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(54) Title: DERIVATIZABLE MONOMERS AND POLYMERS, AND METHODS FOR MAKING AND USING SAME

(57) Abstract: The invention provides UV-sensitive monomers, comprising a cyclopropanone- containing group, which acts as a masked dibenzocyclooctyne (DBCO) / dibenzozocyclooctyne (DIBAC) group. The monomers of the invention can be polymerized for example via reversible addition fragmentation chain transfer (RAFT) polymerization techniques to yield a polymer comprising the masked DBCO/DIBAC group. In certain embodiments, the DBCO/DIBAC group can be unmasked under controlled conditions, allowing conjugation of small molecules and/or macromolecules to the polymer through highly selective and efficient strain-promoted azide alkyne click chemistry (SPAAC).



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TITLE OF THE INVENTION

Derivatizable Monomers and Polymers, and Methods of Making and Using Same

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 62/756,177, filed November 6, 2018, which is hereby incorporated by reference in its entirety herein.

BACKGROUND OF THE INVENTION

10 Bioorthogonal “click” chemistry has revolutionized the field of bioconjugate chemistry, because it allows for controlled introduction of multiple bioactive groups in a single molecule. The “click” reaction paradigm is centered on the development and implementation of robust reactions that proceed with reliable control over the products formed. A “click” reaction should have the following characteristics: the reaction involves
15 minimal set-up effort and the starting materials are readily available; the reaction is high yielding, proceeding with high stereospecificity and high atom economy; the reaction is run solvent-free or in an environmentally benign solvent (preferably water); the product can be easily isolated by crystallization or distillation, and preparative chromatography is not required; the by-products are easily removed and non-toxic; the reaction is physiologically
20 compatible; and there is a large thermodynamic driving force (> 84 kJ/mol) to favor the formation of a single reaction product.

 One reaction that meets most of these criteria is the azide-alkyne Huisgen cycloaddition, which is a 1,3-dipolar cycloaddition between an azide and a terminal or internal alkyne to give a 1,2,3-triazole. A notable variant of the Huisgen 1,3-dipolar
25 cycloaddition is the copper(I) catalyzed variant, in which organic azides and terminal alkynes are united to afford 1,4-regioisomers of 1,2,3-triazoles as sole products. This copper(I)-catalyzed variant reaction is formally not a 1,3-dipolar cycloaddition and thus should not be termed a Huisgen cycloaddition. Instead, this reaction is known as the copper(I)-catalyzed
Azide-Alkyne Cycloaddition (CuAAC).

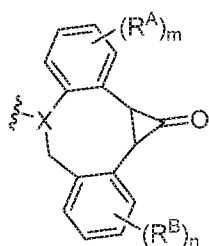
30 Strain promoted alkyne-azide cycloaddition (SPAAC) is particularly attractive as a click reaction, since SPAAC does not require the presence toxic catalysts for bioconjugation. For this reason, a variety of dibenzozacyclooctyne groups (DIBACs; also known as azadibenzocyclooctynes or ADIBOs) and their derivatives have been developed for efficient

bioconjugation to azides. Unfortunately, the intolerance of these functional groups to radical polymerization reactions necessitates multi-step post-polymerization modifications.

There is thus a need in the art to identify monomers and/or polymers that allow for controlled derivatization of a polymer with bioactive molecules. Such monomers and/or polymers should be used for applications such as therapeutic treatments, site-directed drug delivery, and/or bioengineering. The present invention addresses this unmet need in the art.

BRIEF SUMMARY OF THE INVENTION

The invention provides a compound comprising an α,β -unsaturated carboxamide group or α,β -unsaturated sulfonamide group conjugated to a group of formula (I):



(I), wherein the variables therein are defined elsewhere herein.

The invention further provides polymers comprising the at least partially polymerized compound of the invention.

The invention further provides a method of derivatizing at least a portion of the compound of the invention with a small molecule, therapeutic agent, or macromolecule.

The invention further provides a method of derivatizing at least a portion of the polymer of the invention with a small molecule, therapeutic agent, or macromolecule.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of selected embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, specific embodiments are illustrated in the drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments illustrated in the drawings.

FIG. 1 is a graph illustrating UV-Vis of cp-DIBAC monomer (M1) before and after deprotection showing blue-shift of the characteristic peak, followed by a loss in absorbance after click to 2kDa PEG-N₃ (SPAAC).

FIG. 2 illustrates an exemplary copolymerization with a masked DIBAC (cp-DIBAC).

FIG. 3 illustrates an exemplary UV deprotection and SPAAC bioconjugation of polymers of the invention.

FIG. 4A-4B illustrate a non-limiting synthesis of an exemplary monomer of the invention.

5 FIG. 5 illustrates conjugation of cp-DIBAC with a thiol (such as propanethiol).

FIGs 6A-6C illustrate UV-Vis spectra of cp-DIBAC and polymerization kinetics.

FIG. 6A: UV-Vis traces of pDMA with 5% (mol) cp-DIBAC monomer (M1) incorporated at DP 200 and 400 before and after deprotection, and following click to 2kDa PEG-N₃. FIG. 6B: Kinetics of PET-RAFT polymerization with and without cp-DIBAC monomer (M1) showing complete conversion in both cases after 16h. FIG. 6C: GPC traces for pDMA with 5% (mol) cp-DIBAC monomer (M1) after 8, 12 and 16h of polymerization, showing incorporation of the cp-DIBAC by UV detection at 265 nm.

FIGs. 7A-7D illustrate GPC traces showing the copolymerization of cp-DIBAC monomer (1) into side-chain functionalized linear polymers followed by click with 2k PEG-N₃. FIG. 7A: DMA with 5% cp-DIBAC. FIG. 7B: HEA (2-hydroxyethyl acrylate) with 5% cp-DIBAC. FIG. 7C: DMA DP 200 with 2.5 – 7.5% cp-DIBAC. FIG. 7D: UV signal at 265 nm from GPC showing increasing incorporation of cp-DIBAC as a function of feed ratio.

FIGs. 8A-8B illustrate dual functionalization by SPAAC. FIG. 8A: GPC traces after copolymerization of DMA / NHS-acrylate / cp-DIBAC (95 / 2.5 / 2.5 mol%) and addition of DBCO-NH₂ to create dual SPAAC-ready polymers. Subsequent click to DBCO, deprotection and secondary click of 2kDa PEG-N₃ to cp-DIBAC are also shown. FIG. 8B: UV-Vis traces of the protected polymer before and after the first DBCO click, after deprotection of the cp-DIBAC and after subsequent click to the DIBAC.

FIGs. 9A-9E comprise a schematic showing dual functionalization capabilities using DBCO and cp-DIBAC via SPAAC.

FIG. 10 comprises a graph illustrating the compatibility of copolymers of 4-acryloyl morpholine (NAM) and N-isopropylacrylamide (NIPAM) & 5% cp-DIBAC monomer.

DETAILED DESCRIPTION OF THE INVENTION

30 The invention provides UV sensitive monomers, comprising a cyclopropanone group, which is radical-stable, acts as a masked dibenzoazacyclooctyne (DIBAC) or dibenzocyclooctyne (DBCO) group, and can be deprotected to a DIBAC or DBCO group through use of radiation. The monomers of the invention further comprise a polymerizable

group, such as an α,β -unsaturated carbonyl or sulfonyl group, such as a (meth)acrylamide group.

As used herein, any description of a DIBAC group within the invention can be used interchangeably with a DBCO group, and any description of a DBCO group within the invention can be used interchangeably with a DIBAC group.

The monomers of the invention can be polymerized, in a non-limiting embodiment, using radical chemistry, for example, via reversible addition fragmentation chain transfer (RAFT) polymerization. The polymerized monomers afford a polymer comprising the masked DBCO/DIBAC group. In certain embodiments, the polymer of the invention further comprises unmasked DBCO/DIBAC groups as well; such unmasked DBCO/DIBAC groups can be introduced in the polymer through coupling reactions of DBCO/DIBAC-containing reagents and appropriate reactive groups on the polymer. These unmasked DBCO/DIBAC groups can be reacted with azido-containing molecules through highly selective and efficient strain-promoted azide alkyne click chemistry (SPAAC), thus allowing for the conjugation of small molecules and/or macromolecules to the polymer in the presence of the masked DIBAC groups.

In certain embodiments, the masked DIBAC groups in the polymer can be unmasked under controlled conditions and reacted with azido-containing molecules using SPAAC, allowing for conjugation of additional small molecules and/or macromolecules to the polymer.

In certain embodiments, the deprotected (unmasked) monomers and/or polymers of the invention can be reacted with azido-derivatized small molecules, therapeutic agents, and/or macromolecules (such as, but not limited to, antibiotics, antivirals, antifungals, polypeptides, proteins, antibodies, aptamers, saccharides, lipids, proteoglycans, chemotherapeutics, polymers, and so forth).

The monomers of the invention can also be conjugated with small molecules and/or macromolecules comprising reactive thiol groups (-SH), through a Michael addition of the thiol group into the α,β -unsaturated carbonyl or sulfonyl group. The monomers of the invention can also be conjugated with small molecules and/or macromolecules comprising reactive thiol groups (-SH) through thiol-ene free radical chemistry as appropriate.

In certain embodiments, the polymers of the invention can be conjugated with small molecules and/or macromolecules after the monomers are at least partially polymerized.

In certain embodiments, the monomers and/or polymers of the invention can be used for drug delivery. In certain embodiments, the monomers and/or polymers of the invention

can be structurally modified so as to adjust the physical and/or chemical characteristics of the drug-containing compositions.

As described herein, an exemplary dibenzocyclooctyne monomer masked with a cyclopropenone group (cp-DIBAC) was prepared and directly copolymerized into a polymer backbone. Subsequent addition of the clickable azide with UV deprotection of the cyclopropenone results in rapid and efficient bioconjugation via SPAAC.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

The following abbreviations are used herein: ADIBO, azadibenzocyclooctyne; CRP, controlled radical polymerization; cp-DIBAC, cyclopropenone-masked dibenzoazacyclooctyne; CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition; DBCO, dibenzocyclooctyne; DIBAC, dibenzoazacyclooctyne; DP, degree of polymerization; pDMA, polymerized dimethylacrylamide; PET-RAFT, porphyrin-catalyzed photoinduced electron/energy transfer-reversible addition-fragmentation chain-transfer; SPAAC, strain promoted azide-alkyne cycloaddition.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, the term “abnormal” when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (*e.g.*, age, treatment, or time of day) from those organisms, tissues, cells or components thereof that display the “normal”

(expected) respective characteristic. Characteristics that are normal or expected for one cell or tissue type might be abnormal for a different cell or tissue type.

As used herein, the term “acrylate group” corresponds to the group $-C(R')=C(R'')-C(=O)-$, wherein R' and R'' are independently hydrogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_3-C_8 cycloalkyl, which may be present in a monomer, an oligomer, and/or a polymer. In a non-limiting embodiment, the term “acrylate” encompass a methacrylate, wherein R'' is methyl. In certain embodiments, the acrylate is part of an acrylamide, wherein the carbonyl of the acrylate is coupled to a primary or secondary amide.

In certain embodiments, the acrylate can be acrylamide, methacrylamide, dimethyl acrylamide, methyl methacrylamide, benzyl methacrylamide (BnMA), isobutyl methacrylamide (BMA), and isododecyl methacrylamide (IDMA). In other embodiments, the acrylate is selected from the group consisting of ethylene glycoldi(meth)acrylate, ethoxylated bisphenol-A dimethacrylate (EBPADMA), tetraethyleneglycoldi(meth)acrylate (TEGDMA), poly(ethylene glycol) dimethacrylates, the condensation product of bisphenol A and glycidyl methacrylate, 2,2'-bis-[4-(3-methacryloxy-2-hydroxy propoxy)-phenyl] propane (BisGMA), hexanediol di(meth)acrylate, tripropylene glycol di(meth)acrylate, butanediol di(meth)acrylate, neopentyl glycol di(meth)acrylate, diethylene glycol di(meth)acrylate, triethylene glycol di(meth)acrylate, dipropylene glycol di(meth)acrylate, allyl (meth)acrylate, the corresponding amide, and any combinations thereof.

As used herein, the term “effective amount” or “therapeutically effective amount” of a compound is that amount of compound that is sufficient to provide a beneficial effect to the subject to which the compound is administered.

As used herein, the term “electromagnetic radiation” includes radiation of one or more frequencies encompassed within the electromagnetic spectrum. Non-limiting examples of electromagnetic radiation comprise gamma radiation, X-ray radiation, UV radiation, visible radiation, infrared radiation, microwave radiation, radio waves, and electron beam (e-beam) radiation. In one aspect, electromagnetic radiation comprises ultraviolet radiation (wavelength from about 10 nm to about 400 nm), visible radiation (wavelength from about 400 nm to about 750 nm) or infrared radiation (radiation wavelength from about 750 nm to about 300,000 nm). Ultraviolet or UV light as described herein includes UVA light, which generally has wavelengths between about 320 and about 400 nm, UVB light, which generally has wavelengths between about 290 nm and about 320 nm, and UVC light, which generally has wavelengths between about 200 nm and about 290 nm. UV light may include UVA, UVB, or UVC light alone or in combination with other type of UV light. In one embodiment,

the UV light source emits light between about 350 nm and about 400 nm. In some embodiments, the UV light source emits light between about 400 nm and about 500 nm.

As used herein, an “instructional material” includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of a composition or method of the invention in the kit for performing the methods recited herein. Optionally, or alternately, the instructional material can describe one or more methods recited herein. The instructional material of the kit of the invention can, for example, be affixed to a container that contains the identified composition or delivery system of the invention or be shipped together with a container that contains the identified composition or delivery system. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the composition be used cooperatively by the recipient.

As used herein, the term “polymer” refers to a molecule composed of repeating structural units typically connected by covalent chemical bonds. The term “polymer” is also meant to include the terms copolymer and oligomers. In one embodiment, a polymer comprises a backbone (*i.e.*, the chemical connectivity that defines the central chain of the polymer, including chemical linkages among the various polymerized monomeric units) and a side chain (*i.e.*, the chemical connectivity that extends away from the backbone).

As used herein, the term “polymerization” or “crosslinking” refers to at least one reaction that consumes at least one functional group in a monomeric molecule (or monomer), oligomeric molecule (or oligomer) or polymeric molecule (or polymer), to create at least one chemical linkage between at least two distinct molecules (*e.g.*, intermolecular bond), at least one chemical linkage within the same molecule (*e.g.*, intramolecular bond), or any combinations thereof. A polymerization or crosslinking reaction may consume between about 0% and about 100% of the at least one functional group available in the system. In one embodiment, polymerization or crosslinking of at least one functional group results in about 100% consumption of the at least one functional group. In other embodiments, polymerization or crosslinking of at least one functional group results in less than about 100% consumption of the at least one functional group.

As used herein, reversible addition-fragmentation chain transfer or “RAFT” polymerization is a type of reversible-deactivation radical polymerization. It uses a chain transfer agent in the form of a RAFT agent, such as a thiocarbonylthio compound (such as dithioesters, thiocarbamates, and xanthates), to afford control over the generated molecular weight and polydispersity during a free-radical polymerization. RAFT polymerization is a

living or controlled radical polymerization techniques, others examples being atom transfer radical polymerization (ATRP) and nitroxide-mediated polymerization (NMP). RAFT polymerizations can be performed with conditions to favor low dispersity (molecular weight distribution) and a pre-chosen molecular weight.

5 As used herein, the term “reaction condition” refers to a physical treatment, chemical reagent, or combination thereof, which is required or optionally required to promote a reaction. Non-limiting examples of reaction conditions are electromagnetic radiation, heat, a catalyst, a chemical reagent (such as, but not limited to, an acid, base, electrophile or nucleophile), and a buffer.

10 As used herein, the term “alkenyl,” employed alone or in combination with other terms, means, unless otherwise stated, a stable monounsaturated or diunsaturated straight chain or branched chain hydrocarbon group having the stated number of carbon atoms. Examples include vinyl, propenyl (or allyl), crotyl, isopentenyl, butadienyl, 1,3-pentadienyl, 1,4-pentadienyl, and the higher homologs and isomers. A functional group representing an
15 alkene is exemplified by $-\text{CH}_2-\text{CH}=\text{CH}_2$.

 As used herein, the term “alkoxy” employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined elsewhere herein, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (or isopropoxy), and the higher
20 homologs and isomers. A specific example is $(\text{C}_1-\text{C}_3)\text{alkoxy}$, such as, but not limited to, ethoxy and methoxy.

 As used herein, the term “alkyl,” by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain hydrocarbon having the number of carbon atoms designated (*i.e.*, C_1-C_{10} means one to ten carbon atoms) and includes straight,
25 branched chain, or cyclic substituent groups. Examples include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, neopentyl, hexyl, and cyclopropylmethyl. Most preferred is $(\text{C}_1-\text{C}_6)\text{alkyl}$, such as, but not limited to, ethyl, methyl, isopropyl, isobutyl, *n*-pentyl, *n*-hexyl and cyclopropylmethyl.

 As used herein, the term “alkynyl” employed alone or in combination with other
30 terms means, unless otherwise stated, a stable straight chain or branched chain hydrocarbon group with a triple carbon-carbon bond, having the stated number of carbon atoms. Non-limiting examples include ethynyl and propynyl, and the higher homologs and isomers. The term “propargylic” refers to a group exemplified by $-\text{CH}_2-\text{C}\equiv\text{CH}$. The term “homopropargylic” refers to a group exemplified by $-\text{CH}_2\text{CH}_2-\text{C}\equiv\text{CH}$.

As used herein, the term “aromatic” refers to a carbocycle or heterocycle with one or more polyunsaturated rings and having aromatic character, *i.e.*, having $(4n+2)$ delocalized π (pi) electrons, where ‘n’ is an integer.

As used herein, the term “aryl” employed alone or in combination with other terms means, unless otherwise stated, a carbocyclic aromatic system containing one or more rings (typically one, two or three rings) wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl, anthracyl, and naphthyl. Aryl groups also include, for example, phenyl or naphthyl rings fused with one or more saturated or partially saturated carbon rings (*e.g.*, bicyclo[4.2.0]octa-1,3,5-trienyl, or indanyl), which can be substituted at one or more carbon atoms of the aromatic and/or saturated or partially saturated rings.

As used herein, the term “aryl-(C₁-C₆)alkyl” refers to a functional group wherein a one to six carbon alkanediyl chain is attached to an aryl group, *e.g.*, -CH₂CH₂-phenyl or -CH₂-phenyl (or benzyl). Specific examples are aryl-CH₂- and aryl-CH(CH₃)-. The term “substituted aryl-(C₁-C₆)alkyl” refers to an aryl-(C₁-C₆)alkyl functional group in which the aryl group is substituted. A specific example is [substituted aryl]-(CH₂)-. Similarly, the term “heteroaryl-(C₁-C₆)alkyl” refers to a functional group wherein a one to three carbon alkanediyl chain is attached to a heteroaryl group, *e.g.*, -CH₂CH₂-pyridyl. A specific example is heteroaryl-(CH₂)-. The term “substituted heteroaryl-(C₁-C₆)alkyl” refers to a heteroaryl-(C₁-C₆)alkyl functional group in which the heteroaryl group is substituted. A specific example is [substituted heteroaryl]-(CH₂)-.

As used herein, the term “cycloalkyl” by itself or as part of another substituent refers to, unless otherwise stated, a cyclic chain hydrocarbon having the number of carbon atoms designated (*i.e.*, C₃-C₆ refers to a cyclic group comprising a ring group consisting of three to six carbon atoms) and includes straight, branched chain, or cyclic substituent groups. Examples of (C₃-C₆)cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. Cycloalkyl rings can be optionally substituted. Non-limiting examples of cycloalkyl groups include: cyclopropyl, 2-methyl-cyclopropyl, cyclopropenyl, cyclobutyl, 2,3-dihydroxycyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctanyl, decalanyl, 2,5-dimethylcyclopentyl, 3,5-dichlorocyclohexyl, 4-hydroxycyclohexyl, 3,3,5-trimethylcyclohex-1-yl, octahydropentalenyl, octahydro-1*H*-indenyl, 3a,4,5,6,7,7a-hexahydro-3*H*-inden-4-yl, decahydroazulenyl, bicyclo[6.2.0]decanyl, decahydronaphthalenyl, and dodecahydro-1*H*-fluorenyl. The term “cycloalkyl” also includes bicyclic hydrocarbon rings, non-limiting

examples of which include bicyclo-[2.1.1]hexanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.1.1]heptanyl, 1,3-dimethyl[2.2.1] heptan-2-yl, bicyclo[2.2.2]octanyl, and bicyclo[3.3.3]undecanyl.

As used herein, the term “halide” refers to a halogen atom bearing a negative charge.

5 The halide anions are fluoride (F^-), chloride (Cl^-), bromide (Br^-), and iodide (I^-).

As used herein, the term “halo” or “halogen” alone or as part of another substituent refers to, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom.

As used herein, the term “heteroalkyl” by itself or in combination with another term refers to, unless otherwise stated, a stable straight or branched chain alkyl group consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S, and wherein the nitrogen and sulfur atoms may be optionally oxidized and the nitrogen heteroatom may be optionally quaternized. The heteroatom(s) may be placed at any position of the heteroalkyl group, including between the rest of the heteroalkyl group and the fragment to which it is attached, as well as attached to the most distal carbon atom in the heteroalkyl group. Examples include: $-OCH_2CH_2CH_3$, $-CH_2CH_2CH_2OH$, $-CH_2CH_2NHCH_3$, $-CH_2SCH_2CH_3$, and $-CH_2CH_2S(=O)CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2NH-OCH_3$, or $-CH_2CH_2SSCH_3$.

As used herein, the term “heteroaryl” or “heteroaromatic” refers to a heterocycle having aromatic character. A polycyclic heteroaryl may include one or more rings that are partially saturated. Examples include tetrahydroquinoline and 2,3-dihydrobenzofuryl.

As used herein, the term “heterocycle” or “heterocyclyl” or “heterocyclic” by itself or as part of another substituent refers to, unless otherwise stated, an unsubstituted or substituted, stable, mono- or multi-cyclic heterocyclic ring system that comprises carbon atoms and at least one heteroatom selected from the group consisting of N, O, and S, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen atom may be optionally quaternized. The heterocyclic system may be attached, unless otherwise stated, at any heteroatom or carbon atom that affords a stable structure. A heterocycle may be aromatic or non-aromatic in nature. In certain embodiments, the heterocycle is a heteroaryl.

30 Examples of non-aromatic heterocycles include monocyclic groups such as aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, pyrroline, imidazoline, pyrazolidine, dioxolane, sulfolane, 2,3-dihydrofuran, 2,5-dihydrofuran, tetrahydrofuran, thiophane, piperidine, 1,2,3,6-tetrahydropyridine, 1,4-dihydropyridine, piperazine, morpholine, thiomorpholine, pyran, 2,3-dihydropyran, tetrahydropyran, 1,4-dioxane, 1,3-

dioxane, homopiperazine, homopiperidine, 1,3-dioxepane, 4,7-dihydro-1,3-dioxepin and hexamethyleneoxide.

Examples of heteroaryl groups include pyridyl, pyrazinyl, pyrimidinyl (such as, but not limited to, 2- and 4-pyrimidinyl), pyridazinyl, thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,3,4-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl.

Examples of polycyclic heterocycles include indolyl (such as, but not limited to, 3-, 4-, 5-, 6- and 7-indolyl), indolinyl, quinolyl, tetrahydroquinolyl, isoquinolyl (such as, but not limited to, 1- and 5-isoquinolyl), 1,2,3,4-tetrahydroisoquinolyl, cinnolinyl, quinoxalinyl (such as, but not limited to, 2- and 5-quinoxalinyl), quinazolinyl, phthalazinyl, 1,8-naphthyridinyl, 1,4-benzodioxanyl, coumarin, dihydrocoumarin, 1,5-naphthyridinyl, benzofuryl (such as, but not limited to, 3-, 4-, 5-, 6- and 7-benzofuryl), 2,3-dihydrobenzofuryl, 1,2-benzisoxazolyl, benzothienyl (such as, but not limited to, 3-, 4-, 5-, 6-, and 7-benzothienyl), benzoxazolyl, benzothiazolyl (such as, but not limited to, 2-benzothiazolyl and 5-benzothiazolyl), purinyl, benzimidazolyl, benztriazolyl, thioxanthinyl, carbazolyl, carbolinyl, acridinyl, pyrrolizidinyl, and quinolizidinyl.

The aforementioned listing of heterocyclyl and heteroaryl moieties is intended to be representative and not limiting.

As used herein, the term “substituted” refers to that an atom or group of atoms has replaced hydrogen as the substituent attached to another group.

As used herein, the term “substituted alkyl,” “substituted cycloalkyl,” “substituted alkenyl” or “substituted alkynyl” refers to alkyl, cycloalkyl, alkenyl, or alkynyl, as defined elsewhere herein, substituted by one, two or three substituents independently selected from the group consisting of halogen, -OH, alkoxy, tetrahydro-2-H-pyranyl, -NH₂, -NH(C₁-C₆ alkyl), -N(C₁-C₆ alkyl)₂, 1-methyl-imidazol-2-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, -C(=O)OH, -C(=O)O(C₁-C₆ alkyl), trifluoromethyl, -C≡N, -C(=O)NH₂, -C(=O)NH(C₁-C₆ alkyl), -C(=O)N((C₁-C₆ alkyl)₂), -SO₂NH₂, -SO₂NH(C₁-C₆ alkyl), -SO₂N(C₁-C₆ alkyl)₂, -C(=NH)NH₂, and -NO₂, in certain embodiments containing one or two substituents independently selected from halogen, -OH, alkoxy, -NH₂, trifluoromethyl, -N(CH₃)₂, and -C(=O)OH, in certain embodiments independently selected from halogen, alkoxy, and -OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

For aryl, aryl-(C₁-C₃)alkyl and heterocyclyl groups, the term “substituted” as applied to the rings of these groups refers to any level of substitution, namely mono-, di-, tri-, tetra-,

or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. In certain embodiments, the substituents vary in number between one and four. In other embodiments, the substituents vary in number between one and three. In yet another
5 embodiments, the substituents vary in number between one and two. In yet other embodiments, the substituents are independently selected from the group consisting of C₁-C₆ alkyl, -OH, C₁-C₆ alkoxy, halo, amino, acetamido, and nitro. As used herein, where a substituent is an alkyl or alkoxy group, the carbon chain may be branched, straight or cyclic.

Unless otherwise noted, when two substituents are taken together to form a ring
10 having a specified number of ring atoms (*e.g.*, two groups taken together with the nitrogen to which they are attached to form a ring having from 3 to 7 ring members), the ring can have carbon atoms and optionally one or more (*e.g.*, 1 to 3) additional heteroatoms independently selected from nitrogen, oxygen, or sulfur. The ring can be saturated or partially saturated, and can be optionally substituted.

15 Whenever a term or either of their prefix roots appear in a name of a substituent the name is to be interpreted as including those limitations provided herein. For example, whenever the term “alkyl” or “aryl” or either of their prefix roots appear in a name of a substituent (*e.g.*, arylalkyl, alkylamino) the name is to be interpreted as including those limitations given elsewhere herein for “alkyl” and “aryl” respectively.

20 In certain embodiments, substituents of compounds are disclosed in groups or in ranges. It is specifically intended that the description include each and every individual subcombination of the members of such groups and ranges. For example, the term “C₁₋₆ alkyl” is specifically intended to individually disclose C₁, C₂, C₃, C₄, C₅, C₆, C₁-C₆, C₁-C₅, C₁-C₄, C₁-C₃, C₁-C₂, C₂-C₆, C₂-C₅, C₂-C₄, C₂-C₃, C₃-C₆, C₃-C₅, C₃-C₄, C₄-C₆, C₄-C₅, and
25 C₅-C₆ alkyl.

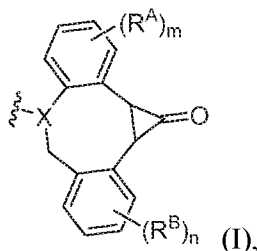
Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have
30 specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 and the like, as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

The invention provides a compound comprising an α,β -unsaturated carboxamide

[—C(R')=C(R'')-C(=O)NH—] group or α,β -unsaturated sulfonamide

5 [—C(R')=C(R'')-S(=O)₂NH—] group conjugated to a group of formula (I):



wherein:

each occurrence of R' and R'' is independently hydrogen, optionally substituted C₁-C₆ alkyl, or optionally substituted C₃-C₈ cycloalkyl;

10 X is N or CH;

each occurrence of R^A is independently H, F, Cl, Br, I, -CN, -NO₂, -OR^C, -SR^C, -S(=O)₁₋₂R^C, -N(R^C)₂, -NR^CC(=O)R^C, -C(=O)N(R^C)₂, -NR^CC(=O)N(R^C)₂, -S(=O)₂N(R^C)₂, -C(=O)R^C, -C(=O)OR^C, C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, C₃-C₈ cycloalkyl, phenyl, heterocyclyl, and heteroaryl,

15 wherein each occurrence of R^C is independently selected from the group consisting of hydrogen, -OR^C, -SR^C, -C(=O)R^C, -C(=O)OR^C, -C(=O)N(R^C)₂, -S(=O)₁₋₂R^C, -N(R^C)₂, -NR^CC(=O)R^C, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₃₋₆ cycloalkyl, and phenyl, or two R^C units taken together with the atom(s) to which they are bound form a 3-7 membered ring, wherein the alkyl, haloalkyl, alkenyl, alkynyl, 20 cycloalkyl, or phenyl is independently optionally substituted with at least one substituent independently selected from -OR^E, -C(=O)R^E, -C(=O)OR^E, -C(=O)N(R^E)₂, N(R^E)₂, halogen; -CH_eX_g, wherein X is independently halogen, e is 0, 1, or 2, and e+g = 3; -S(=O)₂R^E; C₁-C₆ alkyl; -CN; -N(R^E)C(=O)R^E; oxo (=O); heterocyclyl; and heteroaryl;

25 wherein each R^E is independently selected from the group consisting of hydrogen, optionally substituted C₁-C₆ alkyl, and optionally substituted C₃-C₈ cycloalkyl; or two R^E units can be taken together to form a ring comprising 3-7 ring atoms;

each occurrence of R^B is independently H, F, Cl, Br, I, $-\text{CN}$, $-\text{NO}_2$, $-\text{OR}^D$, $-\text{SR}^D$, $-\text{S}(=\text{O})_{1-2}\text{R}^D$, $-\text{N}(\text{R}^D)_2$, $-\text{NR}^D\text{C}(=\text{O})\text{R}^D$, $-\text{C}(=\text{O})\text{N}(\text{R}^D)_2$, $-\text{NR}^D\text{C}(=\text{O})\text{N}(\text{R}^D)_2$, $-\text{S}(=\text{O})_2\text{N}(\text{R}^D)_2$, $-\text{C}(=\text{O})\text{R}^D$, $-\text{C}(=\text{O})\text{OR}^D$, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_1\text{-C}_6$ haloalkyl, $\text{C}_1\text{-C}_6$ alkoxy, $\text{C}_1\text{-C}_6$ haloalkoxy, $\text{C}_3\text{-C}_8$ cycloalkyl, phenyl, heterocyclyl, and heteroaryl,

5 wherein each occurrence of R^D is independently selected from the group consisting of hydrogen, $-\text{OR}^F$, $-\text{SR}^F$, $-\text{C}(=\text{O})\text{R}^F$, $-\text{C}(=\text{O})\text{OR}^F$, $-\text{C}(=\text{O})\text{N}(\text{R}^F)_2$, $-\text{S}(=\text{O})_{1-2}\text{R}^F$, $-\text{N}(\text{R}^F)_2$, $-\text{NR}^F\text{C}(=\text{O})\text{R}^F$, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-6} cycloalkyl, and phenyl, or two R^F units taken together with the atom(s) to which they are bound form a 3-7 membered ring, wherein the alkyl, haloalkyl, alkenyl, alkynyl, 10 cycloalkyl, or phenyl is independently optionally substituted with at least one substituent independently selected from $-\text{OR}^F$; $-\text{C}(=\text{O})\text{R}^F$; $-\text{C}(=\text{O})\text{OR}^F$; $-\text{C}(=\text{O})\text{N}(\text{R}^F)_2$; $\text{N}(\text{R}^F)_2$; halogen; $-\text{CH}_e\text{X}_g$, wherein X is independently halogen, e is 0, 1, or 2, and $e+g = 3$; $-\text{S}(=\text{O})_2\text{R}^F$; $\text{C}_1\text{-C}_6$ alkyl; $-\text{CN}$; $-\text{N}(\text{R}^F)\text{C}(=\text{O})\text{R}^F$; oxo ($=\text{O}$); heterocyclyl; and heteroaryl;

15 wherein each R^F is independently selected from the group consisting of hydrogen, optionally substituted $\text{C}_1\text{-C}_6$ alkyl, and optionally substituted $\text{C}_3\text{-C}_8$ cycloalkyl; or two R^F units can be taken together to form a ring comprising 3-7 ring atoms;

m is 0, 1, 2, 3, or 4;

20 n is 0, 1, 2, 3, or 4.

In certain embodiments, X is N.

In certain embodiments, X is CH.

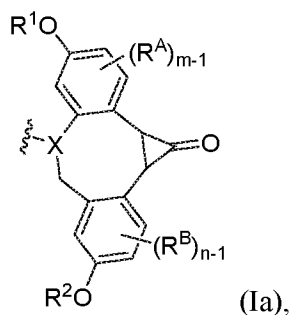
In certain embodiments, at least one R^A is $-\text{OR}^C$. In certain embodiments, the R^A is para to the carbon to which the cyclopropanone group is bound. In certain embodiments, at 25 least one R^A is $-\text{OR}^C$ and is para to the carbon to which the cyclopropanone group is bound.

In certain embodiments, at least one R^B is $-\text{OR}^D$. In certain embodiments, the R^B is para to the carbon to which the cyclopropanone group is bound. In certain embodiments, at least one R^B is $-\text{OR}^D$ and para to the carbon to which the cyclopropanone group is bound.

In certain embodiments, m is 0. In certain embodiments, m is 1. In certain 30 embodiments, m is 2. In certain embodiments, m is 3. In certain embodiments, m is 4.

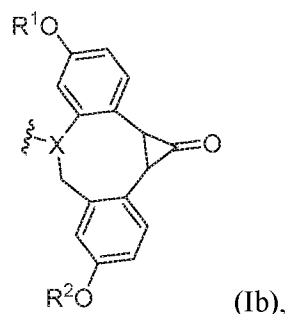
In certain embodiments, n is 0. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4.

In certain embodiments, (I) is:



wherein R^1 and R^2 are independently selected from the group consisting of H, optionally substituted C_1 - C_6 alkyl, and optionally substituted C_3 - C_8 cycloalkyl, m is 1, 2, 3, or 4, and n is 1, 2, 3, or 4.

In certain embodiments, (I) is:



- 5 wherein R^1 and R^2 are independently selected from the group consisting of H, optionally substituted C_1 - C_6 alkyl, and optionally substituted C_3 - C_8 cycloalkyl.

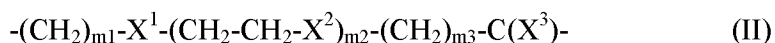
In certain embodiments, R^1 and R^2 are independently selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_8 cycloalkyl. In certain embodiments, R^1 and R^2 are both methyl.

- 10 In certain embodiments, the α,β -unsaturated carboxamide comprises $C(R')_2=C(R'')$ - $C(=O)NH$ —.

In certain embodiments, the α,β -unsaturated sulfonamide comprises $C(R')_2=C(R'')$ - $S(=O)_2NH$ —.

- 15 In certain embodiments, (I) is conjugated by a linker to the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group.

In certain embodiments, the linker comprises the formula:



wherein:

- 20 the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group is covalently bonded to $-(CH_2)_{m1}$, and (I) is covalently bonded to $C(X^3)$ —;

each m_1 , m_2 , and m_3 is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

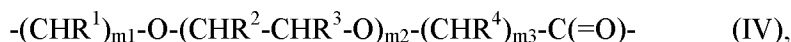
each X^1 , X^2 , and X^3 is independently absent (a bond), O, or $N-R^3$;

each R^3 is independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_3 - C_8 cycloalkyl, and optionally substituted C_3 - C_8 cycloheteroalkyl.

In certain embodiments, the linker comprises the formula:



In certain embodiments, the linker comprises the formula:



wherein:

the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group is covalently bonded to $-(CHR^1)_{m_1}$, and (I) is covalently bonded to $C(O)-$;

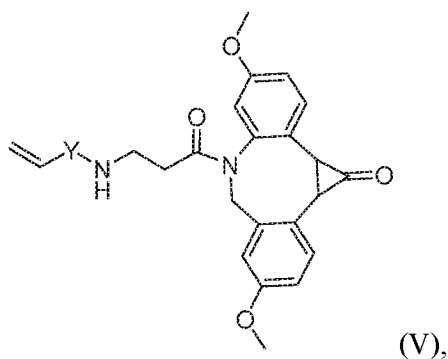
each m_1 , m_2 , and m_3 is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each R^1 , R^2 , R^3 , and R^4 is independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_3 - C_8 cycloalkyl, and optionally substituted C_3 - C_8 cycloheteroalkyl.

In certain embodiments, the linker comprises a polyethylene glycol chain ranging in size from about 1 to about 12 ethylene glycol units.

In certain embodiments, the linker comprises 1-20 optionally substituted methylene groups.

In certain embodiments, the compound is:

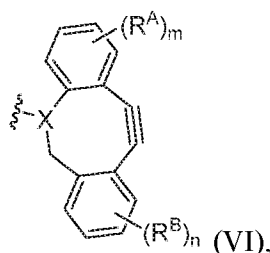


wherein Y is $C(=O)$ or $S(=O)_2$. In certain embodiments, the compound is a monomer.

The invention further provides a polymer comprising the at least partially polymerized compound of the invention. In certain embodiments, the polymer is a hydrogel. In certain embodiments, the at least partially polymerized compound is not cross-linked

therein, and thus the polymer is linear. In certain embodiments, the at least partially polymerized compound is at least partially cross-linked. In certain embodiments, the at least partially polymerized compound is at least partially cross-linked by a bis-acrylate cross-linking agent. In certain embodiments, the bis-acrylate cross-linking agent is selected from the group consisting of ethylene glycol dimethacrylate, tetraethyleneglycol-dimethacrylate, poly(ethylene glycol) dimethacrylates, the condensation product of bisphenol A and glycidyl methacrylate, 2,2'-bis [4-(3-methacryloxy-2-hydroxypropoxy)-phenyl]propane, hexanediol dimethacrylate, tripropylene glycol dimethacrylate, butanediol dimethacrylate, neopentyl glycol dimethacrylate, diethylene glycol dimethacrylate, triethylene glycol dimethacrylate, dipropylene glycol dimethacrylate, allyl methacrylate trimethylolpropane triacrylate, tricyclodecane dimethanol diacrylate, and any combinations thereof.

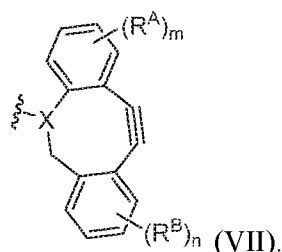
In certain embodiments, the polymer of the invention further comprises at least one group comprising:



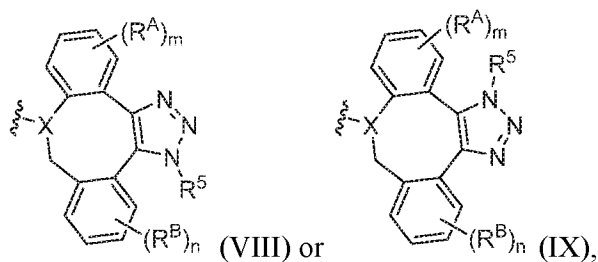
wherein X, R^A , m, R^B , and n in (VI) are the same as in (I), but independently selected therefrom. (VI) can be introduced in the polymer by reacting a DBCO/DIBAC reagent with a reactive group in the polymer, whereby (VI) becomes covalently attached to the polymer. In non-limiting example, a DBCO/DIBAC reagent comprising (VI) linked through a linker to a primary or secondary amino group can be reacted with a free carboxylic group on the polymer using an amide coupling agent (such as but not limited to DCC, DIC, EDC, BOP, COMU, HATU, HBTU, HCTU, PyAOP, PyBOP, PyBrOP, PyClock, PyOxim, TOTU, and so forth) whereby (VI) becomes incorporated in the polymer. In non-limiting example, a DBCO/DIBAC reagent comprising (VI) linked through a linker to a primary or secondary amino group can be reacted with an activated carboxylic group on the polymer (such as but not limited to a N-hydroxysuccinamide ester) whereby (VI) becomes incorporated in the polymer. In non-limiting example, a DBCO/DIBAC reagent comprising (VI) linked through a linker to a carboxylic acid can be reacted with a free primary or secondary amino group on the polymer using an amide coupling agent (such as but not limited to DCC, DIC, EDC, BOP, COMU, HATU, HBTU, HCTU, PyAOP, PyBOP, PyBrOP, PyClock, PyOxim, TOTU,

and so forth) whereby (VI) becomes incorporated in the polymer. In non-limiting example, a DBCO/DIBAC reagent comprising (VI) linked through a linker to an activated carboxylic acid (such as but not limited to a N-hydroxysuccinamide ester) can be reacted with a primary or secondary amino group on the polymer whereby (VI) becomes incorporated in the
 5 polymer. Methods of coupling (VI) to the polymer are known in the art and are incorporated herein by reference.

In certain embodiments, at least a fraction of the partially polymerized compound is converted to



10 In certain embodiments, at least a fraction of the partially polymerized compound is converted to:



wherein R^5 is a small molecule, therapeutic agent, or macromolecule.

15 The invention further provides a method of derivatizing at least a portion of a polymer of the invention with a small molecule or a macromolecule.

In certain embodiments, the polymer comprises at least one group (I). In certain embodiments, the method comprises irradiating the polymer to convert at least one cyclopropenone group (I) to the corresponding cycloalkyne group, and contacting the
 20 resulting material with an azido-containing small molecule or macromolecule. In certain embodiments, the radiation used is around 365 nm.

In certain embodiments, the polymer comprises at least one group (I) and at least one group (VI). In certain embodiments, the method comprises contacting the polymer with an azido-containing small molecule, therapeutic agent, or macromolecule, under conditions that
 25 allow for reaction between the azido-containing small molecule, therapeutic agent, or

macromolecule with (VI). In certain embodiments, the method further comprises irradiating the polymer to convert at least one cyclopropanone group (I) to the corresponding cycloalkyne group (VII). In certain embodiments, the method further comprises contacting the resulting material with an independently selected azido-containing small molecule, therapeutic agent, or macromolecule under conditions that allow for reaction between the azido-containing small molecule, therapeutic agent, or macromolecule with (VII). In certain embodiments, the radiation used is around 365 nm.

In certain embodiments, the derivatized polymer does not release significant amounts of the small molecule or a macromolecule when contacted with a cell or tissue *in vitro* or *in vivo*.

The invention further provides a method of contacting a small molecule, therapeutic agent, and/or macromolecule with a cell, tissue, and/or subject. In certain embodiments, the method comprises contacting the cell, tissue, and/or subject with a polymer comprising (VIII) or (IX).

In certain embodiments, the group (VIII) or (IX) is derived from a monomer comprising (I).

In certain embodiments, the irradiation comprises ultraviolet electromagnetic radiation (wavelength about 10-400 nm), visible electromagnetic radiation (wavelength about 400-750 nm) or infrared electromagnetic radiation (radiation wavelength about 750-300,000 nm). In other embodiments, the electromagnetic radiation comprises ultraviolet or visible electromagnetic radiation.

Ultraviolet or UV light as described herein includes UVA light, which generally has wavelengths between about 320 and about 400 nm, UVB light, which generally has wavelengths between about 290 nm and about 320 nm, and UVC light, which generally has wavelengths between about 200 nm and about 290 nm. UV light may include UVA, UVB, or UVC light alone or in combination with other type of UV light. In certain embodiments, the UV light source emits light between about 350 nm and about 400 nm. In some embodiments, the UV light source emits light between about 400 nm and about 500 nm.

The photo-initiator contemplated within the invention is a molecule that, upon irradiation with a given wavelength at a given intensity for a given period of time, generates at least one species capable of catalyzing, triggering or inducing a polymerization or crosslinking. A photo-initiator known in the art may be employed, such as AIBN (azobisisobutyronitrile), AMBN (2-methylbutyronitrile), a benzoin ether, and a phenone derivative such as benzophenone or diethoxyacetophenone.

Non-limiting examples of the photo-initiator contemplated within the invention are:

AIBN, AMBN, 1-hydroxy-cyclohexyl-phenyl-ketone (IRGACURE® 184; Ciba, Hawthorne, NJ); a 1:1 mixture of 1-hydroxy-cyclohexyl-phenyl-ketone and benzophenone (IRGACURE® 500; Ciba, Hawthorne, NJ); 2-hydroxy-2-methyl-1-phenyl-1-propanone (DAROCUR® 1173; Ciba, Hawthorne, NJ); 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (IRGACURE® 2959; Ciba, Hawthorne, NJ); methyl benzoylformate (DAROCUR® MBF; Ciba, Hawthorne, NJ); oxy-phenyl-acetic acid 2-[2-oxo-2-phenyl-acetoxy-ethoxy]-ethyl ester; oxy-phenyl-acetic 2-[2-hydroxy-ethoxy]-ethyl ester; a mixture of oxy-phenyl-acetic acid 2-[2-oxo-2-phenyl-acetoxy-ethoxy]-ethyl ester and oxy-phenyl-acetic 2-[2-hydroxy-ethoxy]-ethyl ester (IRGACURE® 754; Ciba, Hawthorne, NJ); alpha,alpha-dimethoxy-alpha-phenylacetophenone (IRGACURE® 651; Ciba, Hawthorne, NJ); 2-benzyl-2-(dimethylamino)-1-[4-(4-morpholinyl)-phenyl]-1-butanone (IRGACURE® 369; Ciba, Hawthorne, NJ); 2-methyl-1-[4-(methylthio)phenyl]-2-(4-morpholinyl)-1-propanone (IRGACURE® 907; Ciba, Hawthorne, NJ); a 3:7 mixture of 2-benzyl-2-(dimethylamino)-1-[4-(4-morpholinyl) phenyl]-1-butanone and alpha,alpha-dimethoxy-alpha-phenylacetophenone per weight (IRGACURE® 1300; Ciba, Hawthorne, NJ); diphenyl-(2,4,6-trimethylbenzoyl) phosphine oxide (DAROCUR® TPO; Ciba, Hawthorne, NJ); a 1:1 mixture of diphenyl-(2,4,6-trimethylbenzoyl)-phosphine oxide and 2-hydroxy-2-methyl-1-phenyl-1-propanone (DAROCUR® 4265; Ciba, Hawthorne, NJ); phenyl bis(2,4,6-trimethyl benzoyl) phosphine oxide, which may be used in pure form (IRGACURE® 819; Ciba, Hawthorne, NJ) or dispersed in water (45% active, IRGACURE® 819DW; Ciba, Hawthorne, NJ); a 2:8 mixture of phosphine oxide, phenyl bis(2,4,6-trimethyl benzoyl) and 2-hydroxy-2-methyl-1-phenyl-1-propanone (IRGACURE® 2022; Ciba, Hawthorne, NJ); IRGACURE® 2100, which comprises phenyl-bis(2,4,6-trimethylbenzoyl)-phosphine oxide); bis-(eta 5-2,4-cyclopentadien-1-yl)-bis-[2,6-difluoro-3-(1H-pyrrol-1-yl) phenyl]-titanium (IRGACURE® 784; Ciba, Hawthorne, NJ); (4-methylphenyl) [4-(2-methylpropyl) phenyl]-iodonium hexafluorophosphate (IRGACURE® 250; Ciba, Hawthorne, NJ); 2-(4-methylbenzyl)-2-(dimethylamino)-1-(4-morpholinophenyl)-butan-1-one (IRGACURE® 379; Ciba, Hawthorne, NJ); 4-(2-hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone (IRGACURE® 2959; Ciba, Hawthorne, NJ); bis-(2,6-dimethoxybenzoyl)-2,4,4-trimethylpentylphosphine oxide ; a mixture of bis-(2,6-dimethoxybenzoyl)-2,4,4-trimethylpentylphosphine oxide and 2-hydroxy-2-methyl-1-phenyl-propanone (IRGACURE® 1700; Ciba, Hawthorne, NJ); titanium dioxide; and mixtures thereof.

In certain embodiments, one or more accelerators are utilized in the photopolymerization. Amine accelerators can be used as polymerization accelerators, as well as other accelerators. Polymerization accelerators suitable for use are various organic tertiary amines well known in the art. In visible light curable compositions, the tertiary amines are generally acrylate derivatives such as dimethylaminoethyl methacrylate and, particularly, diethylaminoethyl methacrylate (DEAEMA), EDAB and the like, in an amount of about 0.05 to about 0.5 wt %. The tertiary amines are generally aromatic tertiary amines, such as tertiary aromatic amines such as EDAB, 2-[4-(dimethylamino)phenyl]ethanol, N, N-dimethyl-p-toluidine (commonly abbreviated DMPT), bis(hydroxyethyl)-p-toluidine, triethanolamine, and the like. Such accelerators are generally present at about 0.5 to about 4.0 wt % in the polymeric component. In one embodiment, 0.8 wt % EDAB is used in visible light polymerization.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXPERIMENTAL EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to

these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Materials and instruments:

5

Materials and instrumentation for cp-DIBAC monomer synthesis:

All the raw materials for cp-DIBAC synthesis were purchased either from Sigma, VWR or Fisher Scientific and used as supplied. m-Anisidine, m-anisaldehyde, Boc-beta-alanine-OH, hydroxybenzotriazole (HOBt), aluminium chloride trace metal basis (AlCl₃), and triethylamine (TEA) were purchased from Sigma Aldrich, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) from VWR international, and trifluoroacetic acid (TFA), acryloyl chloride, and tetrachlorocyclopropene were purchased from Fisher Scientific. Solvents DCM, hexane, ethyl acetate, and methanol (MeOH) were purchased from VWR international and used as supplied.

¹H NMR spectra were obtained on a Varian VNMRS 500 MHz spectrometer and processed using Mestrenova 11.0.4.

General procedure to prepare linear polymer with cp-DIBAC:

Stock solutions of monomer (2M), cp-DIBAC (45 mM), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (RAFT agent, 0.05 M) and ZnTPP (0.002 M) were prepared in DMSO and pipetted into 384-well clear flat-bottom white plates (greiner bio-one) while keeping CTA/ZnTPP ratio at 100:1, monomer/CTA agent ratio at either 200:1 or 400:1; mono-mer/cp-DIBAC ratio was varied depending on the amount of incorporation desired in the final polymer composition. The mixtures were then diluted with DMSO to a final volume of 100 µl and final monomer concentration at 0.5 M. Mixtures were covered with well-plate sealing tape to prevent evaporation and irradiated under 560 nm LED light (B12R30DY, Bulbs.com) for 18 h.

Polymer purification:

Polymers were purified using Sephadex G-25 spin columns to remove any unreacted cp-DIBAC before deprotection and click addition. Briefly, Sephadex G-25 super fine powder (Sigma Aldrich) was dissolved in DMSO (37.5 mg/mL) and allowed to sit for at least 3 h at RT for the resin to swell. 0.5 mL Zeba spin columns (Thermo Fisher) were loaded manually with the resin and washed three times with DMSO at 1000 g for 1 min. After washing, 60 µL

of polymer crude was added on top of the resin and centrifuged at 1000 g for 2 min to obtain the purified sample. UV-VIS spectral absorbance (250-400 nm) of polymer sample before and after purification (2 μ L in 98 μ L DMSO for measurement) were collected to calculate the percent of cp- DIBAC removed.

5

Deprotection:

Purified polymer was further diluted in DMSO to a final cp-DIBAC concentration of 5 mM into a 96-well polystyrene flat bottom plate (Denville) with a total volume of 40 μ L. Deprotection of cp-DIBAC was completed by placing the 96-well plate under a 365 nm spectroline (R) E-series UV-lamp (Sigma Aldrich) for 15 mins. Deprotection was confirmed by UV-VIS spectroscopy.

10

Clicking:

Conjugation of 2k PEG-N₃ onto polymers was achieved in similar procedure as deprotection; 1 equivalence of PEG-N₃ (0.05 M in DMSO), with respect to cp-DIBAC, was added into polymer solutions and left overnight at room temperature to allow complete conjugation. UV-VIS spectrum was collected at the end of reaction time to confirm PEG addition.

15

Example 1:

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Cp-DIBAC is synthesized via a five step process and is characterized by UV VIS spectroscopy, mass spectrometry and NMR.

Copolymerization of the monomer was performed in a 96 well plate along with dimethyl acrylamide (DMA monomer) by PET-RAFT in DMSO for incorporation into the polymer backbone. After polymerization, the crude polymer was purified using Sephadex G25 resin to remove any unreacted monomer. The purified polymer was characterized by size exclusion chromatography with multi-angle light scattering (SEC-MALS).

25

The purified polymer was then irradiated under UV light (365 nm) for 15 mins to unmask the cyclopropanone group and generate the azide-reactive alkyne. PEG-N₃ (polyethylene glycol-azide) was then added to the polymer (1:1 azide:alkyne equiv) to enable SPAAC. This reaction proceeded in less than 1 hour but can generally be left overnight. Molecular weight shifts and conjugation were confirmed by SEC-MALS.

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Cyclopropanone-masked Cp-DIBAC did not react with azides under ambient conditions, but very efficiently produced reactive dibenzoazacyclooctynes with UV

deprotection. After synthesis, the presence of the cyclopropanone group was confirmed by UV-VIS spectrophotometry (FIG. 1). The UV spectra of Cp-DIBAC in DMSO (FIG. 1, red) showed two close-lying intense bands ($\lambda_{\text{max}} = 331$ and 347 nm). UV deprotection resulted in the loss of cyclopropanone group and formation of acetylenes (FIG. 1, blue). After
5 deprotection, sodium azide addition resulted in complete loss of the spectrum indicating the formation of triazole, which has no absorbance above 340 nm (FIG. 1, yellow).

In certain embodiments, an exemplary Cp-DIBAC of the invention (0-10 mol%) can be copolymerized into the polymer backbone with good reaction control. Molecular weight shifted after PEG-azide addition are observed confirming SPAAC. Thus, the monomers of
10 the invention are valuable tools for bioconjugation because they can be polymerized and then modified using few synthetic steps.

Example 2:

A non-limiting illustrative cp-DIBAC functionalized monomer was synthesized and
15 incorporated into linear polymers by a statistical copolymerization. The cp-DIBAC monomer was prepared by addition of acryloyl chloride to a precursor, followed by cyclization of the dibenzocyclooctynes with tetrachlorocyclopropene. The linker between cp-DIBAC and the acrylate group allowed for enhancing DMSO solubility and providing space from the polymer chain.

The spectra of cp-DIBAC and deprotected DIBAC allow for simple following of
20 these steps by UV-Vis. Cp-DIBAC has a characteristic, two-hump peak between 340-360 nm which quickly drops off above 370 nm (FIG. 1). Deprotection of the cyclopropanone group with UV (290-350 nm) results in a 30 nm blue shift of this spectrum with a new peak between 310-330 nm. Complete conversion to the deprotected DIBAC can be followed by
25 monitoring for remaining absorbance above 350 nm. Subsequent addition of azide results in a fast click reaction and near complete loss of these peaks (FIG. 1). This convenient method therefore allows for online monitoring of the click reaction and for calculating the final concentration of cp-DIBAC against a calibrated concentration ladder.

Linear polymers with cp-DIBAC in the side chains were prepared in 384 or 96 well
30 plates by PET-RAFT copoly-merization using ZnTPP as the photoinitiator in DMSO. Monomer concentration was varied between 1.0 or 0.5 M, and ZnTPP/CTA ratio was 0.01 or 0.02 depending on de-sired kinetics and conversion. The stock concentration of cp-DIBAC monomer (**1**) was 45 mM as this is the solubility limit in DMSO. PET-RAFT polymerization was photoinitiated under a yellow lamp and allowed to polymerize overnight or at selected

time points. If desired, relative polymerization conversion can be followed by measuring ZnTPP's fluorescence emission intensities at 632 and 615 nm whereby their ratio is directly related to conversion. After polymerization, polymers are purified to remove free cp-DIBAC and deprotected under UV for 15-25 minutes. During this time, deprotection was followed every five minutes by UV-Vis to verify complete deprotection. After deprotection, the concentration of DIBAC was calculated by UV-Vis and the 1:1 equivalent azide was added for SPAAC. Click was verified by UV-Vis and is typically complete within one hour.

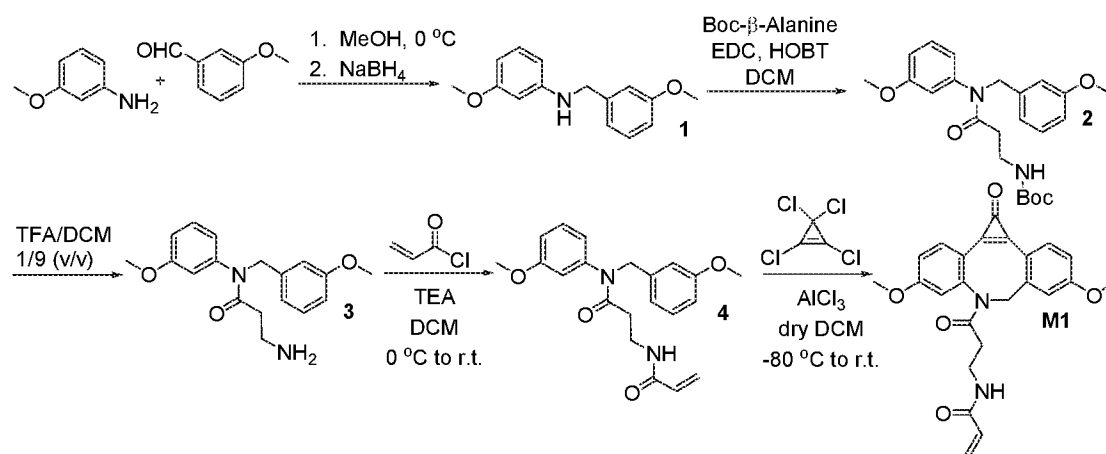
PET-RAFT copolymerization had no effect on cp-DIBAC and its subsequent deprotection and click as verified by UV-Vis (FIG. 6A) and NMR. Copolymerization showed adequate evolution of molecular weight with slightly delayed kinetics. (FIG. 6B). If left for sufficient time, copolymerization with cp-DIBAC proceeds to >90% conversion. For example, when *N,N*-dimethylacrylamide (DMA) was copolymerized (DP 200 or 400) with 5 mol% cp-DIBAC, molecular weights were 29,091 ($\bar{D} = 1.15$) and 55,032 ($\bar{D} = 1.27$) Daltons, respectively (FIG. 7A, Table 1). After clicking with 2k PEG-N₃, these molecular weights shifted to 41,758 ($\bar{D} = 1.29$) and 82,041 ($\bar{D} = 1.34$) Daltons, respectively. This indicates that approximately 7 and 14 PEGs were clicked to the DP 200 and DP 400 polymers, respectively. These click additions match the theoretical number of PEGs that would be clicked at 70% functionalization, indicating remarkable control of post-polymerization modification. It was also found that cp-DIBAC was tolerant to a number of monomers including 2-hydroxyethyl acrylate (HEA) (FIG. 7B), 4-acryloyl morpholine (NAM), and *N*-isopropylacrylamide respectively (FIG. 10). Evaluation of 2.5, 5, and 7.5% cp-DIBAC copolymerization also showed a reasonable range of obtainable valency with increasing comonomer input (FIG. 7C). It was not possible to go higher than 7.5% due to solubility limitations in DMSO, however, sulfonation of the cp-DIBAC could be used to facilitate greater solubility if desired. Because of the strong UV extinction coefficient of cp-DIBAC, the UV trace from the GPC provided a reliable indicator for incorporation into the polymer chain (FIG. 7D). By NMR, all polymers (0, 2.5, 5, and 7.5%) proceeded to full conversion with narrow dispersity and should thus have similar molecular weights.

Taking advantage of the cp protected DIBAC, it was explored if this strategy could allow for dual ligand functionalization using the same SPAAC chemistry. In the present case, polymer-DBCO is SPAAC ready. Therefore, in this study 95% monomer, 2.5% NHS-acrylate, and 2.5% cp-DIBAC were copolymerized to make a random heteropolymer with two different DIBACs (one protected and one unprotected). First addition of PEG-azide and clicking to DBCO provided a predictable shift in molecular weight (FIG. 8A) as well as a

drop in the UV signal for DBCO at 318 nm (FIG. 8B). Deprotection of cp-DIBAC with UV followed by second PEG-azide addition resulted in another molecular weight shift and corresponding shift then reduction in UV absorption by DIBAC. This indicates that dual functional-ize polymers using SPAAC took place in both cases. This capability is very interesting as this allows for polymers to be functionalized with multiple ligands using by SPAAC. For example, when designing mucin mimetics, it is often desirable to label multivalent polymers with several different glycans in order to mimic the complex chemistry of most glycoproteins.

In conclusion, the present disclosure presents a simplified, dual wave-length, one pot strategy for making multivalent polymers. It takes advantage of the tolerance of cp-DIBAC to radical polymerizations as well as well plate format PET-RAFT to make a highly versatile synthetic platform. This approach works with linear polymers whose ligands are in the side chains. Additional use of DBCO provides a simple avenue for functionalizing multiple ligands to single polymers using the same SPAAC click chemistry for each ligand. Thus, using this synthetic technique, one can imagine making very large and diverse libraries of multivalent polymers with relative ease.

Example 3: cp-DIBAC monomer synthesis



Synthesis of 1:

m-Anisidine (5 g, 40.6 mmol, 1.1 eq), m-anisaldehyde (5.02 g, 36.9 mmol, 1 eq), 100 mL methanol and a magnetic stir bar were added to a 250 ml RBF and allowed to mix for 2 h on an ice bath (0 °C). Sodium borohydride (4.2 g, 220, 3 eq) was added slowly for over a period of 1 h and the mixture was allowed to stir for an additional 2 h on ice prior to work up. 150 mL of deionized water (DI H₂O) was added slowly to quench the reaction causing a solid

to form and precipitate out. The mixture was then extracted using ethyl acetate (3 x 100 mL) to separate the organic phase. The organic phase was then washed with 0.5M HCl (2x 100 mL) to remove unreacted aniline followed by 2M NaOH (2x 100 mL), DI H₂O (2x 50 mL), brine (1x 100 mL) and finally dried over Na₂SO₄ for 10 minutes, filtered, and concentrated in vacuum to obtain a brown liquid. The liquid was air dried overnight to obtain the pure compound **1** (7.9 g, 80% yield). ¹H NMR (500 MHz, *d*₆-DMSO) δ 7.28 – 7.16 (m, 1H), 6.91 (tdd, *J* = 7.5, 6.7, 5.6, 2.8 Hz, 3H), 6.80 – 6.73 (m, 1H), 6.24 – 6.13 (m, 2H), 6.13 – 6.04 (m, 2H), 4.20 (dd, *J* = 6.2, 2.1 Hz, 2H), 3.70 (q, *J* = 2.7, 2.2 Hz, 3H), 3.61 (q, *J* = 2.0 Hz, 3H).

Synthesis of 2:

Product **1** (4.5 g, 18.5 mmol, 1 eq) was mixed with Boc-Beta-alanine-OH (4.19 g, 22.14 mmol, 1.2 eq), EDC (4.97 g, 25.89 mmol, 1.4 eq), and HOBT (0.34 g, 2.2 mmol, 0.12 eq) in 100 mL DCM with a stir bar and allowed to mix for 30 h. The organic mixture was then washed with DI water (2x 100 mL), 2M NaOH (1x 100 mL), DI water (1x 100 mL), and finally with brine (1x 100 mL) before being dried over Na₂SO₄ and concentrated in vacuum. The crude was purified using column chromatography over silica (30:70 ethyl acetate) to yield the product as a brownish-red liquid **2** (3.44 g, 45%). ¹H NMR (500 MHz, *d*₆-DMSO) δ 7.22 (dt, *J* = 35.0, 7.6 Hz, 2H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.82 – 6.65 (m, 6H), 4.82 (s, 2H), 4.03 (t, *J* = 7.2 Hz, 1H), 3.70 (s, 6H), 3.41 (s, 1H), 2.28 (s, 2H), 1.34 (s, 9H).

Synthesis of 3:

Product **2** (3.44 g, 8.3 mmol) was dissolved in 50 mL of TFA and DCM (1:9, v:v) and allowed to stir for 4 h at room temperature for Boc deprotection. The resulting solution was washed with 0.1 M NaHCO₃ (2x 50 mL), DI water (1x 50 mL) and finally with brine (1x 50 mL) before being dried over Na₂SO₄ and concentrated in vacuum to yield the pure product as a dark pink liquid **3** (1.82 g, 70%). Sodium bicarbonate wash was used to completely remove any TFA present. ¹H NMR (500 MHz, *d*₆-DMSO) δ 7.26 (t, *J* = 8.1 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 6.89 – 6.84 (m, 1H), 6.79 – 6.68 (m, 5H), 4.80 (s, 2H), 3.68 (d, *J* = 5.6 Hz, 6H), 2.73 (t, *J* = 6.6 Hz, 2H), 2.21 (s, 2H).

Synthesis of 4:

Product **3** (1.82 g, 5.79 mmol, 1 eq) was mixed with 50 mL of DCM in a 100 mL RBF with a magnetic stir bar and the opening was closed with a rubber septum and the setup was placed on an ice bath at 0 °C. TEA (1.61 mL, 11.58 mmol, 2 eq) was added dropwise to the mixture using a syringe and was allowed to stir for half an hour at 0 °C. Acryloyl chloride (470 μL, 5.79 mmol, 1 eq) was then added dropwise carefully and the mixture was allowed to stir on the ice bath for an additional 2 h. After removing from ice bath, the

reaction was allowed to proceed for additional 16 h before quenching it with 10 mL of methanol. The resulting solution was then washed with 0.1 M HCl (1x 50 mL), DI water (2x 50 mL) and finally with brine (1x 50 mL) before being dried over Na₂SO₄ and concentrated in vacuum. The crude was then purified with column chromatography over silica using 5% MeOH in DCM to obtain the product as a light pink liquid (1.109 g, 52%). ¹H NMR (500 MHz, *d*₆-DMSO) δ 8.08 (s, 1H), 7.21 (dtd, *J* = 34.6, 8.0, 1.5 Hz, 2H), 6.90 – 6.83 (m, 1H), 6.79 – 6.66 (m, 5H), 6.15 (ddd, *J* = 17.1, 10.2, 1.5 Hz, 1H), 6.00 (dt, *J* = 17.2, 2.0 Hz, 1H), 5.51 (dt, *J* = 10.1, 1.9 Hz, 1H), 4.81 (s, 2H), 3.68 (d, *J* = 1.5 Hz, 6H), 2.30 (t, *J* = 7.0 Hz, 2H).

Synthesis of M1:

50 mL of anhydrous DCM was added to a 100 ml two neck round bottom flask with a stir bar (one port with N₂ inlet and the other closed with a septum) and was cooled in an acetone-dry ice bath to -80 °C. N₂ exhaust was removed after 10 mins and AlCl₃ (1.60g, 12.04 mmol, 4 eq) was added very quickly and the inlet was closed once again. Tetrachlorocyclopropene (591 μL, 4.82 mmol, 1.6 eq) was added dropwise to the mixture through the septum via syringe-needle system and was allowed to stir for an hour at -80 °C. Product **4** (1.109 g, 3.01 mmol, 1 eq) was dissolved in 5 mL of anhydrous DCM and was added dropwise very slowly for over a period of one hour. After the addition of **4**, the reaction mixture was allowed to stir at -80 °C for another 2.5 h and later at room temperature overnight (16 h). The reaction was quenched by adding 10% HCl (10 ml) and was allowed to stir for another 5 min. The organic mixture was extracted with 50 mL of hexane and then washed with DI water (2x 100 mL) and brine (1x 50) before being dried over Na₂SO₄ and concentrated in vacuum. The crude was purified by column chromatography over silica (10 % methanol in DCM) to yield **M1** as a pale white solid (138 mg, 11%). ¹H NMR (500 MHz, *d*₆-DMSO) δ 7.89 (d, *J* = 5.6 Hz, 2H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 11.1 Hz, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.06 – 5.89 (m, 2H), 5.48 (d, *J* = 9.6 Hz, 1H), 5.04 (d, *J* = 14.6 Hz, 1H), 4.23 (d, *J* = 14.8 Hz, 1H), 3.89 (s, 6H), 3.16 (s, 1H), 3.01 (s, 1H), 2.04 – 1.90 (m, 1H).

Table 1: ¹H NMR and GPC results of cp-DIBAC copolymerized linear polymers before and after clicking.

#	Monomer	DP _{targ} (total)	% (mol) 1	X (%)	polymerised			clicked	
					$M_{n,theo}$	$M_{n,GPC}$	$\bar{D}$	$M_{n,GPC}$	$\bar{D}$
1	DMA	200	2.5	98	21.9	27.7	1.15	37.7	1.29
2	DMA	200	5	95	23.5	29.1	1.15	41.7	1.30
3	DMA	200	7.5	91	25.3	23.6	1.11	46.1	1.26
4	DMA	400	5	92	46.7	55.1	1.27	82.1	1.34
5	HEA	200	5	93	26.6	40.4	1.27	51.2	1.26
6	HEA	400	5	89	52.9	70.6	1.44	94.3	1.35

Table 2: GPC characterisation of NAM and NIPAM linear polymers prepared with 0% and 5% cp-DIBAC (M1).

Monomer	% cp-DIBAC	DP	$M_{n,theo}$	$M_{n,GPC}$	$M_{w,GPC}$	$\bar{D}$
NAM	0	200	28603	19893	22386	1.12
NAM	5	200	31183	24006	29026	1.20
NIPAM	0	200	23003	26974	30068	1.11
NIPAM	5	200	26053	25199	28420	1.12

5

Table 3: Dual conjugation study molecular weight data for DMA DP 200 2.5% DBCO 2.5% cp-DIBAC composition.

Polymer	$M_{n,theo}$	$M_{n,GPC}$	$M_{w,GPC}$	$\bar{D}$
DMA 0% NHS 0% cp-DIBAC	20203	23128	24997	1.08
DMA 2.5% NHS 2.5% cp-DIBAC	22735	23605	26163	1.10
First PEG addition		36027	45534	1.26
Second PEG addition		43220	53774	1.24

10 **Table 4: DMA DP 200 5% cp-DIBAC molecular weight data at various time points.**

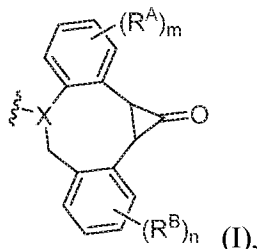
DMA 5% cp-DIBAC (DP 200)	$M_{n,GPC}$	$M_{w,GPC}$	$\bar{D}$
t = 8 h	14601	15746	1.078
t = 12 h	19149	21865	1.14
t = 16 h	22976	27272	1.18

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing
5 from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A compound comprising an α,β -unsaturated carboxamide [$-\text{C}(\text{R}')=\text{C}(\text{R}'')-\text{C}(=\text{O})\text{NH}-$] group or α,β -unsaturated sulfonamide [$-\text{C}(\text{R}')=\text{C}(\text{R}'')-\text{S}(=\text{O})_2\text{NH}-$] group conjugated to a group of formula (I):



wherein:

each occurrence of R' and R'' is independently hydrogen, optionally substituted $\text{C}_1\text{-C}_6$ alkyl, or optionally substituted $\text{C}_3\text{-C}_8$ cycloalkyl;

X is N or CH;

each occurrence of R^A is independently H, F, Cl, Br, I, $-\text{CN}$, $-\text{NO}_2$, $-\text{OR}^C$, $-\text{SR}^C$, $-\text{S}(=\text{O})_{1-2}\text{R}^C$, $-\text{N}(\text{R}^C)_2$, $-\text{NR}^C\text{C}(=\text{O})\text{R}^C$, $-\text{C}(=\text{O})\text{N}(\text{R}^C)_2$, $-\text{NR}^C\text{C}(=\text{O})\text{N}(\text{R}^C)_2$, $-\text{S}(=\text{O})_2\text{N}(\text{R}^C)_2$, $-\text{C}(=\text{O})\text{R}^C$, $-\text{C}(=\text{O})\text{OR}^C$, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_1\text{-C}_6$ haloalkyl, $\text{C}_1\text{-C}_6$ alkoxy, $\text{C}_1\text{-C}_6$ haloalkoxy, $\text{C}_3\text{-C}_8$ cycloalkyl, phenyl, heterocyclyl, and heteroaryl,

wherein each occurrence of R^C is independently selected from the group consisting of hydrogen, $-\text{OR}^C$, $-\text{SR}^C$, $-\text{C}(=\text{O})\text{R}^C$, $-\text{C}(=\text{O})\text{OR}^C$, $-\text{C}(=\text{O})\text{N}(\text{R}^C)_2$, $-\text{S}(=\text{O})_{1-2}\text{R}^C$, $-\text{N}(\text{R}^C)_2$, $-\text{NR}^C\text{C}(=\text{O})\text{R}^C$, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-6} cycloalkyl, and phenyl, or two R^C units taken together with the atom(s) to which they are bound form a 3-7 membered ring, wherein the alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, or phenyl is independently optionally substituted with at least one substituent independently selected from $-\text{OR}^E$, $-\text{C}(=\text{O})\text{R}^E$, $-\text{C}(=\text{O})\text{OR}^E$, $-\text{C}(=\text{O})\text{N}(\text{R}^E)_2$, $\text{N}(\text{R}^E)_2$, halogen, $-\text{CH}_e\text{X}_g$, wherein X is independently halogen, e is 0, 1, or 2, and $e+g=3$; $-\text{S}(=\text{O})_2\text{R}^E$, $\text{C}_1\text{-C}_6$ alkyl; $-\text{CN}$; $-\text{N}(\text{R}^E)\text{C}(=\text{O})\text{R}^E$; oxo $(=\text{O})$; heterocyclyl; and heteroaryl;

wherein each R^E is independently selected from the group consisting of hydrogen, optionally substituted $\text{C}_1\text{-C}_6$ alkyl, and optionally substituted $\text{C}_3\text{-C}_8$ cycloalkyl; or two R^E units can be taken together to form a ring comprising 3-7 ring atoms;

each occurrence of R^B is independently H, F, Cl, Br, I, $-\text{CN}$, $-\text{NO}_2$, $-\text{OR}^D$, $-\text{SR}^D$, $-\text{S}(=\text{O})_{1-2}\text{R}^D$, $-\text{N}(\text{R}^D)_2$, $-\text{NR}^D\text{C}(=\text{O})\text{R}^D$, $-\text{C}(=\text{O})\text{N}(\text{R}^D)_2$, $-\text{NR}^D\text{C}(=\text{O})\text{N}(\text{R}^D)_2$, $-\text{S}(=\text{O})_2\text{N}(\text{R}^D)_2$, $-\text{C}(=\text{O})\text{R}^D$, $-\text{C}(=\text{O})\text{OR}^D$, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_1\text{-C}_6$ haloalkyl, $\text{C}_1\text{-C}_6$ alkoxy, $\text{C}_1\text{-C}_6$ haloalkoxy, $\text{C}_3\text{-C}_8$ cycloalkyl, phenyl, heterocyclyl, and heteroaryl,

wherein each occurrence of R^D is independently selected from the group consisting of hydrogen, $-\text{OR}^F$, $-\text{SR}^F$, $-\text{C}(=\text{O})\text{R}^F$, $-\text{C}(=\text{O})\text{OR}^F$, $-\text{C}(=\text{O})\text{N}(\text{R}^F)_2$, $-\text{S}(=\text{O})_{1-2}\text{R}^F$, $-\text{N}(\text{R}^F)_2$, $-\text{NR}^F\text{C}(=\text{O})\text{R}^F$, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-6} cycloalkyl, and phenyl, or two R^F units taken together with the atom(s) to which they are bound form a 3-7 membered ring, wherein the alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, or phenyl is independently optionally substituted with at least one substituent independently selected from $-\text{OR}^F$; $-\text{C}(=\text{O})\text{R}^F$; $-\text{C}(=\text{O})\text{OR}^F$; $-\text{C}(=\text{O})\text{N}(\text{R}^F)_2$; $\text{N}(\text{R}^F)_2$; halogen; $-\text{CH}_e\text{X}_g$, wherein X is independently halogen, e is 0, 1, or 2, and $e+g = 3$; $-\text{S}(=\text{O})_2\text{R}^F$; $\text{C}_1\text{-C}_6$ alkyl; $-\text{CN}$; $-\text{N}(\text{R}^F)\text{C}(=\text{O})\text{R}^F$; oxo ($=\text{O}$); heterocyclyl; and heteroaryl;

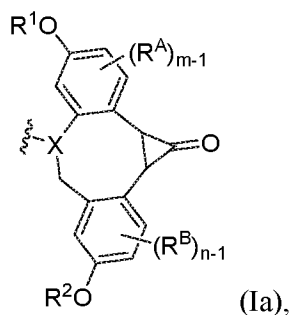
wherein each R^F is independently selected from the group consisting of hydrogen, optionally substituted $\text{C}_1\text{-C}_6$ alkyl, and optionally substituted $\text{C}_3\text{-C}_8$ cycloalkyl; or two R^F units can be taken together to form a ring comprising 3-7 ring atoms;

m is 0, 1, 2, 3, or 4;

n is 0, 1, 2, 3, or 4;

or a salt or solvate thereof.

2. The compound of claim 1, wherein X is N.
3. The compound of claim 1, wherein at least one applies:
at least one R^A is $-\text{OR}^C$,
at least one R^B is $-\text{OR}^D$.
4. The compound of claim 1, wherein (I) is:



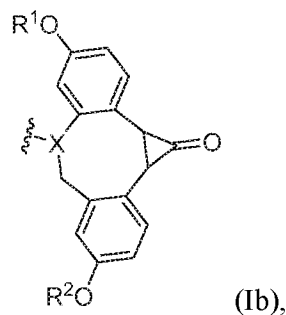
wherein:

R^1 and R^2 are independently selected from the group consisting of H, optionally substituted C_1 - C_6 alkyl, and optionally substituted C_3 - C_8 cycloalkyl;

m is 1, 2, 3, or 4; and

n is 1, 2, 3, or 4.

5. The compound of claim 4, wherein (I) is:



wherein R^1 and R^2 are independently selected from the group consisting of H, optionally substituted C_1 - C_6 alkyl, and optionally substituted C_3 - C_8 cycloalkyl.

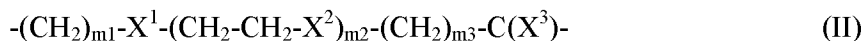
6. The compound of claim 4 or 5, wherein R^1 and R^2 are independently selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_8 cycloalkyl.

7. The compound of claim 4, 5, or 6, wherein R^1 and R^2 are both methyl.

8. The compound of claim 1, wherein the α,β -unsaturated carboxamide group comprises $C(R')_2=C(R'')-C(=O)NH-$, or wherein the α,β -unsaturated sulfonamide group comprises $C(R')_2=C(R'')-S(=O)_2NH-$.

9. The compound of claim 1, wherein (I) is conjugated by a linker to the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group.

10. The compound of claim 9, wherein the linker comprises the formula:



wherein:

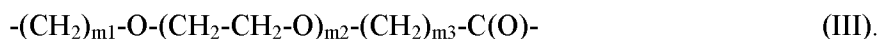
the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group is covalently bonded to $-(\text{CH}_2)_{m1}$, and (I) is covalently bonded to $\text{C}(\text{X}^3)-$;

each $m1$, $m2$, and $m3$ is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each X^1 , X^2 , and X^3 is independently absent (a bond), O, or $\text{N}-\text{R}^3$,

each R^3 is independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_3 - C_8 cycloalkyl, and optionally substituted C_3 - C_8 cycloheteroalkyl.

11. The compound of claim 10, wherein the linker comprises the formula:



12. The compound of claim 10, wherein the linker comprises the formula:



wherein:

the amide nitrogen of the α,β -unsaturated carboxamide or sulfonamide group is covalently bonded to $-(\text{CHR}^1)_{m1}$, and (I) is covalently bonded to $\text{C}(=\text{O})-$;

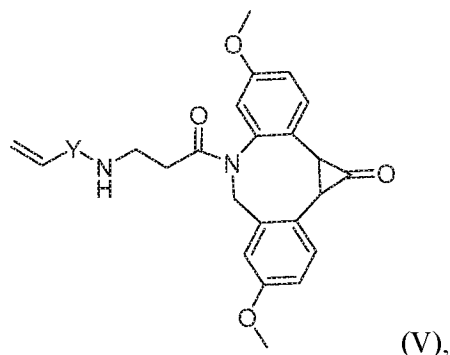
each $m1$, $m2$, and $m3$ is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each R^1 , R^2 , R^3 , and R^4 is independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl, optionally substituted C_3 - C_8 cycloalkyl, and optionally substituted C_3 - C_8 cycloheteroalkyl.

13. The compound of claim 9, wherein the linker comprises a polyethylene glycol chain ranging in size from about 1 to about 12 ethylene glycol units.

14. The compound of claim 9, wherein the linker comprises 1-20 optionally substituted methylene groups.

15. The compound of claim 1, which is:



wherein Y is C(=O) or S(=O)₂.

16. A polymer comprising the at least partially polymerized compound of claim 1.

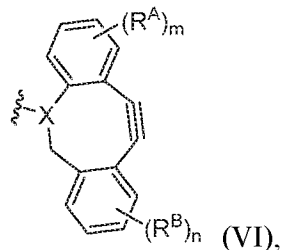
17. The polymer of claim 16, wherein the at least partially polymerized compound is not cross-linked therein and the polymer is linear.

18. The polymer of claim 16, wherein the at least partially polymerized compound is at least partially cross-linked.

19. The polymer of claim 18, wherein the at least partially polymerized compound is at least partially cross-linked by a bis-acrylate cross-linking agent.

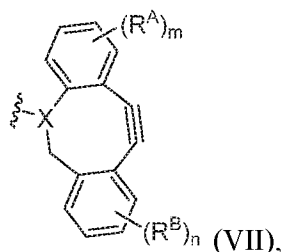
20. The polymer of claim 19, wherein the bis-acrylate cross-linking agent is selected from the group consisting of ethylene glycol dimethacrylate, tetraethyleneglycol-dimethacrylate, poly(ethylene glycol) dimethacrylates, the condensation product of bisphenol A and glycidyl methacrylate, 2,2'-bis [4-(3-methacryloxy-2- hydroxypropoxy)-phenyl]propane, hexanediol dimethacrylate, tripropylene glycol dimethacrylate, butanediol dimethacrylate, neopentyl glycol dimethacrylate, diethylene glycol dimethacrylate, triethylene glycol dimethacrylate, dipropylene glycol dimethacrylate, allyl methacrylate trimethylolpropane triacrylate, tricyclodecane dimethanol diacrylate, and any combinations thereof.

21. The polymer of claim 16, which further comprises at least one group of formula (VI):



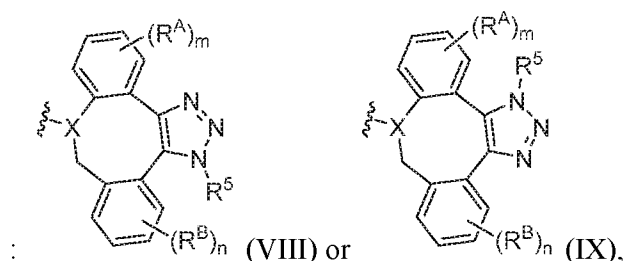
wherein X, R^A , m, R^B , and n in (VI) are independently defined in claim 1.

22. The polymer of claim 16, wherein at least a fraction of (I) is converted by irradiation to (VII):



wherein X, R^A , m, R^B , and n in (VII) are independently defined in claim 1.

23. The polymer of claim 21 or 22, which is reacted with an azido-containing compound to generate one of the following:



wherein R^5 is a small molecule, therapeutic agent, or macromolecule.

24. A method of derivatizing at least a portion of the polymer of claim 16 with a small molecule, therapeutic agent, or macromolecule, the method comprising
 irradiating the polymer to convert at least one group (I) to the corresponding group (VII); and
 contacting the resulting material with N_3-R^5 , wherein R^5 is the small molecule, therapeutic agent, or macromolecule.

25. A method of derivatizing at least a portion of the polymer of claim 21 with one or more optionally distinct small molecules, therapeutic agents, or macromolecules, the method comprising

contacting the polymer of claim 21 with N_3-R^{5A} , wherein R^{5A} is a small molecule, therapeutic agent, or macromolecule, under conditions that allow for reaction between (VI) and N_3-R^{5A} ;

irradiating the resulting polymer to convert at least one group (I) to the corresponding group (VII), and

contacting the irradiated material with N_3-R^{5B} , wherein R^5 is an independently selected small molecule, therapeutic agent, or macromolecule, under conditions that allow for reaction between (VII) and N_3-R^{5B} .

26. The method of claim 24 or 25, wherein the radiation used is around 365 nm.

27. The method of claim 24 or 25, wherein the derivatized polymer does not release significant amounts of the small molecule, therapeutic agent, or macromolecule when contacted with a cell or tissue *in vitro* or *in vivo*.

28. A method of derivatizing a small molecule, therapeutic agent, or a macromolecule with at least one compound of claim 1, the method comprising contacting the small molecule, therapeutic agent, or a macromolecule comprising a thiol group with the at least one compound of claim 1.

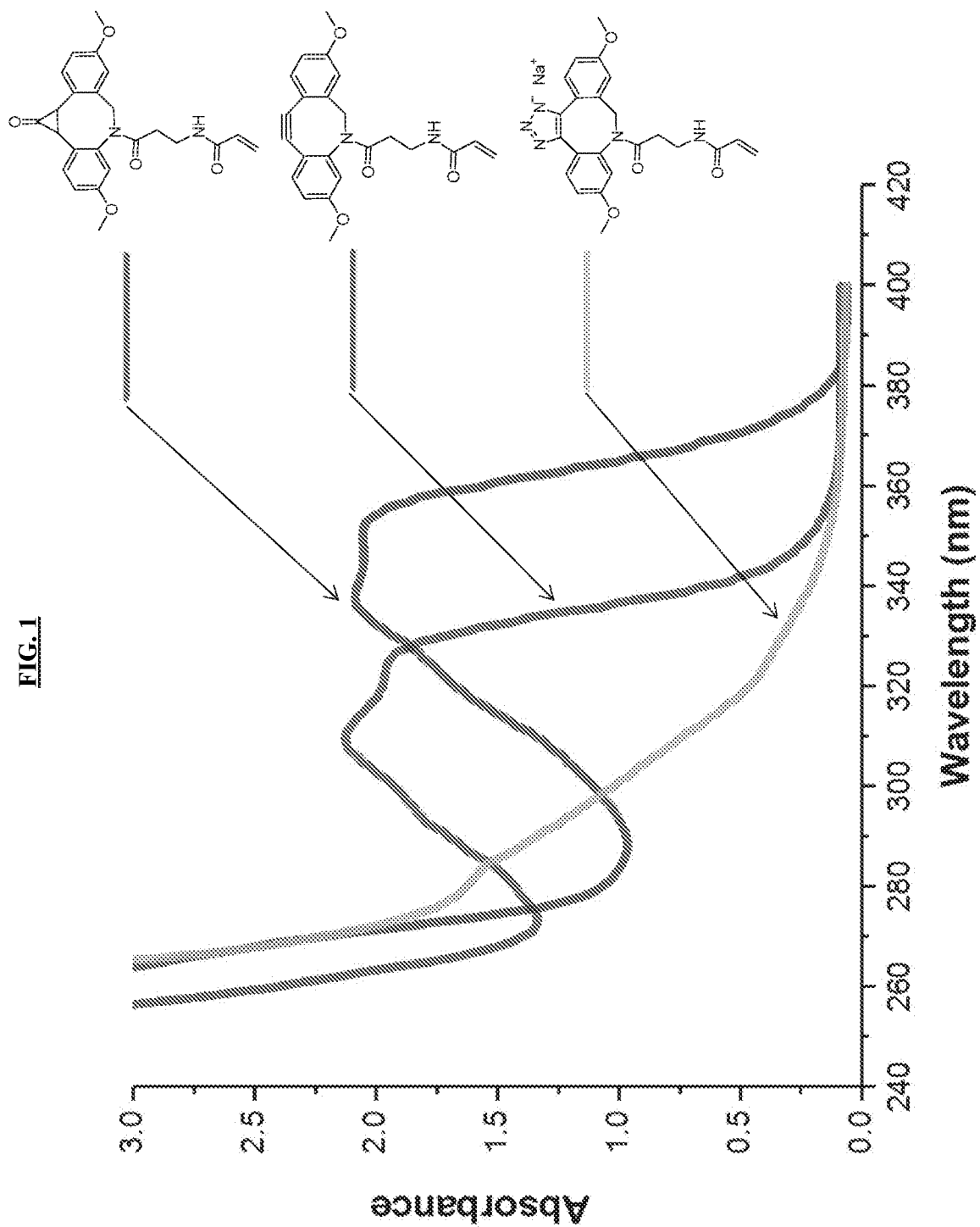


FIG. 2

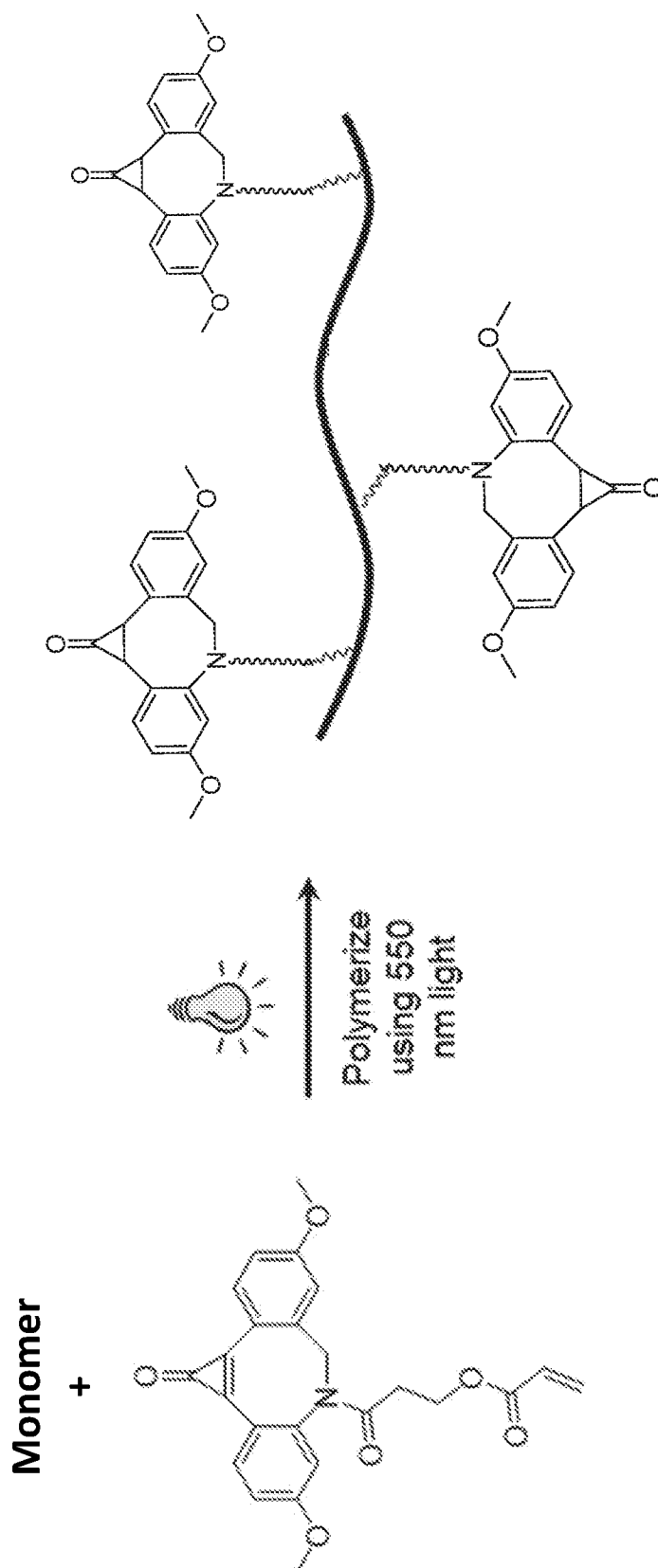


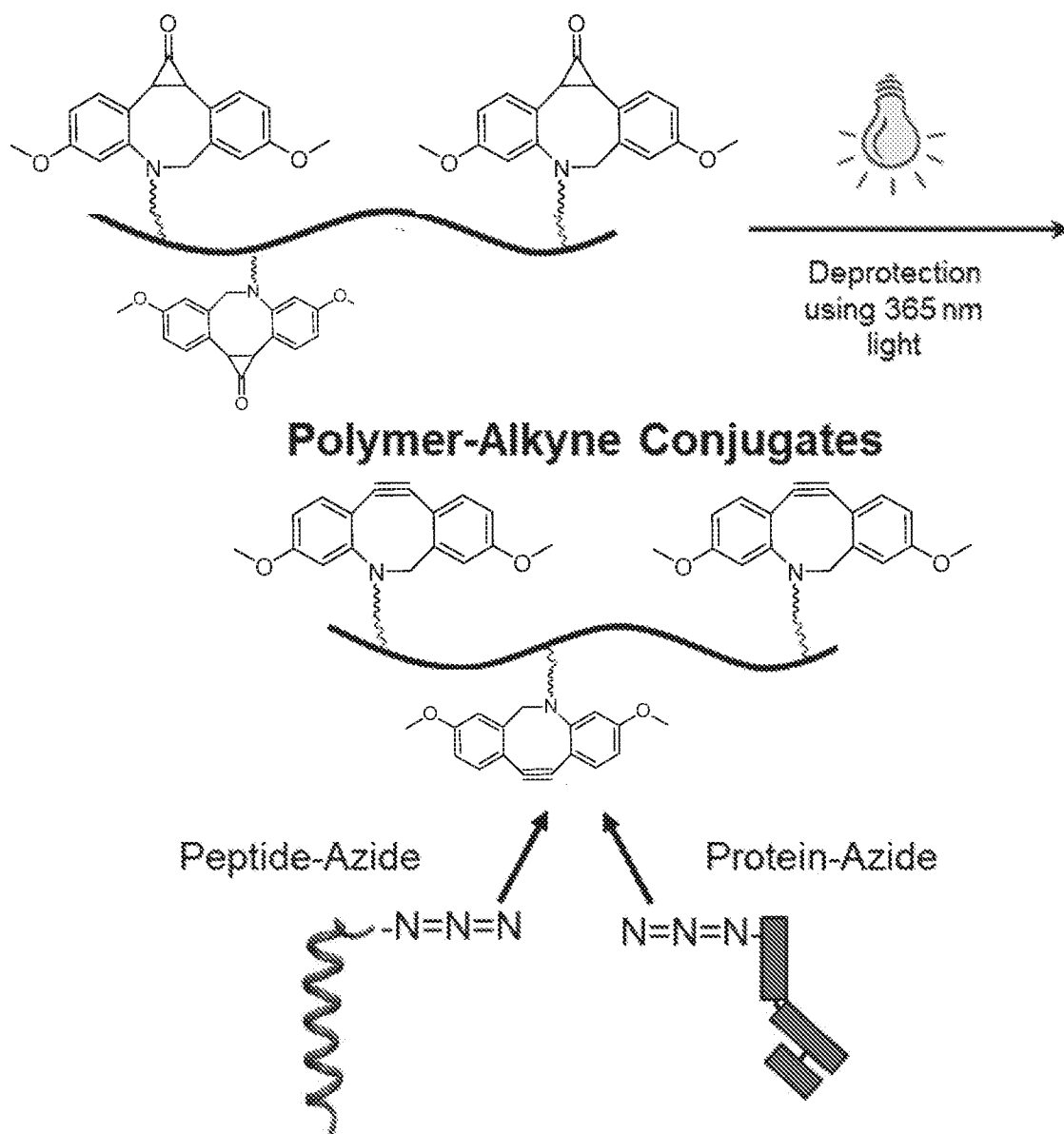
FIG. 3

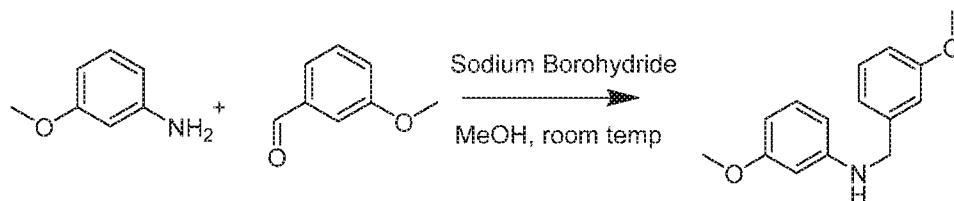
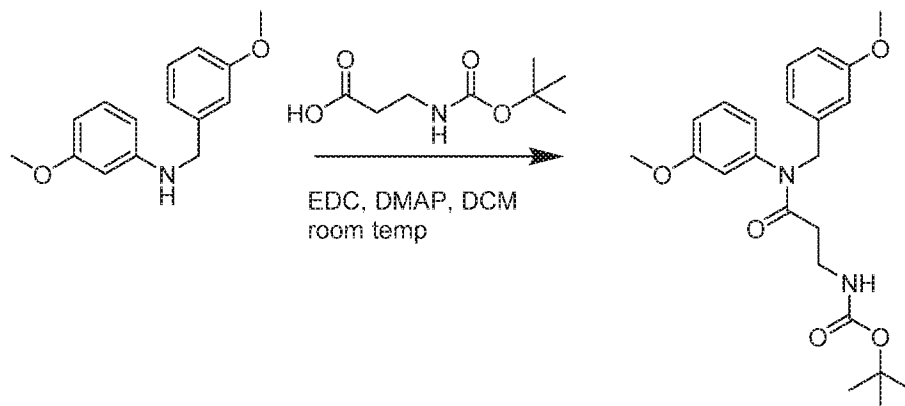
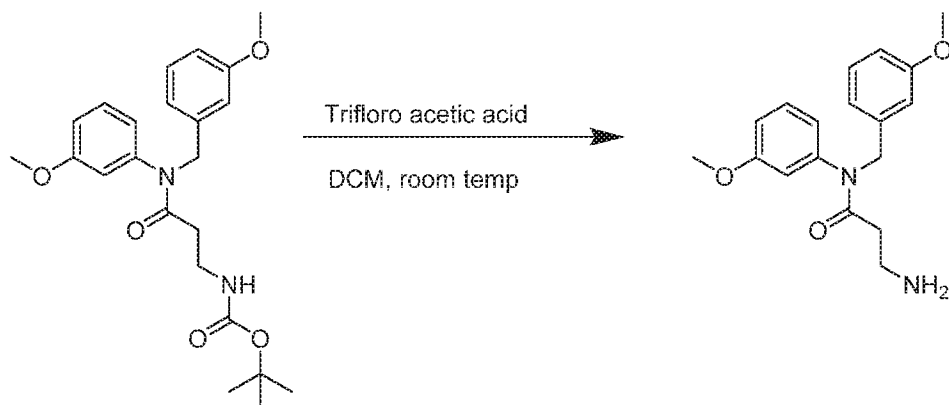
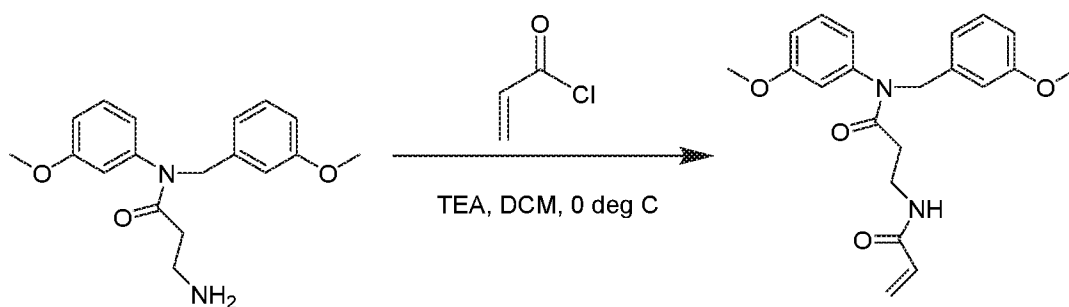
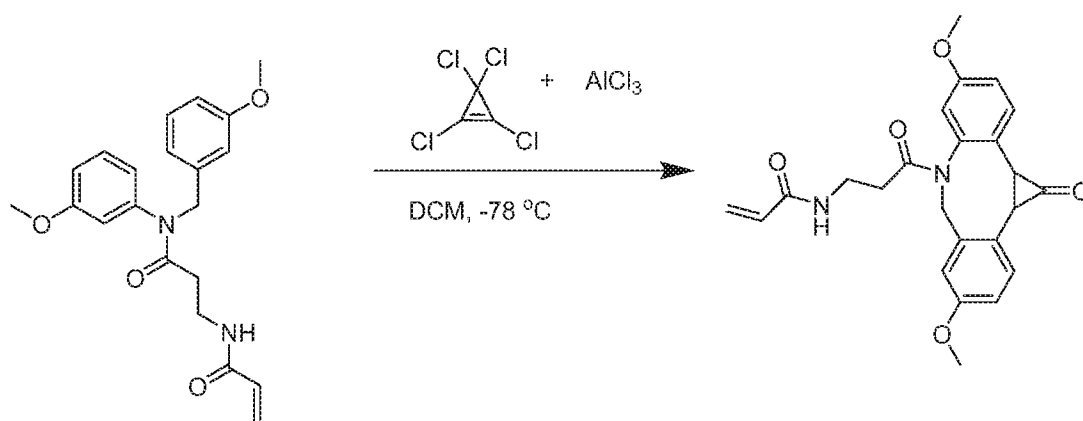
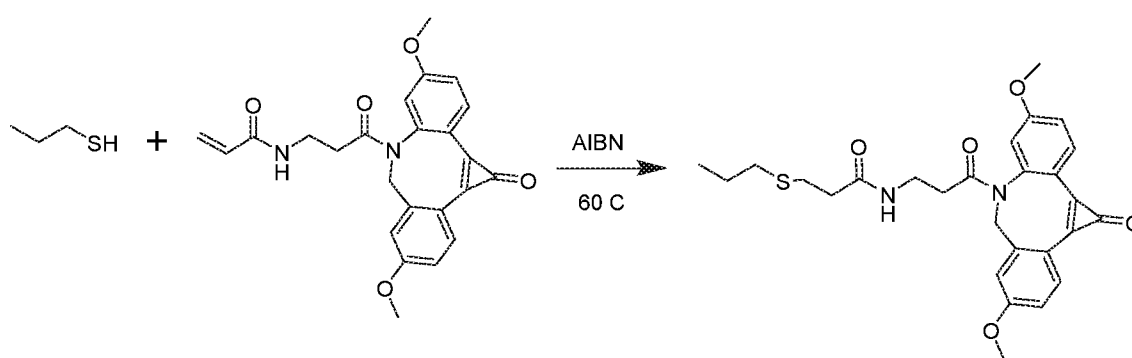
FIG. 4A**Step 1****Step 2****Step 3**

FIG. 4B**Step 4****Step 5****FIG. 5**

propanethiol

cp-DIBAC

Michael adduct

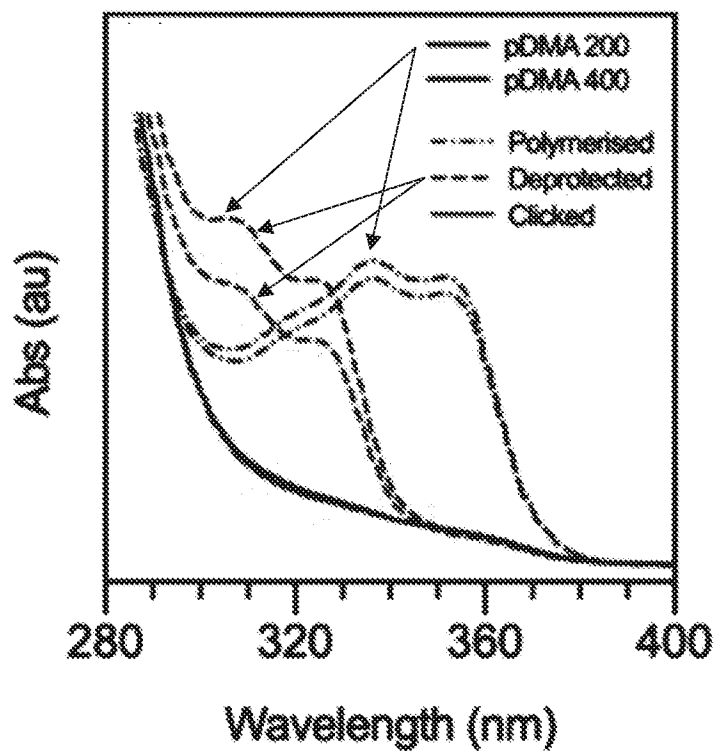
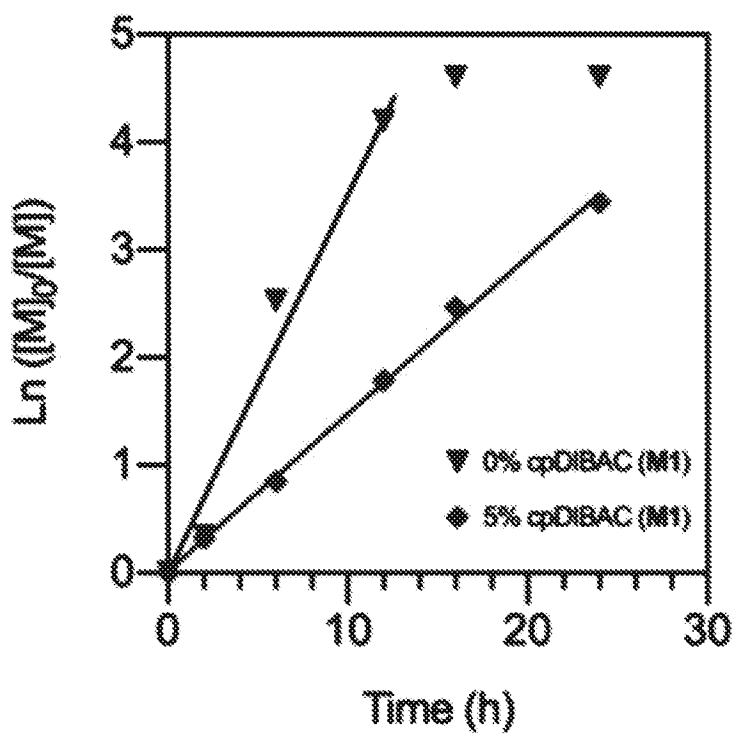
FIG. 6A**FIG. 6B**

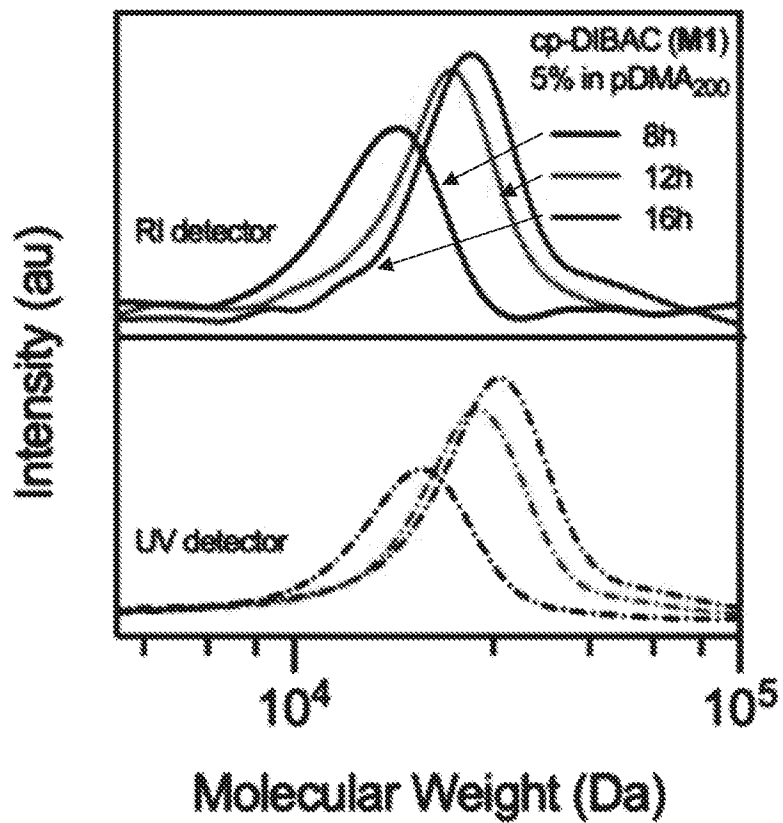
FIG. 6C

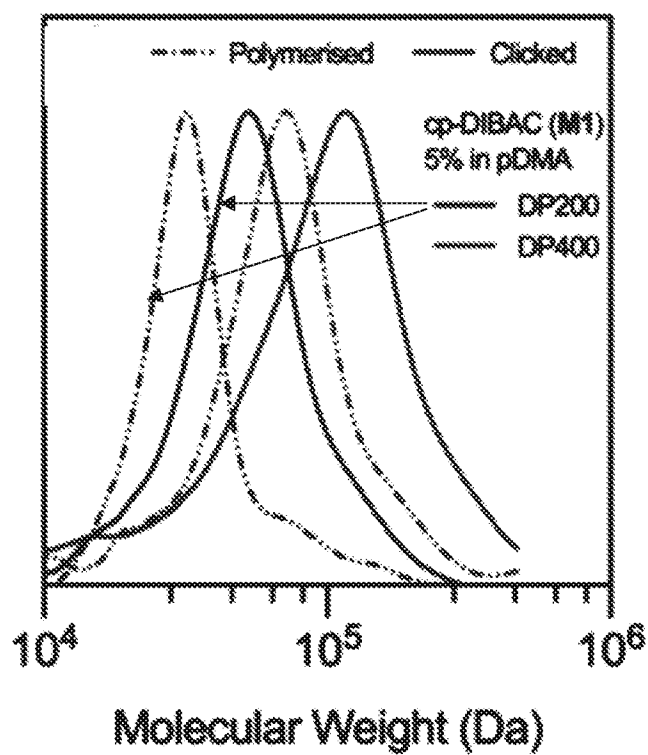
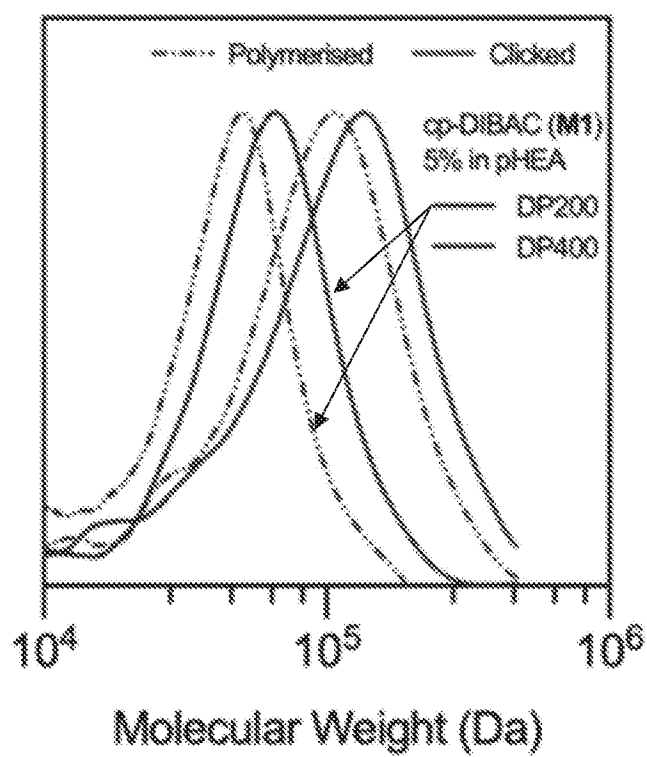
FIG. 7A**FIG. 7B**

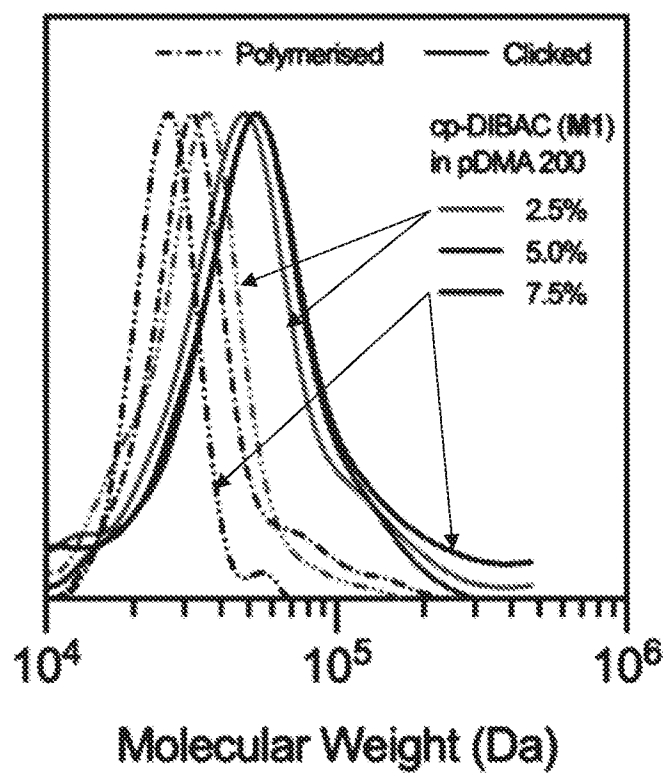
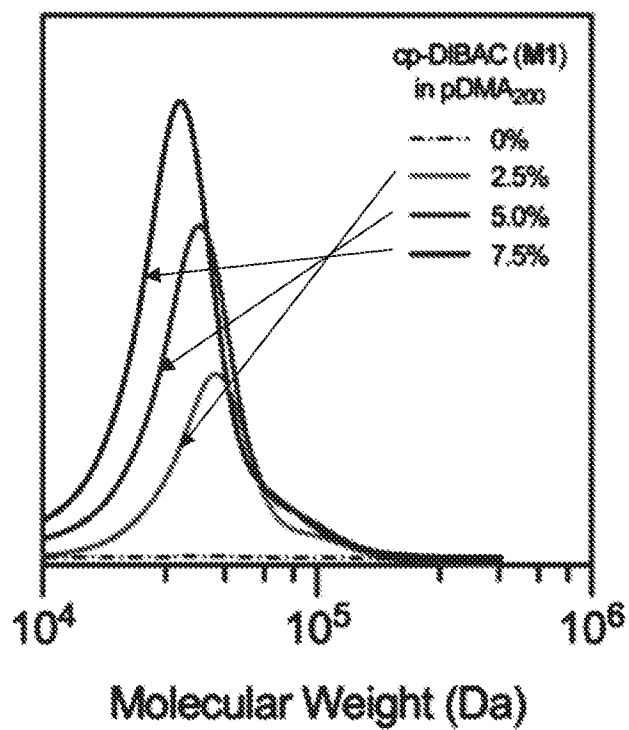
FIG. 7C**FIG. 7D**

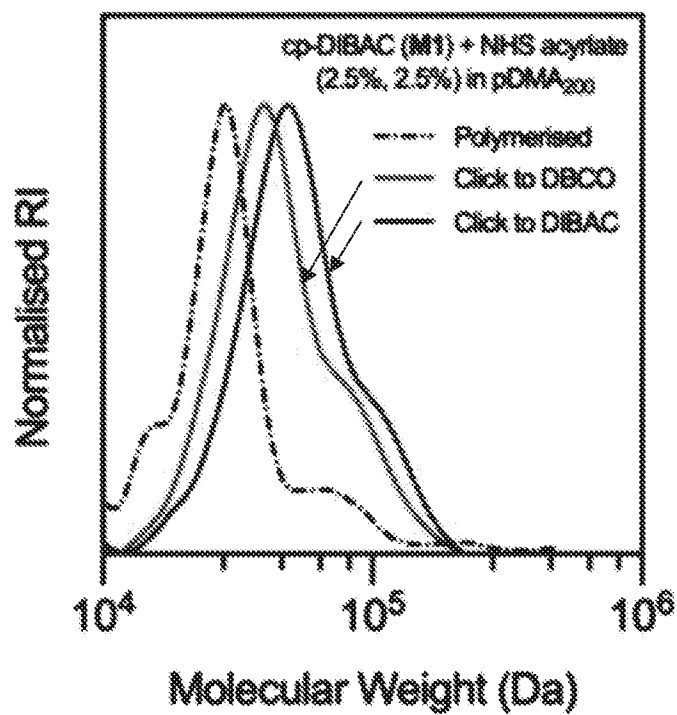
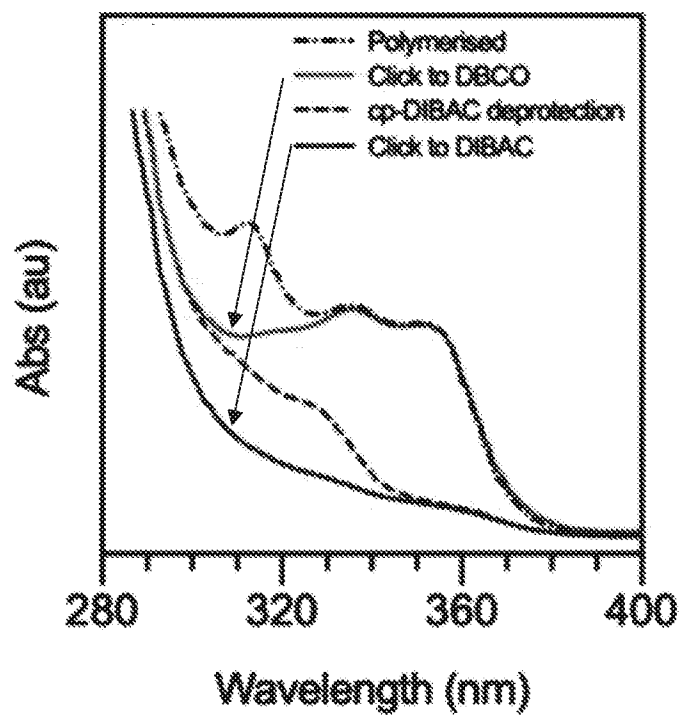
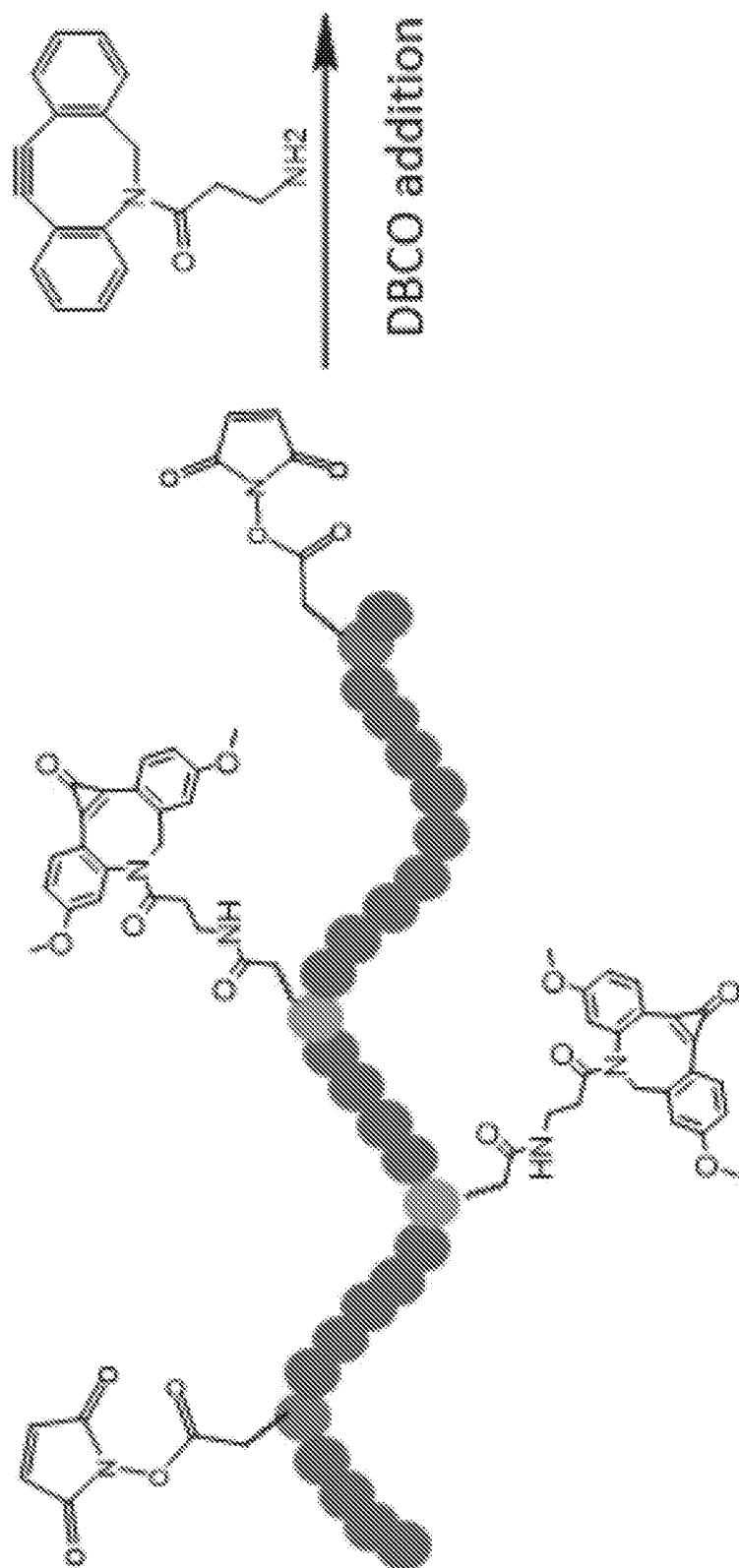
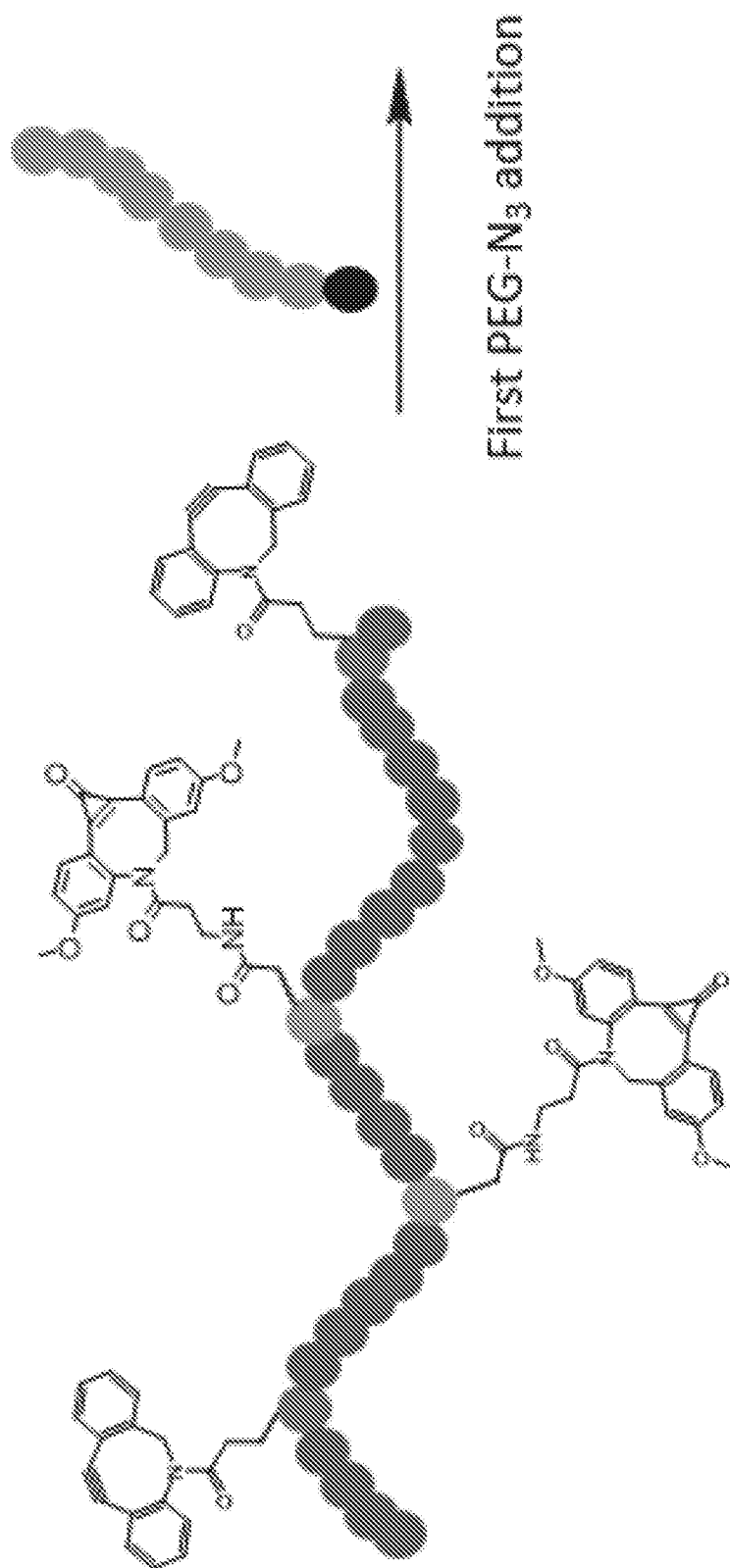
FIG. 8A**FIG. 8B**

FIG. 9A



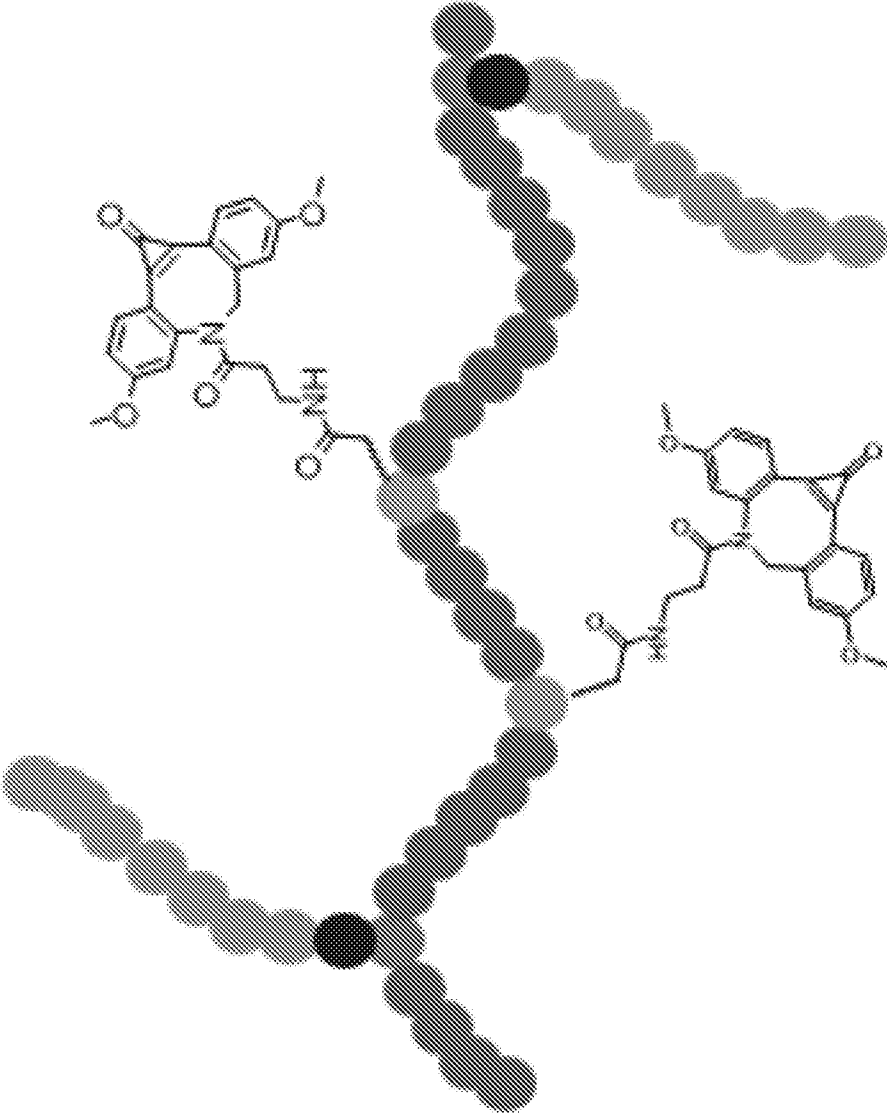
NHS ester and cp-DIBAC incorporated

FIG. 9B



DBCO and cp-DIBAC functional handles on polymer backbone

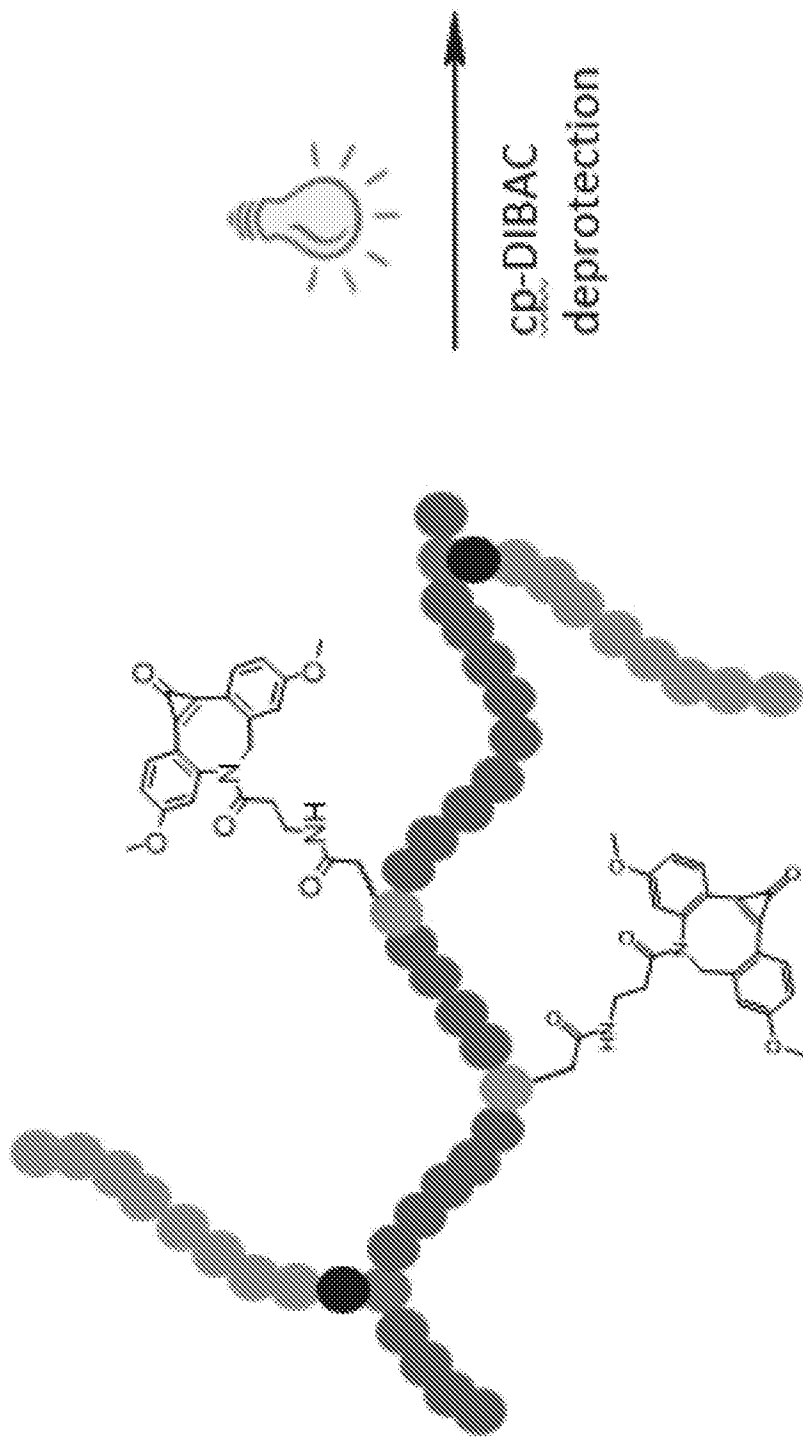
FIG. 9C



First conjugation

FIG. 9D

Step2: Conjugation via cp-DIBAC deprotection



cp-DIBAC functional handle available

FIG. 9E

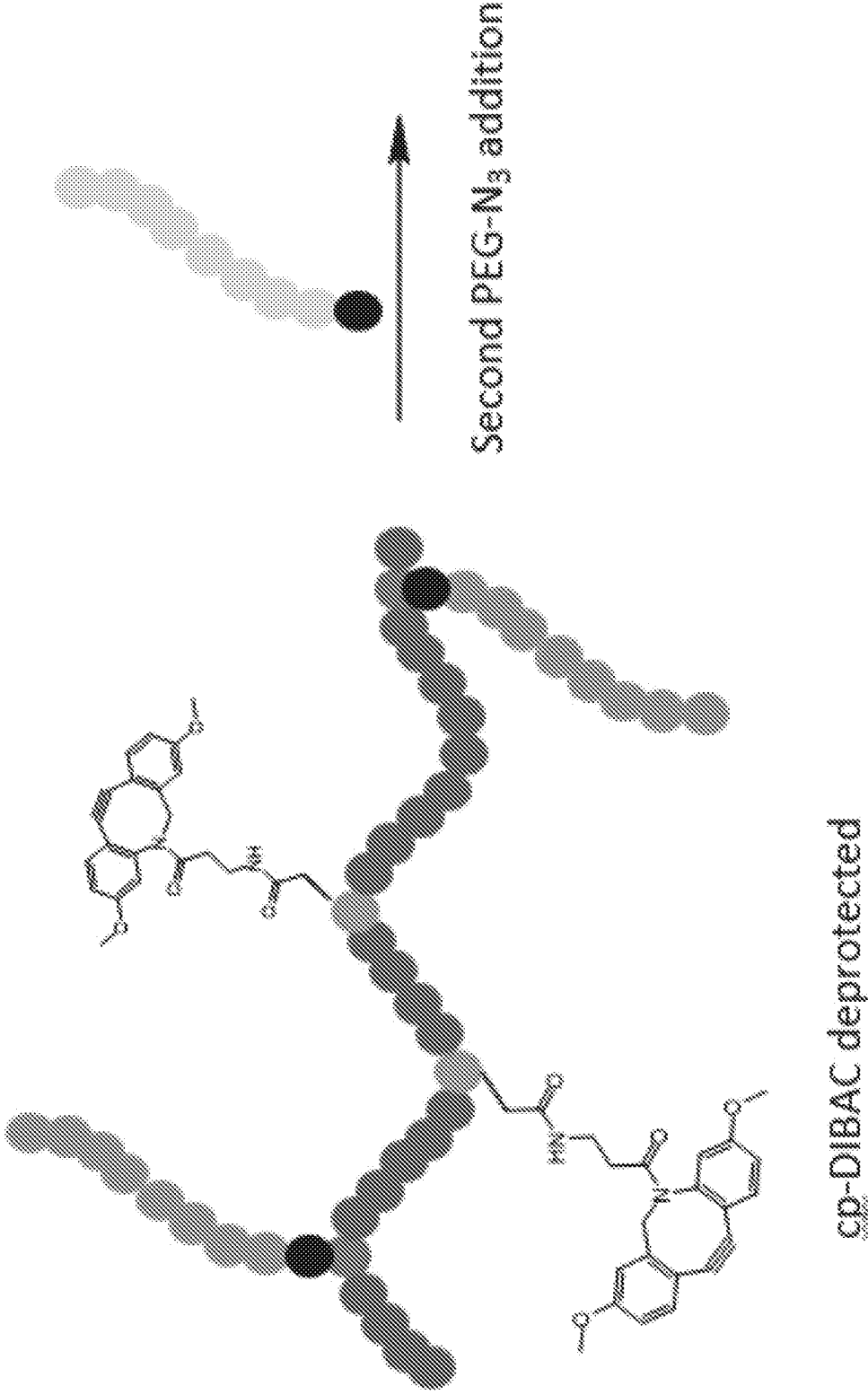
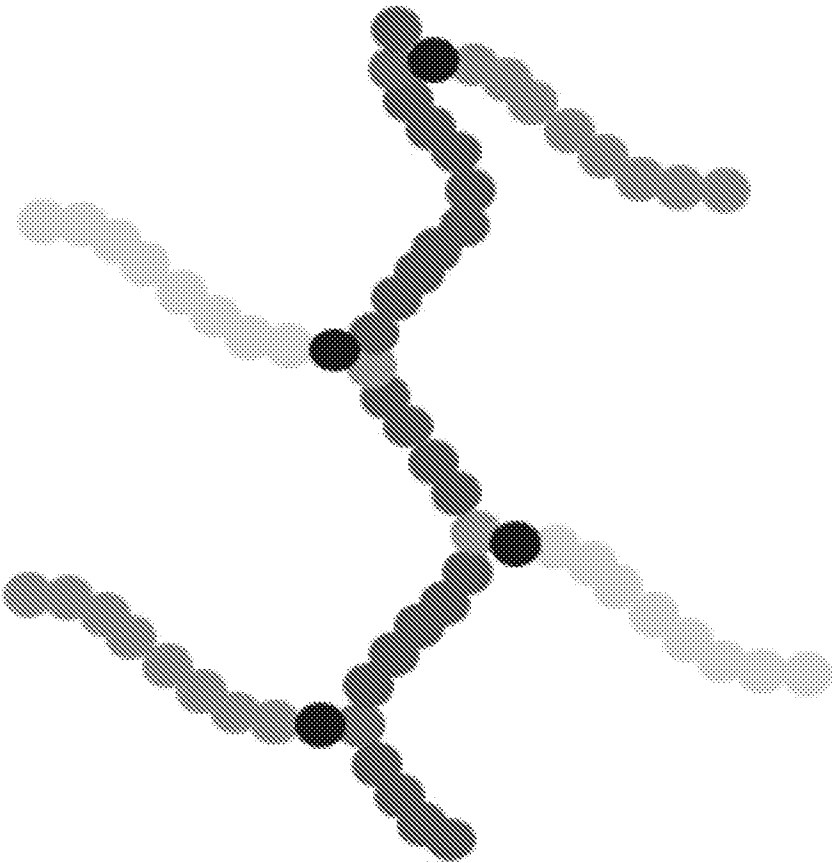
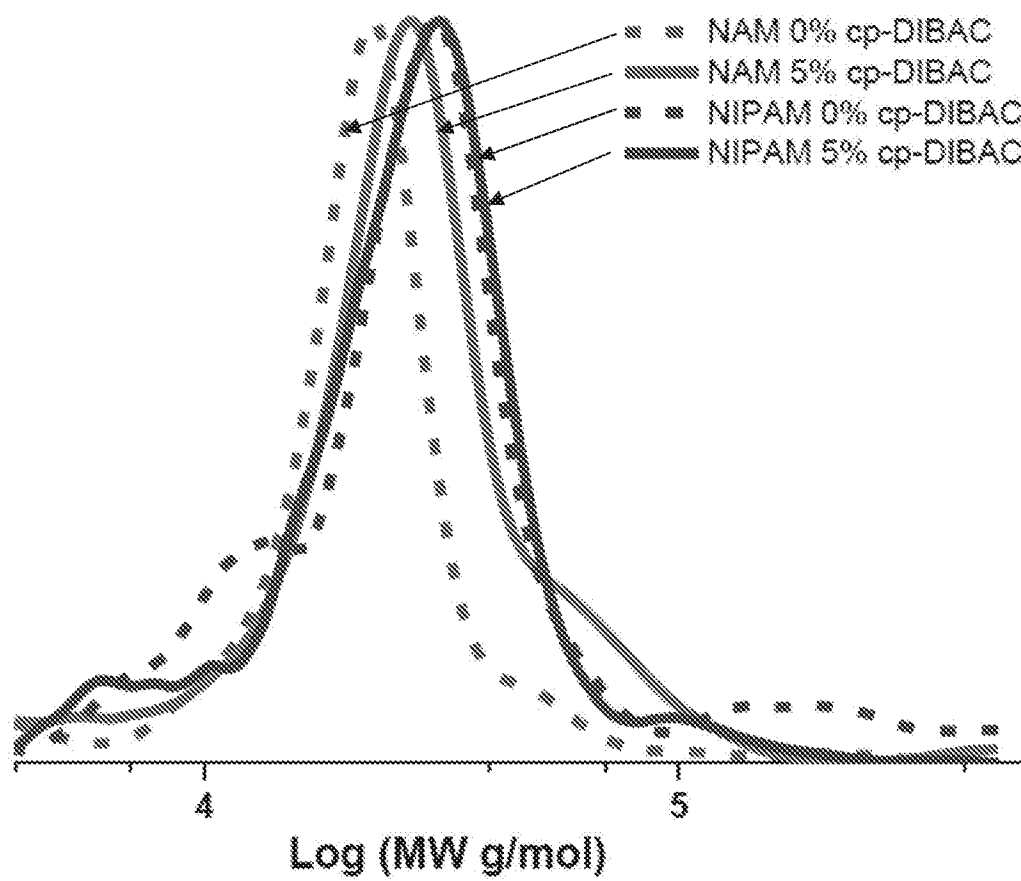


FIG. 9F



Second conjugation

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/59755

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C08F 112/32; C08F 12/26; C08F 212/08 (2019.01)

CPC - C08F 112/32; C08F 12/26; C08F 212/08; C08F 212/14

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US2012/0053299 A1 (POPIK et.al.) 01 March 2012 (01.03.2012) Figure 13, Compound 5c	1-6; 8-28
A	• XU et.al. Cytocompatible Poly(ethylene glycol)-co-polycarbonate Hydrogels Cross-Linked by Copper-Free, Strain-Promoted Click Chemistry in Chem. Asian J. 2011, Vol. 6, pp. 2730 ? 2737. pg. 2733, Figure 2	1-6; 8-28
A	• CHUPAKHIN et.al. Achievements in the synthesis of cyclooctynes for ring strain-promoted [3+2] azide-alkyne cycloaddition in Chemistry of Heterocyclic Compounds, May 2018, Vol. 54(5), pp. 483-501. pg. 493, Scheme 22, Compound 87; pg. 495, Figure 10, Compound 112	1-6; 8-28
A	US 7,417,040 B2 (FINK et.al.) 26 August 2008 (26.08.2008) Col 117, Example 214	1-6; 8-28
A	• DOMMERHOLT et.al. Highly accelerated inverse electron-demand cycloaddition of electron-deficient azides with aliphatic cyclooctynes in Nature Communications, 2014, pp. 1-7. ENTIRE DOCUMENT	1-6; 8-28
A	US 2018/0023084 A1 (ARROWHEAD PHARMACEUTICALS, INC) 25 January 2018 (25.01.2018) ENTIRE DOCUMENT	1-6; 8-28

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 DECEMBER 2019

Date of mailing of the international search report

21 JAN 2020

Name and mailing address of the ISA/US

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Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/59755

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.