III is n, o, cc, dd, ee, ff, hh, h, ii, jj, ll, or uu. In still other embodiments, the R^1 group of any of formulae I, II, II', and III is h, aa, yy, zz, or aa.

According to another aspect of the present invention, the R^1 group of any of formulae I, II, II', and III is q, r, s, t, www, xxx, or yyy.

In certain embodiments, methods of the present invention are performed in a suitable medium. According to one embodiment, a suitable medium for the preparation of compounds of formula III includes a polar aprotic solvent or a mixture thereof. Examples of polar aprotic solvents include, but are not limited to, DMF, DMSO, THF, hexamethylphosphoramide, glyme, diglyme, MTBE, N-methyl pyrrolidone, and acetonitrile.

As defined generally above, the Q group of formula III is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy, -O-, -NH-, =S-, -OC(O)-, =C(O)O-, =C(O)N-, -SO-, -SO_2-, -NHSO_2-, -SO_2NH-, -NHC(O)-, -C(O)NH-, =OC(O)NH-, or =NHC(O)O-, wherein -Cy is an optionally substituted 5-8 membered bivalent saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, Q is a valence bond. In other embodiments, Q is a bivalent, saturated C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy, -O-, -NH-, =S-, -OC(O)-, =C(O)O-, =C(O)N-, wherein -Cy is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

In certain embodiments, Q is -Cy- (i.e. a C_{1} alkylene chain wherein the methylene unit is replaced by -Cy-), wherein -Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to another aspect of the present invention, -Cy- is an optionally substituted bivalent phenyl group. In other embodiments, -Cy- is an optionally substituted 5-8 membered bivalent, saturated carbocyclic ring. In still other embodiments, -Cy- is an optionally substituted 5-8 membered bivalent, saturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Exemplary -Cy- groups include bivalent rings selected from phenyl, pyridyl, pyrimidyl, cyclohexyl, cyclopentyl, or cyclopropyl.

As defined generally above, the R^{2d} group of formula III is a mono-protected amine, di-protected amine, -NR^{3}, -N(R^{3})^{2}, -NHC(O)R^{3}, -NR^{3}C(O)R^{3}, -NHC(O)NR^{3}, -NHC(O)N(R^{3})_{2}, -NR^{3}C(O)NR^{3}, -NHC(O)OR^{3}, -NHC(O)NR^{3}, or -NR^{3}SO_{2}R^{3}, wherein each R^{3} is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, or two R^{3} on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7 membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

In certain embodiments, the R^{2b} group of formula III is -NR^{3}_2 or -N(R^{3})_{2} wherein each R^{3} is an optionally substituted aliphatic group. One exemplary R^{3} group is 5-norbornen-2-yl-methyl. According to yet another aspect of the present invention, the R^{2b} group of formula III is -NH_{2}, wherein R^{2} is a C_{1-5}, aliphatic group substituted with N_{2}. Examples include -CH_{2}N_{2}. In some embodiments, R^{2} is an optionally substituted C_{1-6} alkyl group. Examples include methy1, ethyl, propyl, butyl, pentyl, hexyl, 2-(tetrahydrodipyromethan-2-yl)oxyethyl, pyridin-2-ylsulfanyl)ethyl, methyldisulfanyl)ethyl, (4-acetylphenyl)ethyl, 3-(methoxycarbonyl)-prop-2-ynyl, methoxyacarbomethyl, 2-(N-methyl-N-(4-acetylphenyl)carbonylamino)ethyl, 2-phthalimidoethyl, 4-bromobenzyl, 4-chlorobenzyl, 4-fluorobenzyl, 4-iodobenzyl, 4-propargyloxybenzyl, 2-nitrobenzyl, 4-(bis-4-acetylbenzyl)aminomethyl-benzyl, 4-propargyloxy-benzyl, 4-dipropargylamino-benzyl, 4-(2-propargyloxy-ethyl)disulfanyl)benzyl, 2-propargyloxy-ethyl, 2-propargyldisulfanyl-ethyl, 4-propargyloxy-butyl, 2-(N-methyl-N-propargylamino)ethyl, and 2-(2-dipropargylamino)ethyl.
noethoxy)-ethylin. In other embodiments, R' is an optionall substituted C<sub>2</sub> alkyl group. Examples include vinyl, allyl, crotyl, 2-propenyl, and but-3-enyl. When R<sup>2</sup> group is a substituted aliphatic group, suitable substituents on R<sup>2</sup> include N<sub>2</sub>, CN, and halogen. In certain embodiments, R<sup>2</sup> is —CH<sub>2</sub>CH<sub>2</sub>CN, —CH<sub>2</sub>CH(OCH<sub>2</sub>), 4-(bisbenzoylomethyl)phenylethyl, and the like.

According to another aspect of the present invention, the R<sup>2</sup>' group of formula III is —NHR<sup>4</sup> wherein R<sup>4</sup> is an optionally substituted C<sub>2</sub> alkyl group. Examples include —CO—CH<sub>3</sub>, —CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>—, —CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, and —CH<sub>2</sub>CH<sub>2</sub>CH—CH<sub>2</sub>. In certain embodiments, the R<sup>2</sup>' group of formula III is —NHR<sup>5</sup> wherein R<sup>5</sup> is an optionally substituted 5-8-membered aryl ring. In certain embodiments, R<sup>5</sup> is optionally substituted phenyl or optionally substituted pyridyl. Examples include phenyl, 4-t-butoxycarbonylaminophenol, 4-azidomethyl phenyl, 4-propargyloxyphenol, 2-pyridyl, 3-pyridyl, and 4-pyridyl. In certain embodiments, R<sup>2</sup>' is 4-t-butoxycarbonyloxyphenol, 4-azidomethylphenol, or 4-propargyloxyphenol.

In certain embodiments, the R<sup>2</sup>' group of formula III is —NHR<sup>5</sup> wherein R<sup>5</sup> is an optionally substituted phenyl ring. Suitable substituents on the R<sup>2</sup>' phenyl ring include halogen; —(CH<sub>2</sub>)<sub>m</sub>R<sup>1</sup>; —(CH<sub>2</sub>)<sub>m</sub>OR; —(CH<sub>2</sub>)<sub>m</sub>CO(R<sup>1</sup>); —(CH<sub>2</sub>)<sub>m</sub>Ph, which may be substituted with R<sup>1</sup>; —(CH<sub>2</sub>)<sub>m</sub>0(CH<sub>2</sub>)<sub>n</sub>Ph which may be substituted with R<sup>m</sup>; —CHCH<sub>2</sub>Ph, which may be substituted with R<sup>m</sup>; —CN; —N<sub>2</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>); —N(R<sup>m</sup>) CR<sup>n</sup>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>)<sub>n</sub>; —N(R<sup>m</sup>) CR<sup>n</sup>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>)OR<sup>n</sup>; —N(R<sup>m</sup>) CR<sup>n</sup>; —N(R<sup>m</sup>)CR<sup>n</sup>OR<sup>n</sup>; —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>)OR<sup>n</sup>; —N(R<sup>m</sup>) CR<sup>n</sup>; —N(R<sup>m</sup>)CR<sup>n</sup>OR<sup>n</sup>; —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>)OR<sup>n</sup>; —N(R<sup>m</sup>) CR<sup>n</sup>; —N(R<sup>m</sup>)CR<sup>n</sup>OR<sup>n</sup>; —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>NC(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(OOR<sup>n</sup>)<sub>n</sub>; —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>); —(CH<sub>2</sub>)<sub>m</sub>N(R<sup>m</sup>)C(NR<sup>m</sup>)OR<sup>n</sup>.

In certain embodiments, the R<sup>2</sup>' group of formula III comprises a group suitable for Click chemistry. One of ordinary skill in the art would recognize that certain R<sup>2</sup>' groups of the present invention are suitable for Click chemistry.

Compounds of formula III having R<sup>2</sup>' groups comprising groups suitable for Click chemistry are useful for conjugating said compounds to biological systems such as proteins, viruses, and cells, to name but a few. After conjugation to a biomolecule, drug, cell, substrate, or the like, the other end group functionality, corresponding to the R<sup>2</sup>' moiety of formula III, can be used to attach targeting groups for cell specific delivery including, but not limited to, fluorescent dyes, covalent attachment to surfaces, and incorporation into hydrogels. Thus, another embodiment of the present invention provides a method of conjugating the R<sup>2</sup>' group of a compound of formula III to a macromolecule via Click chemistry. Yet another embodiment of the present invention provides a macromolecule conjugated to a compound of formula III via the R<sup>2</sup>' group.

According to one embodiment, the R<sup>2</sup>' group of formula III is an azide-containing group. According to another embodiment, the R<sup>2</sup>' group of formula III is an alkyne-containing group.

In certain embodiments, the R<sup>2</sup>' group of formula III has a terminal alkyne moiety. In other embodiments, the R<sup>2</sup>' group of formula III is an alkyne-containing moiety having an electron withdrawing group. Accordingly, in such embodiments, the R<sup>2</sup>' group of formula III is
wherein E is an electron withdrawing group, such as a —C(O) O— group and y is 0-6.

According to another embodiment, the present invention provides compounds of formula III, as described above, wherein said compounds have a polydispersity index (“PDI”) of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula III, as described above, wherein said compound has a polydispersity index (“PDI”) of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula III, as described above, wherein said compound has a polydispersity index (“PDI”) of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula III having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula III, as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

Exemplary R^26 groups of formula III are set forth in Table 2, below.

### TABLE 2

<table>
<thead>
<tr>
<th>Representative R^26 Groups</th>
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<tbody>
<tr>
<td>i</td>
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### TABLE 2-continued

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</table>
TABLE 2-continued

<table>
<thead>
<tr>
<th>Representative R&lt;sup&gt;2&lt;/sup&gt; Groups</th>
</tr>
</thead>
</table>

In certain embodiments, the R<sup>2a</sup> group of formula III is selected from any of those R<sup>2a</sup> groups depicted in Table 2, supra. In other embodiments, the R<sup>2</sup> group of formula III is group v, vii, vi, xii, xxx, xxxi, xxxii, xxxiii, xxxiv, xxxv, xxxvi, xxxvii, or xlii. In yet other embodiments, the R<sup>2a</sup> group of formula III is xv, xvii, xx, xxi, xxxviii, or xxxix.

As described above, one step in the preparation of a compound of formula III comprises terminating the living polymer chain-end of the compound of formula II' with a suitable polymerization terminator to afford a compound of formula III. One of ordinary skill in the art would recognize that the polymerization terminator provides the R<sup>2</sup> group of formula III. Accordingly, embodiments directed to the R<sup>2a</sup> group of formula III, as set forth above and herein, are also directed to the suitable polymerization terminator itself, and similarly, embodiments directed to the suitable polymerization terminator, as set forth above and herein, are also directed to the R<sup>2</sup> group of formula III.

As described above, compounds of formula III are prepared from compounds of formula II' by treatment with a suitable terminating agent. One of ordinary skill in the art would recognize that compounds of formula III are also readily prepared directly from compounds of formula II. In such cases, and in certain embodiments, the compound of formula II is treated with a base to form the freebase compound prior to, or concurrent with, treatment with the suitable terminating agent. For example, it is contemplated that a compound of formula II is treated with a base and a suitable terminating agent in the same reaction to form a compound of formula III. In such cases, it is also contemplated that the base may also serve as the reaction medium.

One of ordinary skill in the art would also recognize that the above method for preparing a compound of formula III may be performed as a "one-pot" synthesis of compounds of formula III that utilizes the living polymer chain-end to incorporate the R<sup>2</sup> group of formula III. Alternatively, compounds of formula III may also be prepared in a multi-step fashion. For example, the living polymer chain-end of a compound of formula II may be quenched to afford an amino group which may then be further derivatized, according to known methods, to afford a compound of formula III.

One of ordinary skill in the art will recognize that a variety of polymerization terminating agents are suitable for the present invention. Such polymerization terminating agents include any R<sup>2a</sup>-containing group capable of reacting with the living polymer chain-end of a compound of formula II, or the free-based amino group of formula II', to afford a compound of formula III. Thus, polymerization terminating agents include anhydrides, and other acylating agents, and groups that contain a suitable leaving group that is subject to nucleophilic displacement.

Alternatively, compounds of formula II or II' may be coupled to carboxylic acid-containing groups to form an amide thereof. Thus, it is contemplated that the amine group of formula II or II' may be coupled with a carboxylic acid moiety to afford compounds of formula III wherein R<sup>2</sup>' is —NHC(O)R<sup>4</sup>. Such coupling reactions are well known in the art. In certain embodiments, the coupling is achieved with a suitable coupling reagent. Such reagents are well known in the art and include, for example, DCC and EDC, among others. In other embodiments, the carboxylic acid moiety is activated for use in the coupling reaction. Such activation includes formation of an acyl halide, use of a Mukaiyama reagent, and the like. These methods, and others, are known to one of ordinary skill in the art, e.g., see, "Advanced Organic Chemistry," Jerry March, 5th Ed., pp. 351-357, John Wiley and Sons, N.Y.

A "suitable leaving group that is subject to nucleophilic displacement" is a chemical group that is readily displaced by a desired incoming chemical moiety. Suitable leaving groups are well known in the art, e.g., see, March. Such leaving groups include, but are not limited to, halogen, alkoxy, sulphonyloxy, optionally substituted alkylsulphonyloxy, optionally substituted alkylsulphonfyoxy, or diazonium moieties. Examples of suitable leaving groups include chloro, iodo, bromo, fluoro, methanesulphonyloxy (mesyl), toslyloxy, triflyloxy, nitrophenylsulfonyloxy (nosylox), and bromo-phenylsulfonyloxy (brosylox).

According to an alternate embodiment, a suitable leaving group may be generated in situ within the reaction medium. For example, a leaving group may be generated in situ from a precursor of that compound wherein said precursor contains a group readily replaced by said leaving group in situ.

Alternatively, when the R<sup>2</sup> group of formula III is a mono- or di-protected amine, the protecting group(s) is removed and that functional group may be derivatized or protected with a different protecting group. It will be appreciated that the removal of any protecting group of the R<sup>2</sup>' group of formula III is performed by methods suitable for that protecting group. Such methods are described in detail in Green.

In other embodiments, the R<sup>2a</sup> group of formula III is incorporated by derivatization of the amino group of formula II or II' via anhydride coupling, optionally in the presence of base as appropriate. One of ordinary skill in the art would recognize that anhydride polymerization terminating agents containing an azide, an aldehyde, a hydroxyl, an alkylene, and other groups, or protected forms thereof, may be used to incorporate said azide, said aldehyde, said protected hydroxyl, said alkylene, and other groups into the R<sup>2</sup> group of compounds of formula III. It will also be appreciated that such anhydride polymerization terminating agents are also...
suitable for terminating the living polymer chain-end of a compound of formula II. Such anhydride polymerization terminating agents include, but are not limited to, those set forth in Table 3, below.

**TABLE 3**

Representative Anhydride Polymerization Terminating Agents

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<th>Structure</th>
<th>Number</th>
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<tbody>
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</table>

In other embodiments, the $R^4$ moiety of the R$^{24}$ group of formula III is incorporated by derivatization of the amino group of formula II or II* via reaction with a polymerization terminating agent having a suitable leaving group. It will also be appreciated that such polymerization terminating agents are also suitable for terminating the living polymer chain-end of a compound of formula II. Examples of these polymerization terminating agents include, but are not limited to, those set forth in Table 4, below.
wherein each L is a suitable leaving group as defined above and in classes and subclasses as described above and herein. Exemplary compounds of formula II are set forth in Table 5, below.
Representative compounds of formula II

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</table>

- **II-2**
  - Chemical structure
  - Represents a compound with specific functional groups and structures.

- **II-3**
  - Chemical structure
  - Represents a compound with specific functional groups and structures.

- **II-4**
  - Chemical structure
  - Represents a compound with specific functional groups and structures.

- **II-5**
  - Chemical structure
  - Represents a compound with specific functional groups and structures.
TABLE 5-continued

Representative compounds of formula II

II-10

II-11

II-12
wherein each $R^1$, $n$, $m$, $m'$, and $A$ are as defined above and in classes and subclasses as described above and herein.

According to another embodiment, the present invention provides compounds of formula II, as described above, wherein said compounds have a polydispersity index ("PDI") of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula II, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula II, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.12. According to other embodiments, the present invention provides compounds of formula II having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula II, as described above, wherein $n$ is about 225. In other embodiments, $n$ is about 200 to about 300. In still other embodiments, $n$ is about 200 to about 250. In still other embodiments, $n$ is about 100 to about 150. In still other embodiments, $n$ is about 400 to about 500.

Exemplary compounds of formula II are set forth in Table 6, below.

\begin{table}[h]
\centering
\caption{Representative compounds of formula II'}
\end{table}
wherein each R<sub>1</sub>, n, m, and m' are as defined above and in classes and subclasses as described above and herein.

According to another embodiment, the present invention provides compounds of formula II, as described above, wherein said compounds have a polydispersity index ("PDI") of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula II, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula II', as described above, wherein said compound has a polydispersity index ("PDI") of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula II having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula II', as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

Alternatively, and as described in detail in U.S. Ser. No. 11/256,735, suitably protected PEG-amines may be formed by initiating the polymerization of ethylene oxide with a compound that contains a suitably protected amino moiety. The PEG formed therefrom may be terminated by any manner known in the art, including those described in U.S. Ser. No. 11/256,735. Upon termination of the polymerization, the protected amino moiety is then deprotected and an amine salt formed. This amine salt is then used to initiate the polymerization of NCAs as described herein.

Accordingly, an alternate method of the present invention provides a method of preparing a compound of formula IV:

wherein:
A is a suitable acid anion;
n is 10-2500;
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C<sub>1-12</sub> alkylen chain, wherein 0-6 methylene units of Q are independently replaced by: -Cy, -O-, -NH-, -S-, -OC(O), -C(O) O-, -C(O), -SO-, -SO<sub>2</sub>-, -NH(O)SO<sub>2</sub>-, -SO<sub>2</sub>NH-, -NH(C)O-, -C(O)NH-, -OC(O) NH-, or -NH(C)O(O)O-, wherein:
-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;
R<sup>2</sup> is halogen, N<sub>3</sub>, CN, a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected aldehyde, a protected thiol, -NR<sup>4</sup>-, -N(R<sup>3</sup>)<sub>3</sub>, -SR<sup>4</sup>,-O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>n</sub>(CH<sub>2</sub>)<sub>3</sub>R<sup>5</sup>, -OC(O)R<sup>3</sup>, or -OS(O)R<sup>5</sup>; q and r are each independently 0-4;
each R<sup>4</sup> is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, or:
two R<sup>4</sup> on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7 membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and
R<sup>5</sup> is hydrogen, halogen, CN, a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, or an optionally substituted group selected from aliphatic.
phatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, comprising the steps of:

(a) providing a compound of formula A:

\[
\text{A}
\]

wherein:

- \( n \) is 10-2500;
- \( M \) is the cation of a suitable metal;
- each of \( PG^1 \) and \( PG^2 \) is hydrogen or a suitable amino protecting group, or \( PG^1 \) and \( PG^2 \) are taken together to form a cyclic amino protecting group, provided that at least one of \( PG^1 \) and \( PG^2 \) is a suitable amino protecting group; and
- \( Q \) is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkyne chain, wherein 0-6 methylene units of \( Q \) are independently replaced by -Cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO_2-, -NHSO_2-, -SO_2NH-, -NHC(O)-, -C(O)NH-, -OC(O)NH-, or -NHC(O)O-, wherein:
  - -Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, or:
  - two \( R^4 \) on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7 membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and
  - \( R^4 \) is hydrogen, halogen, CN, a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected aldehyde, a protected thiol, -NHRR', -N(R')_2, -SR', -O(CH_2)_nH_2O_2, -OC(O)R', or -OS(O)_2 R';
- and
- \( q \) and \( r \) are each independently 0-4;
- each \( R^4 \) is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, and
- (c) deprotecting the amino group and forming a salt thereof to form a compound of formula IV.

As described generally above, the \( M \) moiety of formula A is the cation of a metal capable of forming a corresponding anion, of affecting the polymerization of ethylene oxide. In certain embodiments, \( M \) is K', Cs', Na', Al(OM)_3, or Y'. In other embodiments, \( M \) is K' or Na'. According to another aspect of the present invention, \( M \) is K'. In other embodiments \( M \) is a transition metal such as Sn, Pb, Zn, Cd, Cu, Pd, Mn, Cr, Mo, W, Fe, Co or organometallic complexes of these metals. In yet other embodiments, \( M \) is a rare-earth metal such as Sc, La, Pr, Nd, Sm, Eu, Gd, Dy, Yb or organometallic complexes of these metals.

In another embodiment, the present invention provides a method for preparing a compound of formula IV:

\[
\text{IV}
\]

wherein:

- each of \( PG^1 \) and \( PG^2 \) is hydrogen or a suitable amino protecting group, or \( PG^1 \) and \( PG^2 \) are taken together to form a cyclic amino protecting group, provided that at least one of \( PG^1 \) and \( PG^2 \) is a suitable amino protecting group; and
- \( Q \) is a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkyne chain, wherein 0-6 methylene units of \( Q \) are independently replaced by -Cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO_2-, -NHSO_2-, -SO_2NH-, -NHC(O)-, -C(O)NH-, -OC(O)NH-, or -NHC(O)O-, wherein:
  - -Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10-membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and
- \( R^2 \) is halogen, N3, CN, a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected aldehyde, a protected thiol, -NHR', -N(R')_2, -SR', -O(CH_2)_nH_2O_2, -OC(O)R', or -OS(O)_2 R';
- and
- \( q \) and \( r \) are each independently 0-4.
-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

(b) polymerizing ethylene oxide onto said polymerization initiator to provide a compound of formula A:

\[
\text{A} = \begin{array}{c}
\text{PG}^1 \\
\text{PG}^2 \\
\end{array}
\]

wherein:

- n is 10-2500;
- M is the cation of a suitable metal;
- each of PG\(^1\) and PG\(^2\) is hydrogen or a suitable amino protecting group, or PG\(^1\) and PG\(^2\) are taken together to form a cyclic amino protecting group, provided that at least one of PG\(^1\) and PG\(^2\) is a suitable amino protecting group; and
- Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C\(_{1-12}\) alkyne chain, wherein 0-6 methylene units of Q are independently replaced by
  - cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)
  - O-, -C(O)-, -SO-, -SO\(_2\)-, -NH\(_2\),
  - SO\(_2\)NH-, -NH(O)-, -C(O)NH-, -OC(O)
  - NH-, or -NH(O)O-, wherein:
- Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

(c) terminating the living polymer chain-end of the compound of formula A with a suitable polymerization terminator to afford a compound of formula C:

\[
\text{C} = \begin{array}{c}
\text{PG}^1 \\
\text{PG}^2 \\
\end{array}
\]

A is a suitable acid anion;
- n is 10-2500;
- each of PG\(^1\) and PG\(^2\) is hydrogen or a suitable amino protecting group, or PG\(^1\) and PG\(^2\) are taken together to form a cyclic amino protecting group, provided that at least one of PG\(^1\) and PG\(^2\) is a suitable amino protecting group
- Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C\(_{1-12}\) alkyne chain, wherein 0-6 methylene units of Q are independently replaced by
  - cy-, -O-, -NH-, -S-, -OC(O)-, -C(O)
  - O-, -C(O)-, -SO-, -SO\(_2\)-, -NH\(_2\),
  - SO\(_2\)NH-, -NH(O)-, -C(O)NH-, -OC(O)
  - NH-, or -NH(O)O-, wherein:

As defined generally above, PG\(^1\) and PG\(^2\) are suitable amino protecting groups. Suitably protected amines include, but are not limited to, aralkylamines, carbamates, cyclic imides, allyl amines, amidines, and the like. Examples of amino protecting groups include tert-butylcarboxylic acid (BOC), ethylcarboxylic acid, methylcarboxylic acid, chloroethylcarboxylic acid, allylcarboxylic acid, allyloxycarbonyl (Alloc), benzoxycarbonyl (CBZ), allyl, phthalimide, benzyl (Bz), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzyl, and the like. In certain embodiments, the protected amine is phthalimido. In other embodiments, the amino protecting group is benzyl or allyl. In still other embodiments, the amino protecting group is a tert-butyloxycarbonyl (BOC) group. In certain embodiments, PG\(^1\) and PG\(^2\) are taken together to form a cyclic amino protecting group. Such cyclic amino protecting groups include phthalimide, maleimide, succinimide, and the like.

As defined generally above, the R\(^2\) group of formulae IV and C is halogen, N\(_3\), CN, a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected aldehyde, a protected thiol, -NR\(^4\)-, -N(R\(^4\))\(_2\), -SR\(^4\),
-OC(CH\(_3\))\(_2\)O, (CH\(_2\))\(_2\)R\(^5\), -OC(O)R\(^4\), or -OS(O)\(_2\)R\(^4\),

where q and r are each independently 0-4;
- each R\(^5\) is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or a detectable moiety, or
- two R\(^4\) on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7 membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and
- R\(^2\) is hydrogen, halogen, CN, a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected carboxylic acid, a protected aldehyde, a protected thiol, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or a detectable moiety, or
- removal of the PG\(^1\) and/or PG\(^2\) protecting groups; and
- treatment with an acid to form the compound of formula IV.

Embodiments, classes, and subclasses described herein for the Q and groups of formulae I, II, III, and III apply singly and in combination to compounds of formulae IV, A, B, and C.
wherein q and r are each independently 0-4, each R⁴ is independently an optionally substituted group selected from aliphatic, a 5-8-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, or two R⁵ on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7-membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and R⁵ is hydrogen, halogen, CN, a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, or an optionally substituted group selected from aliphatic, a 5-8-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety.

In certain embodiments, the R² group of either of formulae IV and C is —N₃.

In other embodiments, the R² group of either of formulae IV and C is —CN.

In other embodiments, the R² group of either of formulae IV and C is —Br, —Cl, —F, or —I.

In certain embodiments, the R² group of either of formulae IV and C is —OS(O)R³, wherein R³ is an optionally substituted aliphatic group, or an optionally substituted 5-8-membered aryl ring. Exemplary R³ groups include p-tolyl and methyl. In certain embodiments, R² is p-toluenesulfonyl or methanesulfonyl.

In certain embodiments, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is an optionally substituted aliphatic group. One exemplary R⁴ group is 5-norbornene-2-yl-methyl. According to yet another aspect of the present invention, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is a C₅₋₆ aliphatic group substituted with N₃. Examples include —CH₃N₃. In some embodiments, R² is an optionally substituted C₁₋₆ alkyl group. Examples include methyl, ethyl, propyl, butyl, pentyl, hexyl, 2-(tetrahydrofuran-2-yl)ethyl, pyridinyl-2-ylsulfonyl methyl, methyldisulfanyl methyl, (4-acetylenylethylene)methyl, (1-(methoxycarbonyl)-prop-2-ynyl) methoxy carbonylmethyl, 2-(methyl-N-4-(4-acetylenyl)phenyl)carboxylamino)-ethyl, 2-phthalimidomethyl, 4-bromobenzyl, 4-chlorobenzyl, 4-fluorobenzyl, 4-iodobenzyl, 4-propargyloxybenzyl, 2-bromobenzyl, 4-(bis-4-acetylenylbenzyl)aminomethyl benzyl, propargyloxy-4-propargyloxyethylsulfonyl benzyl, 2-propargyloxy-ethylsulfonyl ethyl, 2-propargyloxydisulfanyl ethyl, 4-propargyloxybutyl, 2-(methyl-N-propargylamino) ethyl, and 2-(2-propargyloxyethylsulfonyl) benzyl, 2-propargyloxy-ethylsulfonyl ethyl, 2-propargyloxydisulfanyl ethyl, 4-propargyloxybutyl, 2-(methyl-N-propargylamino) ethyl, and 2-(2-propargyloxyethylsulfonyl) benzyl.

In other embodiments, R² is an optionally substituted C₁₋₆ alkyl group. Examples include vinyl, allyl, crotyl, 2-propenyl, and but-3-enyl. When R² is a group substituted with a substituent on R² include N₃, CN, and halogen. In certain embodiments, R² is —CH₃CN, —CH₂CH₂CN, —CH₂CH(OCH₃)₂, —CH₂CH₂CN, 4-(bisbenzoyl oxyethyl)methylphenyl, and the like.

According to another aspect of the present invention, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is an optionally substituted C₁₋₆ alkyl group. Examples include —CC=CH, —CH₂CH=CH₂, —CH₂CH₂CN, and —CH₂CH₂CN. In certain embodiments, R² is propargyloxy.

In other embodiments, the R² group of either of formulae IV and C is —OC(O)R⁴ wherein R⁴ is an optionally substituted aliphatic group. Examples include methyl, ethyl, propyl, butyl, pentyl, hexyl, acetylenyl, propargyl, but-3-ynyl, vinyl, crotyl, 2-propenyl, azidomethyl, 5-norbornene-2-yl, octen-5-yl, trisopropylsilylethynyl, 4-vinylphenoxy, 4-dipropargylylamino phenyl, 4-propargyloxoyphenyl, 4-(2-propargyldisulfanyl methyl) phenyl, and 2-(propargyloxoy carbony)ethyl.

In certain embodiments, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is an optionally substituted 5-8-membered aryl ring. In certain embodiments, R² is optionally substituted phenyl or optionally substituted pyridyl. Examples include phenyl, 4-butoxy carbonylamino phenyl, 4-azidomethyl phenyl, 4-propargyloxoy phenyl, 2-pyridyl, 3-pyridyl and 4-pyridyl. In certain embodiments, R² is 4-butoxy carbonylamino phenyl, 4-azidomethyl phenoxyl, or 4-propargyloxoy phenoxyl.

In other embodiments, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is an optionally substituted phenyl ring. Suitable substituents on the R² phenyl ring include halogen: —(CH₂)₃—OR⁴; —(CH₂)₉—OR⁴; —(CH₂)₁₀—SR⁴; —(CH₂)₉—Ph, which may be substituted with R⁴; —(CH₂)₉—O(CH₂)₉—Ph which may be substituted with R⁴; —CH=CHPh, which may be substituted with R⁴; —NO₂; —CN; —N₃; —(CH₂)₉—N(R⁴)²; —(CH₂)₉—N(R⁴)N(R⁴)²; —(CH₂)₉—N(R⁴)C(O)N(R⁴)²; —N(R⁴)C(S)N(R⁴)²; —(CH₂)₉—N(R⁴)C(O)N(R⁴)²; —N(R⁴)C(S)N(R⁴)²; —(CH₂)₉—C(O)OR⁴; —(CH₂)₉—C(O)SR⁴; —(CH₂)₉—C(O)OSR⁴; —(CH₂)₉—C(O)OCOR⁴; —(CH₂)₉—SC(O)R⁴; —(CH₂)₉—SC(O)OSR⁴; —(CH₂)₉—SC(O)NR⁴; —(CH₂)₉—SC(O)NR⁴; —(CH₂)₉—SC(O)NR⁴; —(CH₂)₉—SC(O)NR⁴; —(CH₂)₉—SC(O)NR⁴.

In other embodiments, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is phenyl substituted with one or more optionally substituted C₁₋₆ aliphatic groups. In still other embodiments, R² is phenyl substituted with vinyl, allyl, acetylenyl, —CH₂N₃, —CH₂CH₂N₃, —CH₂C=CH₂ or —CH₂C=CH₂.

In other embodiments, the R² group of either of formulae IV and C is —OR⁴ wherein R⁴ is phenyl substituted with N₃, N(R⁴)₂, CO₂R⁴, or CO(O)R⁴ wherein R⁴ is independently as defined herein supra.

In other embodiments, the R² group of either of formulae IV and C is a protected hydroxyl group. In certain embodiments the protected hydroxyl of the R² moiety is an ester, carbonate, sulfonate, allyl ether, ether, silyl ether, alkyl ether, arylalkyl ether, or alkoxyalkyl ether. In certain embodiments, the ester is a formate, acetate, propionate, pentaenoate, crotonate, or benzoate. Exemplary esters include formate, benzyloxycarboxylate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionic acid, 4-oxopentanoate, 4,4-(ethylenedioxy) pentenoate, pivaloate (trimethoxycacetate), crotonate, 4-methoxy-crotonate, benzoate, p-benzybenzoate, 2,4,6-trimethoxybenzoate. Exemplary carbonates include 9-fluorocarbonylethyl, 2,2,2-trichloroethoxy, 2-(trimethoxymethyl) ethyl, 2-(p-phenyloxysulfonyl) ethyl, vinyl, allyl, and p-nitrobenzyl carbonate.

Exemplary silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, trisopropyl silyl ether, and other trialkylsilyl ethers. Exemplary
According to another embodiment, the R2 group of either of formulae IV and C is a protected thiol group. In certain embodiments, the protected thiol of R2 is a disulfide, thioether, silyl thioether, thioester, thiolcarbamate, or a thiothiocarbamate. Examples of such protected thioles include trisopropylsilyl thioether, tert-butyldimethylsilyl thioether, tert-buty l thioether, benzyl thioether, p-methoxybenzyl thioether, triphenylmethy l thioether, and p-methoxyphenylthiophenylmethyl thioether. In other embodiments, R2 is an optionally substituted thioether selected from alkyl, benzyl, or triphenylmethyl, or trichloroethoxy carbonyl thioether. In certain embodiments, R2 is —S—S-pyridin-2-yl, —S—S-Bn, —S—SC6H5, or —S—SP(ethyl)benzyl). In certain embodiments, R1 is —S—S-pyridin-2-yl.

In still other embodiments, the R2 group of either of formulae IV and C is a detectable moiety. According to another aspect of the invention, the R2 group of either of formulae IV and C is a fluorescent moiety. Such fluorescent moieties are well known in the art and include coumarins, quinolones, benzo[a]quinolones, hostasol, and Rhodamine dyes, to name but a few. Exemplary fluorescent moieties comprising R2 include anthracen-9-yl-methoxy, pyren-4-yl-methoxy, 2-(9-H-carbazol-9-yl)-ethoxy, the carboxyate of rhodamine B, and the carboxylate of coumarin 343.

In certain embodiments, the R2 group of either of formulae IV and C is a group suitable for Click chemistry. One of ordinary skill in the art would recognize that certain R2 groups of the present invention are suitable for Click chemistry.

Compounds of either of formulae IV and C having R2 groups suitable for Click chemistry are useful for conjugating said compounds to biological systems such as proteins, viruses, and cells, to name but a few. After conjugation to a biomolecule, drug, cell, substrate, or the like, the other end-group functionality, corresponding to the R1 moiety of either of formulae IV and C, can be used to attach targeting groups for cell specific delivery including, but not limited to, fluorescent dyes, covalent attachment to surfaces, and incorporation into hydrogels. Thus, another embodiment of the present invention provides a method of conjugating the R2 group of a compound of either of formulae IV and C to a macromolecule via Click chemistry. Yet another embodiment of the present invention provides a macromolecule conjugated to a compound of either of formulae IV and C via the R2 group.

According to one embodiment, the R2 group of either of formulae IV and C is an azide-containing group. According to another embodiment, the R2 group of either of formulae IV and C is an alkynyl-containing group.

In certain embodiments, the R2 group of either of formulae IV and C has a terminal alkyn moiety. In other embodiments, the R2 group of either of formulae IV and C is an alkynyl-containing moiety having an electron withdrawing group. Accordingly, in such embodiments, the R2 group of either of formulae IV and C is

\[
\begin{align*}
\text{R}_2 \equiv & \text{(C=C)\text{Et}} \\
\text{wherein } \text{E is an electron withdrawing group and } y = 0-6. \text{Such electron withdrawing groups are known to one of ordinary skill in the art. In certain embodiments, E is an ester. In other embodiments, the R2 group of either of formulae IV and C is}\end{align*}
\]
wherein E is an electron withdrawing group, such as a —C(O)O— group and y is 0-6.

Exemplary R² groups of either of formulae IV and C are set forth in Table 7, below.

**TABLE 7**

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<tr>
<th>Representative R² Groups</th>
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<tr>
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**Representative R² Groups**

- x
- xi
- xii
- xiii
- xiv
- xv
- xvi
- xvii
- xviii
<table>
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<td>Representative $R^2$ Groups</td>
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**TABLE 7-continued**

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**TABLE 7-continued**
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**TABLE 7-continued**

**Representative R<sup>2</sup> Groups**

| ![Structure 5](image) bxxv        |  
| ![Structure 6](image) bxxvi      |  
| ![Structure 7](image) bxxvii     |  
| ![Structure 8](image) bxxviii    |  
| ![Structure 9](image) bxxix      |  
| ![Structure 10](image) bxxx      |  

**TABLE 7-continued**

**Representative R<sup>2</sup> Groups**

| ![Structure 10](image) bxxi      |  
| ![Structure 11](image) bxxii     |  
| ![Structure 12](image) bxxiii    |  
| ![Structure 13](image) bxxiv     |  
| ![Structure 14](image) bxxv      |  
| ![Structure 15](image) bxxvi     |  
| ![Structure 16](image) bxxvii    |  

**TABLE 7-continued**

**Representative R<sup>2</sup> Groups**

| ![Structure 16](image) bxxviii   |  
| ![Structure 17](image) bxxix     |  
| ![Structure 18](image) bxxx      |  
| ![Structure 19](image) bxxi      |  
| ![Structure 20](image) bxxii     |  
| ![Structure 21](image) bxxiii    |  
| ![Structure 22](image) bxxiv     |  

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TABLE 7-continued

Representative R² Groups

In certain embodiments, the R² group of either of formulae IV and C is selected from any of those R² groups depicted in Table 2, supra. In other embodiments, the R² group of either of formulae IV and C is group xlii or xxiv. In yet other embodiments, the R² group of either of formulae IV and C is xix, xvii, xviii, xxix, xxxii, xliv, xlvi, or xlviii.

According to another aspect of the present invention, the R² group of either of formulae IV and C is ix, xxi, xxx, xxxi, xlvi, xlii, lxxi.

As described above, one step in the preparation of a compound of either of formulae IV and C comprises terminating the living polymer chain-end of the compound of formula A with a suitable polymerization terminator to afford a compound of formula C. One of ordinary skill in the art would recognize that the polymerization terminator provides the R² group of either of formulae IV and C. Accordingly, embodiments directed to the R² group of either of formulae IV and C, as set forth above and herein, are also directed to the suitable polymerization terminator itself, and similarly, embodiments directed to the suitable polymerization terminator, as set forth above and herein, are also directed to the R² group of either of formulae IV and C.

One of ordinary skill in the art would also recognize that the above method for preparing a compound of formula C may be performed as a "one-pot" synthesis of compounds of formula C that utilizes the living polymer chain-end to incorporate the R² group of formula IV. Alternatively, compounds of formula C may also be prepared in a multi-step fashion. For example, the living polymer chain-end of a compound of formula A may be quenched to afford a hydroxyl group which may then be further derivatized, according to known methods, to afford a compound of formula C.

One of ordinary skill in the art will recognize that a variety of polymerization terminating agents are suitable for the present invention. Such polymerization terminating agents include any R²-containing group capable of reacting with the living polymer chain-end of a compound of formula A to afford a compound of formula C. Thus, polymerization terminating agents include anhydrides, suitable Mitsunobu reactants, and groups that contain a suitable leaving group, L, that is subject to nucleophilic displacement.

A "suitable leaving group that is subject to nucleophilic displacement" is a chemical group that is readily displaced by a desired incoming chemical moiety. Suitable leaving groups are well known in the art, e.g., see, March. Such leaving groups include, but are not limited to, halogen, alkyl, sulphonyloxy, optionally substituted alkysulphonyloxy, optionally substituted alkenysulfonyloxy, optionally substituted arylsulfonyloxy, and diazonium moieties. Examples of suitable leaving groups include chloro, iodo, bromo, fluoro, methansulfonyloxy (mesyloxy), tosylxyloxy, triflyloxy, nitrophenyloxyloxy (nosyloxy), and bromo-phenylsulfonyloxy (brosyloxy).

According to an alternate embodiment, the suitable leaving group may be generated in situ within the reaction medium. For example, a leaving group may be generated in situ from a precursor of that compound wherein said precursor contains a group readily replaced by said leaving group in situ.

Alternatively, when the R² group of either of formulae IV and C is a protected functional group, such as a protected amine, protected thiol, protected carboxylic acid, protected acetylene, protected aldehyde, etc., the protecting group may be removed and that functional group may be derivatized or protected with a different protecting group. It will be appreciated that the removal of any protecting group of the R² group of either of formulae IV and C is performed by methods suitable for that protecting group. Such methods are described in detail in Green.

In other embodiments, the R² group of formula C is incorporated by derivatization of the hydroxyl group of formula A via anhydride coupling, optionally in the presence of base as appropriate. One of ordinary skill in the art would recognize that anhydride polymerization terminating agents containing an azide, an aldehyde, a hydroxyl, an alkyne, and other groups, or protected forms thereof, may be used to incorporate said azide, said aldehyde, said protected hydroxyl, said alkyne, and other groups into the R² group of compounds of formula C. It will also be appreciated that such anhydride polymerization terminating agents are also suitable for terminating the living polymer chain-end of a compound of formula A. Such anhydride polymerization terminating agents include, but are not limited to, those set forth in Table 8, below.
In other embodiments, the R² group of either of formulae IV and C is incorporated by derivatization of the hydroxyl group of formula A via reaction with a polymerization terminating agent having a suitable leaving group. It will also be appreciated that such polymerization terminating agents are also suitable for terminating the living polymer chain-end of a compound of formula A. Examples of these polymerization terminating agents include, but are not limited to, those set forth in Table 9, below.
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lazodicarboxylate (DEAD) and disopropyldiazodicarboxylate (DIAD). These include dibenzyl azodicarboxylate (DBAD), N,N,N',N'-tetramethylazodicarboxamide (TMAD), and dipiperidyl azodicarboxylate (DPAD). Mitsunobu coupling provides access to terminal groups including, but not limited to, halides, azide, amines, esters, ethers, thioethers and isothiocyanates. Accordingly, it will be appreciated that a variety of compounds of formulae IV and C are obtained by the derivatization of the hydroxyl group of formula A by Mitsunobu reaction.

In certain embodiments, the polymerization terminating agent is one that is capable of Mitsunobu coupling. These include optionally substituted phenols, optionally substituted thiophenols, cyclic imides, carboxylic acids, azide, and other reagents capable of Mitsunobu coupling. Such Mitsunobu terminating agents include, but are not limited to, those set forth in Table 10, below.

TABLE 10
Representative Mitsunobu Polymerization Terminating Agents

wherein each L is a suitable leaving group as defined above and in classes and subclasses as described above and herein.

As described above, a compound of formula A is treated with a polymerization terminating agent to form a compound of formula C. Such terminating agents include those described herein and in detail in U.S. Ser. No. 11/256,735. In certain embodiments, the R² group of formula IV or C is incorporated by derivatization of the hydroxyl group of formula A or B via Mitsunobu coupling. The Mitsunobu reaction is a mild method for achieving formal substitution of the hydroxyl group using azodicarboxylic esters/amides and triphenylphosphine (TPP) or trialkylphosphines or phosphites. In addition, other azo compounds have been developed as alternatives to the traditional azodicarboxylic esters diethyl-
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### Representative Mitsunobu Polymerization Terminating Agents

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According to yet another embodiment, the present invention provides a method for preparing a compound of formula \( V \):
wherein:
\( n \) is 10-2500;
\( m \) is 1 to 1000;
\( m' \) is 0 to 1000;

\( R^1 \) and \( R^2 \) are each independently a natural or unnatural amino acid side-chain group, wherein \( R^1 \) and \( R^2 \) are different from each other;

\( Q \) is a valence bond or a bivalent, saturated or unsaturated, straight or branched C\(_{1-12}\) alkylene chain, wherein 0-6 methylene units of \( Q \) are independently replaced by \(-\text{Cy}-, -\text{O}-, -\text{NH}-, -\text{S}-, -\text{OC(O)}-, -\text{C(O)}-, -\text{SO}-, -\text{SO}_2-, -\text{NHSO}_2-, -\text{SO}_2\text{NH}-, -\text{NH(C(O))}-, -\text{C(O)} \text{NH}-, -\text{OC(O)NH}-, \) or \(-\text{NHC(O)O}-\), wherein:

\(-\text{Cy}- \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

\( R^2 \) is halogen, \( \text{N}_3, \text{CN}, \) a mono-protected amine, a di-protected amine, a protected hydroxyl, a protected aldehyde, a protected thiol, \(-\text{NR}^4-, \text{NH}_2-, \text{SR}^4-, -\text{O(CH}_2\text{CH}_2\text{O)}_q\text{(CH}_2)_r\text{R}^5-, -\text{OC(O)R}^4, \) or \(-\text{OS(O)}_2\text{R}^4,-\)

\( q \) and \( r \) are each independently 0-4;

each \( R^4 \) is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety, or:

two \( R^4 \) on the same nitrogen atom are taken together with said nitrogen atom to form an optionally substituted 4-7 membered saturated, partially unsaturated, or aryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and

\( R^5 \) is hydrogen, halogen, \( \text{CN}, \) a mono-protected amine, a di-protected amine, a protected aldehyde, a protected hydroxyl, a protected carboxylic acid, a protected thiol, or an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety,

wherein said method comprises the steps of:

(a) providing a compound of formula IV:

![Diagram](image)

A is a suitable acid anion;
\( n \) is 10-2500;

(b) polymerizing a first cyclic amino acid monomer onto the amino salt terminal end of formula IV, wherein said first cyclic amino acid monomer comprises \( R^2 \); and

(c) optionally polymerizing a second cyclic amino acid monomer, comprising \( R^2 \), onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer to form a compound of formula V.

Each of the \( R^2 \), \( Q \), \( m' \), \( R^4 \), and \( R^5 \) groups of formula V are as defined above and in various embodiments, classes and subclasses described herein both singly and in combination.

In certain embodiments, the \( m' \) group of formula V is 1-1000. In certain embodiments, the \( m' \) group of formula V is 0. In other embodiments, \( m' \) is 1-1000. According to other embodiments, \( m \) and \( m' \) are independently 10 to 100 repeat units. In still other embodiments, \( m \) is 1-20 repeat units and \( m' \) is 10-50 repeat units.

According to another embodiment, the present invention provides a compound of formula V.
wherein:

n is 10-2500;
m is 1 to 1000;
R<sup>n</sup> and R<sup>r</sup> are each independently a natural or unnatural amino acid side-chain group, wherein R<sup>n</sup> and R<sup>r</sup> are different from each other.

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C<sub>1-12</sub> alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -C<sub>-</sub>-NH-, -SO<sub>-</sub>-,

Each of the R<sup>s</sup>, Q, m, R<sup>r</sup>, R<sup>n</sup>, and R<sup>r</sup> groups of formula V are as defined above and in various embodiments, classes and subclasses described herein both singly and in combination.

In certain embodiments, the m' group of formula V is 1-1000. In certain embodiments, the m' group of formula V is 0. In other embodiments, m' is 1-1000. According to other embodiments, m and m' are independently 10 to 100 repeat units. In still other embodiments, m is 1-20 repeat units and m' is 10-50 repeat units.

According to another embodiment, the present invention provides compounds of formula V, as described above, wherein said compounds have a polydispersity index (“PDI”) of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula V, as described above, wherein said compound has a polydispersity index (“PDI”) of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula V, as described above, wherein said compound has a polydispersity index (“PDI”) of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula V having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula V, as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

It will be appreciated by one of ordinary skill in the art that a compound of formula V may be treated with a base to generate the free amine. Such methods are known to one of ordinary skill in the art and include those described herein. In addition, it will be appreciated that the amino group of formula V may be further derivatized. Such derivatizations include protection, coupling, alkylation, and the like. In certain embodiments, the derivatization of the amino group of formula V incorporates an R<sup>2</sup> group as defined and described herein. Such compounds are of formula VI:

wherein:

n is 10-2500;
m is 1 to 1000;
m' is 0 to 1000;
R<sup>r</sup> and R<sup>r</sup>' are each independently a natural or unnatural amino acid side-chain group, wherein R<sup>r</sup> and R<sup>r</sup>' are different from each other;
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C<sub>1-12</sub> alkylene chain, wherein 0-6 methylene units of Q are independently
133 replaced by -Cy-, -O-, -NH-, -S-, -OC
(O)-, -C(O)-, -C(OH)-, -SO2-, -SO3-,  
NHSO3-, -SO2NH-, -NHC(O)-, -C(O)NH-
, -OC(O)NH-, or -NHC(O)O-, wherein:
-Cy- is an optionally substituted 5-8 membered biva-
 lent, saturated, partially unsaturated, or aryl ring
having 0-4 heteroatoms independently selected from
nitrogen, oxygen, or sulfur, or an optionally
substituted 8-10 membered bivalent saturated, par-
tially unsaturated, or aryl bicyclic ring having 0-5
heteroatoms independently selected from nitrogen,
134 oxygen, or sulfur;
R2 is halogen, N+, CN, a mono-protected amine, a di-
protected amine, a protected hydroxyl, a protected
aldehyde, a protected thiol, -NHR4-, -N(R5)2,
15 -SR5-, -O(CH2CH2O)x(CH2)yR5-, or -OC(O)R4 or
-O(OS(O)R)4,
q and r are each independently 0-4;
15 R2 is a mono-protected amine, a di-protected amine,
-NHRC(O)R5, -NH2, -NH(CO)R5, -NR2C(O)R4,
-NHC(O)NHRC(O)R5, -NH(CO)N(R5)2, -NR2C(O)
NHRC(O)R5, -NR2C(O)N(R5)2, -NH(CO)OR4,
-OR5-, -NRR5SO2R4, or -NRR5SO2R4;
20 each R2 is independently an optionally substituted group
selected from aliphatic, a 5-8 membered saturated,
partially unsaturated, or aryl ring having 0-4 heteroa-
toms independently selected from nitrogen, oxygen,
or sulfur, an 8-10-membered saturated, partially
unsaturated, or aryl bicyclic ring having 0-5 heteroa-
toms independently selected from nitrogen, oxygen,
or sulfur, or a detectable moiety, or:

two R2 on the same nitrogen atom are taken together
25 with said nitrogen atom to form an optionally sub-
stituted 4-7 membered saturated, partially unsat-
urated, or aryl ring having 1-4 heteroatoms inde-
dependently selected from nitrogen, oxygen, or sulfur;
and
R3 is hydrogen, halogen, CN, a mono-protected amine, a
di-protected amine, a protected hydroxyl, a protected
30 carbonyl, a protected carboxylic acid, a protected
thiol, or an optionally substituted group selected from
aliphatic, a 5-8 membered saturated, partially unsat-
urated, or aryl ring having 0-4 heteroatoms inde-
dependently selected from nitrogen, oxygen, or sulfur,
an 8-10 membered saturated, partially unsaturated, or
aryl bicyclic ring having 0-5 heteroatoms inde-
dependently selected from nitrogen, oxygen, or sulfur, or a
detectable moiety.

Each of the R2, R3, Q, m, m', R4, and R5 groups of formula
VI are as defined above and in various embodiments, classes
and subclasses described herein both singly and in combina-
tion.

In certain embodiments, the m' group of formula VI is
1-1000. In certain embodiments, the m' group of formula VI
is 0. In other embodiments, m' is 1-1000. According to other
embodiments, m and m' are independently 10 to 100 repeat
units. In still other embodiments, m is 1-20 repeat units and m'
is 10-50 repeat units.

According to another embodiment, the present invention
provides compounds of formula VI, as described above,
wherein said compounds have a polydispersity index ("PDI")
of about 1.0 to about 1.2. According to another embodiment,
the present invention provides compounds of formula VI, as
described above, wherein said compound has a polydispersity
index ("PDI") of about 1.03 to about 1.15. According to yet
another embodiment, the present invention provides com-
ounds of formula VI, as described above, wherein said com-
ounds has a polydispersity index ("PDI") of about 1.10 to
about 1.12. According to another embodiments, the present
invention provides compounds of formula VI having a PDI of
less than about 1.10.

In certain embodiments, the present invention provides
compounds of formula VI, as described above, wherein n is
about 225. In other embodiments, n is about 200 to about 300.
In still other embodiments, n is about 200 to about 250. In still
other embodiments, n is about 100 to about 150. In still other
embodiments, n is about 400 to about 500.

As described generally herein, and as depicted in Scheme
Supra, a polymer macroinitiator having two terminal amine

45 groups may be used in methods of the present invention.

Accordingly, another aspect of the present invention provides
a method of preparing a compound of formula VII:

wherein:

n is 10-2500;
each m is independently 1 to 1000;
each m' is independently 0 to 1000;
each R4 and R5 are independently a natural or unnatural
amino acid side-chain group, wherein R4 and R5 are
different from each other;
each Q is independently a valence bond or a bivalent,
saturated or unsaturated, straight or branched C1-12
alkylene chain, wherein 0-6 methylene units of Q are
independently replaced by -Cy-, -O-, -NH-, -
S-, -OC(O)-, -C(O)-, -C(OH)-, -SO-, -SO2-, -NHSO3-, -SO2NH-, -
NHC(O)-, -C(O)NH-, -OC(O)NH-, or
-NHC(O)O-, wherein:
-Cy- is an optionally substituted 5-8 membered biva-
lent, saturated, partially unsaturated, or aryl ring
having 0-4 heteroatoms independently selected from
nitrogen, oxygen, or sulfur, or an optionally
substituted 8-10 membered bivalent saturated, par-
tially unsaturated, or aryl bicyclic ring having 0-5
heteroatoms independently selected from nitrogen,
oxygen, or sulfur; and

each A is a suitable acid anion,
wherein said method comprises the steps of:
(a) providing a compound of formula B:

wherein:
- n is 10-2500;
- each Q is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-12} alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -C-O-, -C(O)O-, -C(O)-, -S-, -SO-, -SO_{2-}, -NH_{2}, -SO_{4}, -SO_{4}NH_{2}, -NH(C)O-, -C(O)NH-, -OC(O)NH-, or -NH(C)(O)O-, wherein:
  - Cy- is an optionally substituted 5-8 membered nonvalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and
  - each A is a suitable acid anion,
(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula I, wherein said first cyclic amino acid monomer comprises R^2; and
(c) optionally polymerizing a second cyclic amino acid monomer, comprising R', onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer.

Each of the Q, A, m, n, R', and R'' groups of formula VIII are as defined above and in various embodiments, classes and subclasses described herein both singly and in combination.

In certain embodiments, the m' group of formula VIII is 1-1000. In other embodiments, the m' group of formula VIII is 0. In other embodiments, m' is 1-1000. According to other embodiments, m and m' are independently 10 to 100 repeat units. In still other embodiments, m is 1-20 repeat units and m' is 10-50 repeat units.

According to another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compounds have a polydispersity index ("PDI") of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.1 to about 1.2. According to other embodiments, the present invention provides compounds of formula VIII having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula VIII, as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

In certain embodiments, the m' group of formula VIII is 0. In other embodiments, the m' group of formula VIII is 1-1000.

One of ordinary skill in the art will recognize that compounds of formula VIII may be further derivatized as described herein. In certain embodiments, the present invention provides a compound of formula VIII:
wherein:
- \( n \) is 10-2500;
- each \( m \) is independently 1 to 1000;
- each \( m' \) is independently 0 to 1000;
- each \( R^2 \) and \( R' \) are independently a natural or unnatural amino acid side-chain group, wherein \( R^2 \) and \( R' \) are different from each other;
- each \( Q \) is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \) alkenylene chain, wherein 0-6 methylene units of \( Q \) are independently replaced by -\( \text{O} \), -\( \text{O} \), -\( \text{N} \), -\( \text{S} \), -\( \text{OC} \), -\( \text{OC} \), -\( \text{SO} \), -\( \text{SO} \), -\( \text{SO}_2 \), -\( \text{NH}_{2} \), -\( \text{SO}_2 \), -\( \text{NH} \), -\( \text{OC} \), or -\( \text{NH} \), wherein:
  - \( -\text{O} \) is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;
- each \( R^{2a} \) is independently a mono-protected amine, a di-protected amine, -\( \text{NHR} \), -\( \text{NHR}^{2a} \), -\( \text{NHCO} \) \( R \), -\( \text{NR}^2 \text{C} \) \( \text{OR} \), -\( \text{NHCO} \) \( \text{NH} \), -\( \text{NHCO} \) \( \text{NHR} \), -\( \text{NR}^2 \text{C} \) \( \text{OR} \), -\( \text{NHCO} \) \( \text{OR} \), or \( \text{NHCO} \) \( \text{SO}_2 \), or \( \text{NR}^2 \text{SO}_2 \); and
- each \( R^2 \) is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, an 8-10-membered saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a detectable moiety,

In certain embodiments, the \( m' \) group of formula VIII is 1-1000. In certain embodiments, the \( m' \) group of formula VIII is 0. In other embodiments, \( m' \) is 1-1000. According to other embodiments, \( m' \) and \( m' \) are independently 10 to 100 repeat units. In still other embodiments, \( m' \) is 1-20 repeat units and \( m' \) is 10-50 repeat units.

According to another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compounds have a polydispersity index ("PDI") of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula VIII, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula VIII having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula VIII, as described above, wherein \( n \) is about 225. In other embodiments, \( n \) is about 200 to about 300. In still other embodiments, \( n \) is about 200 to about 250. In still other embodiments, \( n \) is about 100 to about 150. In still other embodiments, \( n \) is about 400 to about 500.

One of ordinary skill in the art will recognize that compounds of formula VIII are prepared by symmetrical polymerization of the two terminal amine salts of formula D. However, it is also contemplated that one of the terminal amine groups of formula D may be protected such that the other terminal amine may be used to initiate ROP in accordance with the present invention. The protecting group may then be removed and ROP can be initiated from the other terminal amine group. Accordingly, another embodiment of the present invention provides a method for preparing a compound of formula IX:

\[ \text{IX} \]

wherein:
- \( n \) is 10-2500;
- each \( m \) and \( m-a \) is independently 1 to 1000;
- each \( m' \) and \( m'-a \) is independently 0 to 1000;
- each of \( R^2, R^{2a}, R' \), and \( R^{2a} \) is independently a natural or unnatural amino acid side-chain group, wherein \( R^2 \) and \( R' \) and \( R^{2a} \) and \( R^{2a} \) are different from each other;
- each \( Q \) is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched \( C_{1-12} \);
alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -O(O)-, -C(O)O-, -C(O)-, -SO-, -SO2-, -NH2SO-, -NH2C(O)-, -C(O)NH-, -OC(O)NH-, or -NH2C(O)O-, wherein:

-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and each A is a suitable acid anion,

wherein said method comprises the steps of:
(a) providing a compound of formula E:

wherein:
- n is 10-2500;
- each of PG¹ or PG² is hydrogen or a suitable amine protecting group, or PG¹ and PG² are taken together to form a cyclic amine protecting group, providing that at least one of PG¹ and PG² is a suitable amine protecting group;
- each Q is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched C₁₋₁₂ alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -O(O)-, -C(O)O-, -C(O)-, -SO-, -SO₂-, -NH₂SO-, -SO₂NH-, -NH2C(O)-, -C(O)NH-, -OC(O)NH-, or -NH₂C(O)O-, wherein:

-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and each A is a suitable acid anion,

(b) polymerizing a first cyclic amino acid monomer onto the amine salt terminal end of formula E, wherein said first cyclic amino acid monomer comprises R¹:
(c) optionally polymerizing a second cyclic amino acid monomer, comprising R², onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer, to form a compound of formula F:

wherein:
- m is 1-1000;
- m² is 0-1000;
- n is 10-2500;
- each R¹ and R² are independently a natural or unnatural amino acid side-chain group, wherein R¹ and R² are different from each other;
- each of PG¹ or PG² is hydrogen or a suitable amine protecting group, or PG¹ and PG² are taken together to form a cyclic amine protecting group, providing that at least one of PG¹ and PG² is a suitable amine protecting group;
- each Q is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched C₁₋₁₂ alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -Cy-, -O-, -NH-, -S-, -O(O)-, -C(O)O-, -C(O)-, -SO-, -SO₂-, -NH₂SO-, -SO₂NH-, -NH₂C(O)-, -C(O)NH-, -OC(O)NH-, or -NH₂C(O)O-, wherein:

-Cy- is an optionally substituted 5-8 membered bivalent, saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and each A is a suitable acid anion,

(d) protecting the living terminal amine of said compound of formula F;
(e) removing the PG¹ and PG² groups and forming a salt thereof;
(f) polymerizing a first cyclic amino acid monomer onto the resulting amine salt terminal end, wherein said first cyclic amino acid monomer comprises R²;
(g) optionally polymerizing a second cyclic amino acid monomer, comprising R³, onto the living polymer end, wherein said second cyclic amino acid monomer is different from said first cyclic amino acid monomer, to form a compound of formula IX:

Each of the Q, A, m, m₂, m₃, m₄, n, R¹, R², R³, and R⁴ groups of formula IX are as defined above and in various embodiments, classes and subclasses described herein both singly and in combination.

In certain embodiments, the m₃ and m₄ groups of formula IX are independently 1-1000. In certain embodiments, one or both of the m³ and m₄ groups of formula IX are 0. According to other embodiments, m, m₃, m₄, and m₅ are independently 10 to 100 repeat units. In still other embodiments, m and m₄ are independently 1-20 repeat units and m₃ and m₄ are independently 10-50 repeat units.

According to another embodiment, the present invention provides compounds of formula IX, as described above, wherein said compounds have a polydispersity index ("PDI") of about 1.0 to about 1.2. According to another embodiment,
the present invention provides compounds of formula IX, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula IX, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula IX having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula IX, as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

One of ordinary skill in the art will recognize that compounds of formula IX may be further derivatized as described herein. In certain embodiments, the present invention provides a compound of formula X:

\[
\text{X} = \text{alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO}_2-, -NH(O)S(O)_, -SO_NH-, -NH(C)O-, -C(O)NH-, -OC(O)NH-, or -NH(O)O-;}
\]

wherein:

- each m and m-a is independently 1 to 1000;
- each m-b-a is independently 0 to 1000;
- each of R\(_1\), R\(_2\), R\(_3\), and R\(_4\) is independently a natural or unnatural amino acid side-chain group, wherein R\(_1\) and R\(_2\) are different from each other, each Q is independently a valence bond or a bivalent, saturated or unsaturated, straight or branched C\(_1\)-C\(_12\) alkylene chain, wherein 0-6 methylene units of Q are independently replaced by -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO\(_2\), -NH(O)S(O), -SO\(_N\)H-, -NH(C)O-, -C(O)NH-, -OC(O)NH-, or -NH(O)O-; and

- each R\(_{21}\) is independently a mono-protected amine, a di-protected amine, -NH\(_R\)O\(_R\), -N(R\(_3\))\(_3\)-, -NH(C)O\(_R\), -NR\(_2\)C(O)\(_R\)\(_3\), -NH(C)OHR\(_3\), -NH(C)O\(_R\)N\(_R\)(R\(_3\))\(_3\), -NR\(_2\)C(O)\(_R\)(N\(_R\))\(_3\), -NH(C)O\(_R\), -NR\(_2\)C(O)\(_R\)\(_3\), -NH\(_2\)SO\(_R\), or -NR\(_2\)SO\(_R\); and

- each R\(_b\) is independently an optionally substituted group selected from aliphatic, a 5-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur; and

of about 1.0 to about 1.2. According to another embodiment, the present invention provides compounds of formula X, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.03 to about 1.15. According to yet another embodiment, the present invention provides compounds of formula X, as described above, wherein said compound has a polydispersity index ("PDI") of about 1.10 to about 1.12. According to other embodiments, the present invention provides compounds of formula X having a PDI of less than about 1.10.

In certain embodiments, the present invention provides compounds of formula X, as described above, wherein n is about 225. In other embodiments, n is about 200 to about 300. In still other embodiments, n is about 200 to about 250. In still other embodiments, n is about 100 to about 150. In still other embodiments, n is about 400 to about 500.

4. Uses, Methods, and Compositions

As discussed above, the present invention provides multi-block copolymers, intermediates thereto, and methods of preparing the same. Such multi-block copolymers are useful for a variety of purposes in the pharmaceutical and biomedical fields. Such uses include using the multi-block copolymers of the present invention, in particular the PEG-poly (amino acid) block copolymers prepared by the methods of the present invention, in the process of PE\(_G\)-glyating other molecules.

For example, U.S. Pat. No. 6,797,257 describes imaging agents prepared by PE\(_G\)-glyating gadolinium oxide albumin microspheres. U.S. Pat. Nos. 6,790,823 and 6,764,853 describe the PE\(_G\)-ylation of proteins by covalently bonding reactive groups, such as, free amines or carboxylates of amino acid residues. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amine group may include lysine residues and the N-terminal amino acid residues; those having a free carboxylate group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching activated polyethylene glycol molecule(s).
Accordingly, another aspect of the present invention provides a method of conjugating a biomolecule with a compound of formula I, II', III', or V. In certain embodiments, the compounds of formula I, II', III', or V are prepared by the methods of the present invention, as described generally above and in classes and subclasses defined above and herein.

In certain embodiments, the present invention provides a method of conjugating a protein, a plasmid, a dye, a peptide, a hydrogel, or a small molecule drug with a compound of formula I, II', III', or V. In certain embodiments, the compounds of formula I, II', III', or V are prepared by the methods of the present invention, as described generally above and in classes and subclasses defined above and herein.

Yet another aspect of the present invention provides a drug-polymer conjugate comprising a compound of formula I, II', III', or V. In certain embodiments, the present invention provides a drug-polymer conjugate comprising a compound of formula I, II', III', or V prepared by the methods of the present invention, and a pharmaceutically active agent. In still another aspect of the present invention, pharmaceutically acceptable compositions are provided, wherein these compositions comprise a drug-polymer conjugate as described herein, and optionally comprise a pharmaceutically acceptable carrier, adjuvant or vehicle. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents.

One of ordinary skill in the art would recognize that the compounds prepared by the methods of the present invention are useful for the conjugation of small molecule drugs. Small molecule drugs suitable for conjugation with the compounds prepared by the methods of the present invention include, but are not limited to, those having a functional group suitable for covalently linking to the PEG-poly(aminoc acid) block copolymers of the present invention prepared by the methods of the present invention. Such drugs include, without limitation, chemotherapeutic agents or other anti-proliferative agents including alkylating drugs (melphalan, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimitobolites (Methotrexate), purine antagonists and pyrimidine antagonists (6-Mercaptopurine, 5-Fluorouracil, Cytosine, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel), podophyllotoxins (Etoposide, Irinotecan, Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin), nitrosoureas (Carmustine, Lomustine), inorganic ions (Cisplatin, Carboplatin), enzymes (Asparaginase), angiogenesis inhibitors (Avastin) and hormones (Tamoxifen, Leuprolide, Flutamide, and Megestrol), Gleevec, dexamethasone, and cyclophosphamide. For more comprehensive discussion of several cancer therapies see, http://www.cancer.gov/, a list of the FDA approved oncology drugs at http://www.fda.gov/Center/cancer/druglist.htm, and The Merck Manual, Seventeenth Ed., 1999, the entire contents of which are hereby incorporated by reference.

Other examples of small molecule drugs that may be conjugated with the compounds prepared by the methods of the present invention include treatments for Alzheimer’s Disease such as Aricept® and Excelon®; treatments for Parkinson’s Disease such as L-DOPA/carbidopa, entacapone, ropinirole, pramipexole, bromocriptine, pergolide, trihexphenidyl, and amantadine; agents for treating Multiple Sclerosis (MS) such as beta interferon (e.g., Avonex® and Rebif®), Copaxone®, mitoxantrone; treatments for asthma such as albuterol and Singulair®; agents for treating schizophrenia such as zyprexa, risperdal, seroquel, and haloperidol; anti-inflammatory agents such as corticosteroids, TNF blockers, IL-1 RA, azathioprine, cyclophosphamide, and sulfasalazine; immunomodulatory and immunosuppressive agents such as cyclosporin, tacrolimus, rapamycin, mycophenolate mofetil, interferons, corticosteroids, cyclophosphamide, azathioprine, and sulfasalazine; neurotrophic factors such as acetylcholinesterase inhibitors, MAO inhibitors, interferons, anti-convulsants, ion channel blockers, rituximab, and anti-Parkinsonian agents; agents for treating cardiovascular disease such as beta-blockers, ACE inhibitors, diuretics, nitrates, calcium channel blockers, and statins; agents for treating liver disease such as corticosteroids, cholestyramine, interferons, and anti-viral agents; agents for treating blood disorders such as corticosteroids, anti-leukemic agents, and growth factors; and agents for treating immunodeficiency disorders such as gamma globulin.

As described above, the pharmaceutically acceptable compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, adjuvant, or vehicle, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington’s Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (McGraw Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this invention.

The pharmaceutically acceptable compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracutaneously, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), buccally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the composition of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

Amphiphilic multi-block copolymers, as described herein, can self-assemble in aqueous solution to form nano- and micron-sized structures, with applications from drug encapsulation to artificial viruses and cells. In water, these amphiphilic copolymers assemble by multi-molecular micelleization when present in solution above the critical micelle concentration (CMC). Without wishing to be bound by any particular theory, it is believed that the hydrophobic poly(aminoc acid) portion or “block” of the copolymer collapses to form the micellar core, while the hydrophilic PEG block forms a peripheral corona and imparts water solubility. Additionally, poly(aminoc acid) blocks capable of chemical crosslinking (e.g., aspartic and glutamic acid, cysteine, or serine) may also be incorporated into the amphiphilic copolymer to further enhance the stability of micellar assemblies. These core-shell polymer micelles can be tuned to encapsulate a variety of therapeutic molecules, including small molecule drugs, polypeptides, and polymeric esters. Use of compounds of the present invention in micellar assemblies is described in detail in U.S. provisional application Ser. No. 60/667,260, filed Apr. 1, 2005, and U.S. provisional application Ser. No. 60/741,780, filed Dec. 1, 2005, the entirety of both of which is hereby incorporated herein by reference.

In order that the invention described herein may be more fully understood, the following examples are set forth. It will be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.
Example 1

To a dried 100 mL round bottom flask equipped with a stir bar, septum and inlet adapter was added PEG-amine hydrochloride (2.13 g, 0.21 mmol) and t-butyl aspartic acid NCA (0.46 g, 2.1 mmol). The contents were dried under vacuum for 1 hour and backfilled with N₂. Anhydrous dimethylformamide (DMF) (25 mL) was added via syringe and the reaction was heated to 80°C. After 48 hours, an aliquot was removed and the polymer molecular weight was determined to be 12,400 g/mol (PDI=1.10) by size exclusion chromatography in dimethylacetamide (DMAc). Phenylalanine NCA (0.98 g, 5.1 mmol) and t-butyl tyrosine NCA (0.34 g, 1.3 mmol) were placed in a 50 mL round bottom flask and dried under vacuum for 1 hour. The two monomers were dissolved in anhydrous DMF (15 mL) then transferred to the reaction vessel containing the PEG-b-poly(aspartic acid) copolymer. The reaction was stirred for 48 hours at 80°C. The solution was allowed to cool to room temperature, precipitated into diethyl ether (500 mL), filtered, and dried in vacuo. The multi-block copolymer was isolated as a white powder with a molecular weight of 15,100 g/mol (PDI=1.13) as determined by size exclusion chromatography in DMAc.

Example 2
To a dried 100 mL round bottom flask equipped with a stir bar, septum and inlet adapter was added acetylene-PEG-aniline hydrochloride (2.13 g, 0.21 mmol) and t-butyl aspartic acid NCA (0.46 g, 2.1 mmol). The contents were dried under vacuum for 1 hour and backfilled with \( \text{N}_2 \). Anhydrous DMF (25 mL) was added via syringe then the reaction heated to 80°C. After 48 hours, an aliquot was removed and the polymer molecular weight was determined to be 11,900 g/mol (PDI=1.12) by size exclusion chromatography in DMF. Phenylalanine NCA (0.98 g, 5.1 mmol) and t-butyl tyrosine NCA (0.34 g, 1.3 mmol) were placed in a 50 mL round bottom flask and dried under vacuum for 1 hour. The two monomers were dissolved in anhydrous DMF (15 mL) and transferred to the reaction vessel containing the PEG-b-poly(aspartic acid) copolymer. The solution was stirred for 48 hours at 80°C. The solution was allowed to cool to room temperature, precipitated into diethyl ether (500 mL), filtered, and dried in vacuo. The multi-block copolymer was isolated as a white powder with a molecular weight of 12,700 g/mol (PDI=1.15) as determined by size exclusion chromatography in DMF. \(^1\)H NMR (8, 400 MHz, DMSO-d$_6$) 9.12, 8.05, 7.96, 7.44, 7.17, 6.94, 6.83, 6.59, 4.73, 4.51, 4.02, 3.54, 1.92, 1.47, 1.38, 1.21, 1.09.
Example 5
Example 6

Example 7
While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example.

We claim:
1. A triblock copolymer of formula III:

\[
\text{III}
\]

wherein:
- \( n \) is 10-2500;
- \( m \) is 10 to 100;
- \( m' \) is 10 to 100;
- \( R^+ \) is selected from tyrosine, serine, cysteine, threonine, aspartic acid, glutamic acid, asparagine, and glutamine either singly or in combination;
- \( R^2 \) is a hydrophobic amino acid side-chain group;
- \( R^3 \) is \(-Z(CH_2CH_2Y)_p(CH_2)_qR^3\), wherein:
  - \( Z \) is \(-O-, -S-, -C=O-, \) or \(-CH_2-\);
  - \( p \) is independently \(-O-\) or \(-S-\);
  - \( q \) is 0-10;
  - \( t \) is 0-10; and
- \( R^4 \) is an optionally substituted aliphatic group;
- \( Q \) is a valence bond;
- \( R^{2a} \) is \(-\text{NHC(O)R}^{2a}\); and
- \( R^* \) is optionally substituted aliphatic.

2. The compound according to claim 1, wherein \( R^{2a} \) is selected from the group consisting of:

\[
\text{II}
\]

3. The compound according to claim 1, wherein \( R^* \) is an aspartic acid side-chain, a glutamic acid side-chain, a lysine side-chain, an arginine side-chain, or protected form or mixture thereof.

4. The compound according to claim 3, wherein \( R^* \) is an aspartic acid side-chain or a glutamic acid side-chain.

5. The compound according to claim 1, wherein \( R^* \) is a hydrophobic amino acid side-chain selected from:
- a phenylalanine side-chain, alanine side-chain, benzyl or alkyl glutamate side-chain, a benzyl or alkyl aspartate side-chain, or leucine side-chain, and optionally
- one or more of a tyrosine side-chain, a serine side-chain, a threonine side-chain, a glutamic acid side-chain, or aspartic acid side-chain,

wherein the overall \( R^* \) block is hydrophobic.

6. The compound according to claim 5, wherein \( R^* \) is a hydrophobic mixed poly(aminoc acid) side-chain selected from:
- a hydrophobic amino acid side-chain selected from a phenylalanine side-chain, an alanine side-chain, a benzyl or alkyl glutamate side-chain, a benzyl or alkyl aspartate side-chain, or a leucine side-chain, and
157  a hydrophilic amino acid side-chain selected from one or
more of a tyrosine side-chain, a serine side-chain, a
threonine side-chain, a glutamic acid side-chain, or
aspartic acid side-chain,
wherein the overall R’ block is hydrophobic.
7. The compound according to claim 1, wherein R’ is an
aliphatic group optionally substituted with CN, a mono-pro-
tected amino group, a di-protected amino group, a protected
aldehyde group, a protected hydroxyl group, a protected car-
boxylic acid group, a protected thiol group, or a detectable
moiety.
8. The compound according to claim 1, wherein R’ is an
optionally substituted alkyl group.
9. The compound according to claim 1, wherein R’ is an
optionally substituted alkenyl group.
10. The compound according to claim 1, wherein R’ is an
optionally substituted alkynyl group.

158  11. The compound according to claim 1, wherein:
n is about 200 to about 300; and
R” is —NHCOCH3.
12. The compound according to claim 11, wherein R’ is
CH3O——.
13. The compound according to claim 12, wherein R’ is an
aspartic acid side-chain or a glutamic acid side-chain.
14. The compound according to claim 13, wherein R’ is a
hydrophilic amino acid side-chain selected from:
a phenylalanine side-chain, alanine side-chain, benzyl or
alkyl glutamate side-chain, a benzyl or alkyl aspartate
side-chain, or leucine side-chain, and optionally
one or more of a tyrosine side-chain, a serine side-chain, a
threonine side-chain, a glutamic acid side-chain, or
aspartic acid side-chain,
wherein the overall R’ block is hydrophobic.