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(54) Title: SYNTHETIC NANOCARRIERS COMPRISING POLYMERS COMPRISING MULTIPLE IMMUNOMODULATORY AGENTS

(57) Abstract: This invention relates to compositions, and related methods, of synthetic nanocarriers that comprise polymers that comprise at least two immunomodulatory agent moieties.



**SYNTHETIC NANOCARRIERS COMPRISING POLYMERS COMPRISING
MULTIPLE IMMUNOMODULATORY AGENTS**

RELATED APPLICATIONS

5 This application claims the benefit under 35 U.S.C. §119 of United States provisional application 61/513,496, 61/513,526 and 61/513,527, each filed July 29, 2011, the entire contents of each of which are incorporated herein by reference.

FIELD OF THE INVENTION

10 This invention relates to compositions, and related methods, of synthetic nanocarriers that comprise polymers that comprise at least two immunomodulatory agent moieties.

BACKGROUND OF THE INVENTION

15 Immunomodulatory agents, such as adjuvants, are useful in enhancing the effectiveness of vaccines. However, effective ways of delivering high concentrations of immunomodulatory agents to target cells, such as dendritic cells, are needed.

SUMMARY OF THE INVENTION

20 In one aspect, a composition comprising synthetic nanocarriers comprising a first type of polymer that comprises at least two immunomodulatory agent moieties is provided. In one embodiment, the immunomodulatory agent moieties are between 2 and 100% of the polymer weight. In another embodiment, the immunomodulatory agent moieties are at least 4% of the polymer weight. In another embodiment, the immunomodulatory agent moieties are at least 8% of the polymer weight. In one embodiment, at least a portion of the immunomodulatory agent moieties are not present at the surface of the synthetic nanocarriers. In another
25 embodiment, at least a portion of the immunomodulatory agent moieties are not at the terminus of a polymer.

30 In one embodiment, the first type of polymer comprises at least three, four, five, six, seven, eight, nine or ten immunomodulatory agent moieties. In another embodiment, the at least two immunomodulatory agent moieties are at at least one terminus of the first type of polymer. In yet another embodiment, the at least two immunomodulatory agent moieties are along the backbone of the first type of polymer.

 In still another embodiment, the at least two immunomodulatory agent moieties are themselves polymerized and form the backbone or portion of the backbone of the first type of

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polymer. In one embodiment, the synthetic nanocarriers further comprise another material in addition to the immunomodulatory agent moieties. In another embodiment, the other material is another type of polymer. In a further embodiment, the polymer backbone comprises at least one type of monomeric residue that is not the immunomodulatory agent moiety.

In another embodiment, the synthetic nanocarriers comprise at least a second type of polymer.

In one embodiment, the first type of polymer and/or the second type of polymer is a linear polymer. In another embodiment, the first type of polymer and/or the second type of polymer is a branched polymer. In yet another embodiment, the first type of polymer and/or the second type of polymer is part of or forms a dendrimer. In still another embodiment, the first type of polymer and/or the second type of polymer is part of or forms a polymeric matrix.

In one embodiment, the immunomodulatory agent moieties are the same type of immunomodulatory agent moiety. In one embodiment, the immunomodulatory agent moieties comprise a toll-like receptor (TLR) agonist. In another embodiment, the TLR agonist is a TLR-7 and/or TLR-8 agonist. In yet another embodiment, the TLR agonist comprises an aminodiazepine, adenine derivative or an imidazoquinoline. In still another embodiment, the imidazoquinoline comprises resiquimod or imiquimod. In a further embodiment, the adenine derivative comprises PF-4171455 or SM-276001.

In another embodiment, the composition further comprises an antigen. In one embodiment, the antigen comprises a B cell or T cell antigen. In another embodiment, the T cell antigen is a T helper cell antigen.

In one embodiment, the first type of polymer and/or the second type of polymer comprises a polyester, polyether, polycarbonate or polyamino acid. In another embodiment, the polyester comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone. In yet another embodiment, the polyester is coupled to a hydrophilic polymer. In still another embodiment, the hydrophilic polymer comprises a polyether. In a further embodiment, the polyether comprises polyethylene glycol. In still a further embodiment, the polyamino acid comprises polyglutamic acid.

In one embodiment, the first type of polymer and/or the second type of polymer has a weight average or number average molecular weight of at least 2000 Da, at least 2500 Da, at least 3000 Da, at least 3500 Da, at least 4000 Da, at least 4500 Da or at least 5000 Da.

In one embodiment, the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 500 nm. In another embodiment, the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 400 nm. In another embodiment, the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 300 nm. In another embodiment, the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 250 nm.

10 In yet another embodiment, the composition further comprises a pharmaceutically acceptable excipient.

In another embodiment, the composition is sterile. In another embodiment, the composition is in lyophilized form.

15 In another aspect, a dosage form comprising any of the compositions provided herein is provided.

In yet another aspect, a vaccine comprising any of the dosage forms provided herein is provided.

In still another aspect, a method comprising administering any of the compositions, dosage forms or vaccines to a subject is provided. In one embodiment, the subject is a human. In another embodiment, the method further comprises administering an antigen. In one embodiment, the antigen comprises a B cell or T cell antigen. In another embodiment, the T cell antigen is a T helper cell antigen. In still another embodiment, the subject has or is at risk of having cancer. In a further embodiment, the subject has or is at risk of having an infection or infectious disease. In yet another embodiment, the subject has or is at risk of having an autoimmune disease, an inflammatory disease, an allergy or graft versus host disease. In a further embodiment, the subject has undergone or will undergo transplantation.

20 In a further aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties at a terminus of a polymer, comprising preparing a ring-opened polyester polymer with polyalcohol, contacting the ring-opened polyester polymer with succinic anhydride, and reacting the polyester polymer with immunomodulatory agent moieties in the presence of a coupling agent and a base is provided. In one embodiment, the polyester comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone. In another embodiment, the coupling agent comprises TBTU, HBTU,

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EDC, DCC or PyBop. In still another embodiment, the base comprises DIPEA, DMAP or Et3N.

In another aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising preparing a polyamino acid polymer with a free side chain acid group, and coupling the polymer with immunomodulatory agent moieties in the presence of a coupling agent and a base is provided. In one embodiment, the polyamino acid polymer comprises polyglutamic acid. In another embodiment, the coupling agent comprises TBTU, HBTU, EDC, DCC or PyBop. In still another embodiment, the base comprises DIPEA, DMAP or Et3N.

In yet another aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, the method comprising polymerizing a monomer in the presence of a polyol to provide a multi-armed polymer, functionalizing the multi-armed polymer with one or more carboxylic acid groups, and coupling the immunomodulatory agent moieties comprising an amino group with the multi-armed polymer in the presence of a coupling agent is provided. In one embodiment, the multi-armed polymer comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone. In another embodiment, the coupling agent comprises TBTU, HBTU, EDC, DCC or PyBop. In still another embodiment, the coupling is performed also in the presence of a base. In yet another embodiment, the base is DIPEA, DMAP or Et3N.

In yet a further aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties, the method comprising providing a linear polymer comprising two or more side chain groups comprising an electrophilic or nucleophilic chemical moiety attached thereto, and coupling the immunomodulatory agent moieties to the side chain group is provided. In one embodiment, the side chain chemical moiety comprises a carboxylic acid and the immunomodulatory agent moieties, comprising an amino group, are coupled to carboxylic acid group in the presence of a coupling agent. In another embodiment, the coupling agent is TBTU, HBTU, EDC, DCC or PyBop. In still another embodiment, the coupling is performed also in the presence of a base. In yet another embodiment, the base is DIPEA, DMAP or Et3N.

In another aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising functionalizing

monomers of a polymer, coupling the functionalized monomers with immunomodulatory agent moieties, and polymerizing the coupled monomers is provided.

In yet another aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, the method comprising
5 providing a monomer functionalized with immunomodulatory agent moieties, and polymerizing the monomer is provided. In one embodiment, the monomer comprises lactide, glycolide or caprolactone monomer.

In still another aspect, a method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising producing or
10 obtaining reactive bifunctional immunomodulatory agent moieties, and reacting the bifunctional immunomodulatory agent moieties such that a polymer is formed is provided. In one embodiment, the immunomodulatory agent moieties comprise a TLR agonist. In another embodiment, the TLR agonist comprises a TLR-7 and/or TLR-8 agonist. In still another embodiment, the TLR agonist comprises an aminodiazepine, adenine derivative or an
15 imidazoquinoline. In a further embodiment, the imidazoquinoline comprises resiquimod or imiquimod. In another embodiment, the adenine derivative comprises PF-4171455 or SM-276001.

In one embodiment, any of the methods provided further comprise producing a synthetic nanocarrier with the polymer.

20 In a further aspect, a method comprising the steps of any of the methods exemplified herein is provided.

In one aspect, a polymer, synthetic nanocarrier or vaccine obtainable by a process comprising the steps of any method provided herein is provided.

25 In another aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in therapy or prophylaxis.

In yet another aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in any of the methods provided herein.

In still another aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in a method of treating or preventing cancer.

30 In a further aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in a method of treating or preventing infection or infectious disease.

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In still a further aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in a method of treating or preventing an autoimmune disease, an inflammatory disease, an allergy or graft versus host disease.

5 In another aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for use in a method of treating a subject that has undergone or will undergo transplantation.

In yet another aspect, any of the compositions, dosage forms, vaccines, polymers or synthetic nanocarriers can be for the manufacture of a medicament for use in any of the methods provided herein.

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DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified materials or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting of the use of alternative terminology to describe the present invention.

15 All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety for all purposes.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a polymer" includes a mixture of two or more such molecules or a mixture of differing molecular weights of a single polymer species, reference to "a synthetic nanocarrier" includes a mixture of two or more such synthetic nanocarriers or a plurality of such synthetic nanocarriers, reference to "a DNA molecule" includes a mixture of two or more such DNA molecules or a plurality of such DNA molecules, reference to "an adjuvant" includes mixture of two or more such adjuvant molecules or a plurality of such adjuvant molecules, and the like.

As used herein, the term "comprise" or variations thereof such as "comprises" or "comprising" are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) but not the exclusion of any other integer or group of integers. Thus, as used herein, the term

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“comprising” is inclusive and does not exclude additional, unrecited integers or method/process steps.

In embodiments of any of the compositions and methods provided herein, “comprising” may be replaced with “consisting essentially of” or “consisting of”. The phrase “consisting essentially of” is used herein to require the specified integer(s) or steps as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term “consisting” is used to indicate the presence of the recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) alone.

A. INTRODUCTION

The inventors have unexpectedly discovered that it is possible to produce polymers comprising multiple immunomodulatory agent moieties (e.g., at least two, three, four, five, six, seven, eight, nine, ten, or more moieties of immunomodulatory agent per polymer) and use such polymers to form synthetic nanocarriers. These polymers can be useful for targeted delivery of high concentrations of immunomodulatory agents to cells, such as dendritic cells, and can be useful in the treatment and prevention of diseases and conditions, such as cancer, infection or infectious disease, addiction, allergy, autoimmune disease, inflammatory disease, etc. The increased functional-density of multiply-loaded polymers (e.g., moles of immunomodulatory agent per weight polymer) permit not only higher concentration dosage forms but, alternatively, equi-loaded dosage forms with reduced amount of volume taken up by the polymer conjugate.

Accordingly, a composition comprising synthetic nanocarriers comprising a first type of polymer that comprises at least two immunomodulatory agent moieties is provided. In embodiments, at least a portion of the immunomodulatory agent moieties are not present at the surface of the synthetic nanocarriers. In other embodiments, at least one of the immunomodulatory agent moieties of a polymer is not at the terminus (or end) of the polymer. In still other embodiments, wherein the polymer is made up of the immunomodulatory agent moieties forming its backbone, the synthetic nanocarrier comprises another material in addition to the immunomodulatory agent moieties or the polymer comprises at least one type of monomeric residue that is not the immunomodulatory agent moiety.

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Dosage forms and vaccines comprising these compositions are further provided.

Additionally, methods of administering the compositions provided herein to a subject are also provided. In one embodiment, the subject is a human.

Finally, methods of producing the polymers and synthetic nanocarriers described
5 herein are also provided.

The invention will now be described in more detail below.

B. DEFINITIONS

“Adjuvant” means an agent that does not constitute a specific antigen, but boosts the
10 strength and longevity of immune response to a concomitantly administered antigen. Such
adjuvants may include, but are not limited to stimulators of pattern recognition receptors,
such as Toll-like receptors, RIG-1 and NOD-like receptors (NLR), etc. In embodiments,
adjuvants comprise agonists for pattern recognition receptors (PRR), including, but not
15 limited to Toll-Like Receptors (TLRs), specifically TLRs 2, 3, 4, 5, 7, 8, 9 and/or
combinations thereof. In other embodiments, adjuvants comprise agonists for Toll-Like
Receptors 3, agonists for Toll-Like Receptors 7 and 8, or agonists for Toll-Like Receptor 9;
preferably the recited adjuvants comprise aminodiazepine, such as those disclosed in WO
2010/054215, WO 2007/040840, US 2008/0306050, US 2008/0234251; imidazoquinolines;
20 such as R848; adenine derivatives, such as those disclosed in US patent 6,329,381 (Sumitomo
Pharmaceutical Company), US Published Patent Application 2010/0075995 to Biggadike et
al., or WO 2010/018132 to Campos et al. In embodiment, the adenine derivative comprises
SM-276001(9-benzyl-2-butoxy-8-hydroxyadenine) and its analogs with different substituents
at C-9 and C-2 positions and 8-oxo-purine derivatives such as PF-4171455 (4-Amino-1-
25 benzyl-6-trifluoromethyl-1,3-dihydroimidazo[4,5-c] pyridin-2-one) and its analogs with
different substituents at C-6 position. In specific embodiments, synthetic nanocarriers
incorporate as adjuvants compounds that are agonists for toll-like receptors (TLRs) 7 & 8
 (“TLR 7/8 agonists”). Of utility are the TLR 7/8 agonist compounds disclosed in US Patent
6,696,076 to Tomai et al., including but not limited to imidazoquinoline amines,
imidazopyridine amines, 6,7-fused cycloalkylimidazopyridine amines, and 1,2-bridged
30 imidazoquinoline amines. Preferred adjuvants comprise imiquimod and resiquimod (also
known as R848). In specific embodiments, an adjuvant may be an agonist for the DC surface
molecule CD40. In certain embodiments, to stimulate immunity rather than tolerance, a
synthetic nanocarrier incorporates an adjuvant that promotes DC maturation (needed for

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priming of naive T cells) and the production of cytokines, such as type I interferons, which promote antibody immune responses. In some embodiments, an adjuvant may be a TLR-4 agonist. In some embodiments, adjuvants may comprise TLR-5 agonists. In specific embodiments, synthetic nanocarriers incorporate a ligand for Toll-like receptor (TLR)-9.

5 Examples of TLR9 antagonists include hydroxychloroquine and its analogs as well as adenine derivatives.

"Administering" or "administration" means providing a material, such as a drug to a subject in a manner that is pharmacologically useful.

10 An "allergy" also referred to herein as an "allergic condition," is any condition where there is an undesired (e.g., a Type I hypersensitive) immune response (i.e., allergic response or reaction) to a substance. Such substances are referred to herein as allergens. Allergies or allergic conditions include, but are not limited to, allergic asthma, hay fever, hives, eczema, plant allergies, bee sting allergies, pet allergies, latex allergies, mold allergies, cosmetic allergies, food allergies, allergic rhinitis or coryza, topic allergic reactions, anaphylaxis, 15 atopic dermatitis, hypersensitivity reactions and other allergic conditions. The allergic reaction may be the result of an immune reaction to any allergen. In some embodiments, the allergy is a food allergy. Food allergies include, but are not limited to, milk allergies, egg allergies, nut allergies, fish allergies, shellfish allergies, soy allergies or wheat allergies.

"Amount effective" is any amount of a composition provided herein that produces one 20 or more desired responses, such as one or more desired immune responses. This amount can be for in vitro or in vivo purposes. For in vivo purposes, the amount can be one that a clinician would believe may have a clinical benefit for a subject in need thereof. In embodiments, clinically effective amounts are effective amounts that can be helpful in the treatment of a subject with a disease or condition. Such subjects include, in some 25 embodiments, those that have or are at risk of having cancer, an infection or infectious disease, a non-autoimmune or degenerative disease or an addiction. In other embodiments, the subjects include those that have or are at risk of having an autoimmune disease, an inflammatory disease, an allergy or graft versus host disease or has undergone or will undergo transplantation.

30 A subject's immune response can be monitored by routine methods. An amount that is effective to produce the desired immune responses as provided herein can also be an amount of a composition provided herein that produces a desired therapeutic endpoint or a desired therapeutic result. Amounts effective will depend, of course, on the particular subject

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being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reason.

In general, doses of the compositions of the invention can range from about 10 $\mu\text{g}/\text{kg}$ to about 100,000 $\mu\text{g}/\text{kg}$. In some embodiments, the doses can range from about 0.1 mg/kg to about 100 mg/kg . In still other embodiments, the doses can range from about 0.1 mg/kg to about 25 mg/kg , about 25 mg/kg to about 50 mg/kg , about 50 mg/kg to about 75 mg/kg or about 75 mg/kg to about 100 mg/kg . Alternatively, the dose can be administered based on the number of synthetic nanocarriers. For example, useful doses include greater than 10^6 , 10^7 , 10^8 , 10^9 or 10^{10} synthetic nanocarriers per dose. Other examples of useful doses include from about 1×10^6 to about 1×10^{10} , about 1×10^7 to about 1×10^9 or about 1×10^8 to about 1×10^9 synthetic nanocarriers per dose.

“Antigen” means a B cell antigen or T cell antigen. In embodiments, antigens are coupled to the synthetic nanocarriers. In other embodiments, antigens are not coupled to the synthetic nanocarriers. “Type(s) of antigens” means molecules that share the same, or substantially the same, antigenic characteristics.

An “at risk” subject is one in which a health practitioner believes has a chance of having a disease or condition as provided herein.

An “autoimmune disease” is any disease where the immune system mounts an undesired immune response against self (e.g., one or more autoantigens). In some embodiments, an autoimmune disease comprises an aberrant destruction of cells of the body as part of the self-targeted immune response. In some embodiments, the destruction of self manifests in the malfunction of an organ, for example, the colon or pancreas. Examples of autoimmune diseases are described elsewhere herein. Additional autoimmune diseases will be known to those of skill in the art and the invention is not limited in this respect.

“Average”, as used herein, refers to the arithmetic mean unless otherwise noted.

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“B cell antigen” means any antigen that is recognized by or triggers an immune response in a B cell (e.g., an antigen that is specifically recognized by a B cell or a receptor thereon). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. B cell antigens include, but are not limited to proteins, peptides, small molecules, oligosaccharides and carbohydrates. In some embodiments, the B cell antigen comprises a non-protein antigen (i.e., not a protein or peptide antigen). In some embodiments, the B cell antigen comprises a carbohydrate associated with an infectious agent. In some embodiments, the B cell antigen comprises a glycoprotein or glycopeptide associated with an infectious agent. The infectious agent can be a bacterium, virus, fungus, protozoan, or parasite. In some embodiments, the B cell antigen comprises a poorly immunogenic antigen. In some embodiments, the B cell antigen comprises an abused substance or a portion thereof. In some embodiments, the B cell antigen comprises an addictive substance or a portion thereof. Addictive substances include, but are not limited to, nicotine, a narcotic, a cough suppressant, a tranquilizer, and a sedative. In some embodiments, the B cell antigen comprises a toxin, such as a toxin from a chemical weapon or natural sources. The B cell antigen may also comprise a hazardous environmental agent. In some embodiments, the B cell antigen comprises a self antigen. In other embodiments, the B cell antigen comprises an alloantigen, an allergen, a contact sensitizer, a degenerative disease antigen, a hapten, an infectious disease antigen, a cancer antigen, an atopic disease antigen, an autoimmune disease antigen, a non-autoimmune disease antigen, an addictive substance, a xenoantigen, or a metabolic disease enzyme or enzymatic product thereof.

“Couple” or “Coupled” or “Couples” (and the like) means to chemically associate one entity (for example a moiety) with another. In some embodiments, the coupling is covalent, meaning that the coupling occurs in the context of the presence of a covalent bond between the two entities. In non-covalent embodiments, the non-covalent coupling is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, π - π stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. In embodiments, encapsulation is a form of coupling.

“Dosage form” means a pharmacologically and/or immunologically active material in a medium, carrier, vehicle, or device suitable for administration to a subject.

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“Encapsulate” means to enclose at least a portion of a substance within a synthetic nanocarrier. In some embodiments, a substance is enclosed completely within a synthetic nanocarrier. In other embodiments, most or all of a substance that is encapsulated is not exposed to the local environment external to the synthetic nanocarrier. In other
5 embodiments, no more than 50%, 40%, 30%, 20%, 10% or 5% (weight/weight) is exposed to the local environment. Encapsulation is distinct from absorption, which places most or all of a substance on a surface of a synthetic nanocarrier, and leaves the substance exposed to the local environment external to the synthetic nanocarrier.

“Immunomodulatory agent” means an agent that modulates an immune response to an
10 antigen but is not the antigen or derived from the antigen. “Modulate”, as used herein, refers to inducing, enhancing, suppressing, directing, or redirecting an immune response. Such agents include immunostimulatory agents, such as adjuvants, that stimulate (or boost) an immune response to an antigen but is not an antigen or derived from an antigen. There are several distinct types of immunomodulatory agents, which include, but are not limited to,
15 Toll-like Receptor (TLR) agonists and Toll-like Receptor (TLR) antagonists. Such agents also include immunosuppressants. “Moieties” are the active portions of a molecule of the immunomodulatory agents and are useful in the practice of the invention. Such moieties can be chemically modified, e.g., for coupling to the synthetic nanocarrier, and still remain immunologically active. In certain embodiments, multiple moieties of the
20 immunomodulatory agent (e.g., two, three, four, five, six, seven, eight, nine, ten, or more moieties) are coupled to a polymer, e.g., at an end (or terminus) of a polymer, to a polymer backbone, or form at least part of the polymer backbone.

Preferably, at least a portion or all of the immunomodulatory agent moieties are incorporated within the synthetic nanocarriers. Some of the immunomodulatory agent
25 moieties may be present at the surface of the synthetic nanocarriers. In some embodiments, not all of the immunomodulatory agent moieties are present at the surface of the synthetic nanocarriers. In some embodiments, all of the immunomodulatory moieties that are attached to or form part of the polymer, or synthetic nanocarrier that comprises the polymer, are the same type of immunomodulatory agent moiety (i.e., are identical to one another in chemical
30 structure). In other embodiments, a polymer, or synthetic nanocarrier that comprises the polymer, as provided herein comprises one or more moieties of a number of different types of immunomodulatory agents (e.g., two, three, four, five, six, seven, eight, nine, or ten different types of immunomodulatory agent moieties). In still other embodiments, a polymer, or

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synthetic nanocarrier that comprises the polymer, as provided herein comprises exactly one type of immunomodulatory agent moiety. In some embodiments, a polymer, or synthetic nanocarrier that comprises the polymer, as provided herein comprises exactly two distinct types of immunomodulatory agent moieties. In some embodiments, a polymer, or synthetic nanocarrier that comprises the polymer, comprises three or more distinct types of immunomodulatory agent moieties (e.g., three, four, five, six, seven, eight, nine, or ten distinct types of immunomodulatory agent moieties).

“Immunosuppressant” means a compound that causes an immunosuppressive (e.g., tolerogenic) effect. An immunosuppressive effect generally refers to the production or expression of cytokines or other factors by immune cells, such as antigen-presenting cells, that reduce, inhibit or prevent an undesired immune response. Immunosuppressants include, but are not limited to, statins; mTOR inhibitors, such as rapamycin or a rapamycin analog; TGF- β signaling agents; TGF- β receptor agonists; histone deacetylase inhibitors, such as Trichostatin A; corticosteroids; inhibitors of mitochondrial function, such as rotenone; P38 inhibitors; NF- κ B inhibitors, such as 6Bio, Dexamethasone, TCPA-1, IKK VII; adenosine receptor agonists; prostaglandin E2 agonists (PGE2), such as Misoprostol; phosphodiesterase inhibitors, such as phosphodiesterase 4 inhibitor (PDE4), such as Rolipram; proteasome inhibitors; kinase inhibitors; G-protein coupled receptor agonists; G-protein coupled receptor antagonists; glucocorticoids; retinoids; cytokine inhibitors; cytokine receptor inhibitors; cytokine receptor activators; peroxisome proliferator-activated receptor antagonists; peroxisome proliferator-activated receptor agonists; histone deacetylase inhibitors; calcineurin inhibitors; phosphatase inhibitors; PI3KB inhibitors, such as TGX-221; autophagy inhibitors, such as 3-Methyladenine; aryl hydrocarbon receptor inhibitors; proteasome inhibitor I (PSI); and oxidized ATPs, such as P2X receptor blockers.

Immunosuppressants also include IDO, vitamin D3, cyclosporins, such as cyclosporine A, aryl hydrocarbon receptor inhibitors, resveratrol, azathiopurine (Aza), 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), FK506, sanglifehrin A, salmeterol, mycophenolate mofetil (MMF), aspirin and other COX inhibitors, niflumic acid, estriol and triptolide. Other exemplary immunosuppressants include, but are not limited, small molecule drugs, natural products, antibodies (e.g., antibodies against CD20, CD3, CD4), biologics-based drugs, carbohydrate-based drugs, nanoparticles, liposomes, RNAi, antisense nucleic acids, aptamers, methotrexate, NSAIDs; fingolimod; natalizumab; alemtuzumab; anti-CD3; tacrolimus

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(FK506), etc. Further immunosuppressants, are known to those of skill in the art, and the invention is not limited in this respect.

An "infection" or "infectious disease" is any condition or disease caused by a microorganism, pathogen or other agent, such as a bacterium, fungus, prion or virus. "An infection or infectious disease antigen" is an antigen associated with an infection or infectious disease. Such antigens include antigens that can be used to generate an immune response against a pathogen or other infectious agent, or component thereof, or that can generate an immune response against infected cells.

"Inflammatory disease" means any disease, disorder or condition in which undesired inflammation occurs.

"Load" is the amount of a component (e.g., immunomodulatory agent) of a synthetic nanocarrier based on the total weight of materials in an entire synthetic nanocarrier (weight/weight). Generally, the load is calculated as an average across a population of synthetic nanocarriers. In one embodiment, the load of the immunomodulatory agent on average across the synthetic nanocarriers is between 0.0001% and 50%. In another embodiment, the load of the immunomodulatory agent on average across the synthetic nanocarriers is between 0.001% and 50%. In yet another embodiment, the load of the immunomodulatory agent is between 0.01% and 20%. In a further embodiment, the load of the immunomodulatory agent is between 0.1% and 10%. In still a further embodiment, the load of the immunomodulatory agent is between 1% and 10%.

In embodiments of any of the compositions and methods provided, the load is calculated as follows: Approximately 3 mg of synthetic nanocarriers are collected and centrifuged to separate supernatant from synthetic nanocarrier pellet. Acetonitrile is added to the pellet, and the sample is sonicated and centrifuged to remove any insoluble material. The supernatant and pellet are injected on RP-HPLC and absorbance is read at 278nm. The μg found in the pellet is used to calculate % entrapped (load), μg in supernatant and pellet are used to calculate total μg recovered.

"Maximum dimension of a synthetic nanocarrier" means the largest dimension of a nanocarrier measured along any axis of the synthetic nanocarrier. "Minimum dimension of a synthetic nanocarrier" means the smallest dimension of a synthetic nanocarrier measured along any axis of the synthetic nanocarrier. For example, for a spheroidal synthetic nanocarrier, the maximum and minimum dimension of a synthetic nanocarrier would be substantially identical, and would be the size of its diameter. Similarly, for a cuboidal

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synthetic nanocarrier, the minimum dimension of a synthetic nanocarrier would be the smallest of its height, width or length, while the maximum dimension of a synthetic nanocarrier would be the largest of its height, width or length. In an embodiment, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm. In an embodiment, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or less than 5 μm . Preferably, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is greater than 110 nm, more preferably greater than 120 nm, more preferably greater than 130 nm, and more preferably still greater than 150 nm. Aspect ratios of the maximum and minimum dimensions of synthetic nanocarriers may vary depending on the embodiment. For instance, aspect ratios of the maximum to minimum dimensions of the synthetic nanocarriers may vary from 1:1 to 1,000,000:1, preferably from 1:1 to 100,000:1, more preferably from 1:1 to 10,000:1, more preferably from 1:1 to 1000:1, still more preferably from 1:1 to 100:1, and yet more preferably from 1:1 to 10:1. Preferably, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample is equal to or less than 3 μm , more preferably equal to or less than 2 μm , more preferably equal to or less than 1 μm , more preferably equal to or less than 800 nm, more preferably equal to or less than 600 nm, and more preferably still equal to or less than 500 nm. In preferred embodiments, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100nm, more preferably equal to or greater than 120 nm, more preferably equal to or greater than 130 nm, more preferably equal to or greater than 140 nm, and more preferably still equal to or greater than 150 nm. Measurement of synthetic nanocarrier dimensions (e.g., diameter) is obtained by suspending the synthetic nanocarriers in a liquid (usually aqueous) media and using dynamic light scattering (DLS) (e.g. using a Brookhaven ZetaPALS instrument). For example, a suspension of synthetic nanocarriers can be diluted from an aqueous buffer into purified water to achieve a final synthetic nanocarrier

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suspension concentration of approximately 0.01 to 0.1 mg/mL. The diluted suspension may be prepared directly inside, or transferred to, a suitable cuvette for DLS analysis. The cuvette may then be placed in the DLS, allowed to equilibrate to the controlled temperature, and then scanned for sufficient time to acquire a stable and reproducible distribution based on
5 appropriate inputs for viscosity of the medium and refractive indices of the sample. The effective diameter, or mean of the distribution, is then reported. "Dimension" or "size" or "diameter" of synthetic nanocarriers means the mean of a particle size distribution obtained using dynamic light scattering.

10 "Not present at the surface of synthetic nanocarriers" refers to an entity that is not exposed to the environment that is external to the synthetic nanocarrier.

"Pharmaceutically acceptable excipient" means a pharmacologically inactive material used together with the recited synthetic nanocarriers to formulate the compositions. Pharmaceutically acceptable excipients comprise a variety of materials known in the art, including but not limited to saccharides (such as glucose, lactose, and the like), preservatives
15 such as antimicrobial agents, reconstitution aids, colorants, saline (such as phosphate buffered saline), and buffers.

"Polymeric monomer" refers to a monomeric unit of a polymer, the polymer generally being made up of a series of linked monomeric residues.

20 "Subject" means animals, including warm blooded mammals such as humans and primates; avians; domestic household or farm animals such as cats, dogs, sheep, goats, cattle, horses and pigs; laboratory animals such as mice, rats and guinea pigs; fish; reptiles; zoo and wild animals; and the like.

"Synthetic nanocarrier(s)" means a discrete object that is not found in nature, and that possesses at least one dimension that is less than or equal to 5 microns in size. Albumin
25 nanoparticles are generally included as synthetic nanocarriers, however in certain embodiments the synthetic nanocarriers do not comprise albumin nanoparticles. In embodiments, synthetic nanocarriers do not comprise chitosan. In certain other embodiments, the synthetic nanocarriers do not comprise chitosan. In other embodiments, synthetic nanocarriers are not lipid-based nanoparticles. In further embodiments, synthetic
30 nanocarriers do not comprise a phospholipid.

A synthetic nanocarrier can be, but is not limited to, one or a plurality of lipid-based nanoparticles (also referred to herein as lipid nanoparticles, i.e., nanoparticles where the majority of the material that makes up their structure are lipids), polymeric nanoparticles,

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metallic nanoparticles, surfactant-based emulsions, dendrimers, buckyballs, nanowires, virus-like particles (i.e., particles that are primarily made up of viral structural proteins but that are not infectious or have low infectivity), peptide or protein-based particles (also referred to herein as protein particles, i.e., particles where the majority of the material that makes up
5 their structure are peptides or proteins) (such as albumin nanoparticles) and/or nanoparticles that are developed using a combination of nanomaterials such as lipid-polymer nanoparticles. Synthetic nanocarriers may be a variety of different shapes, including but not limited to spheroidal, cuboidal, pyramidal, oblong, cylindrical, toroidal, and the like. Synthetic nanocarriers according to the invention comprise one or more surfaces. Exemplary synthetic
10 nanocarriers that can be adapted for use in the practice of the present invention comprise: (1) the biodegradable nanoparticles disclosed in US Patent 5,543,158 to Gref et al., (2) the polymeric nanoparticles of Published US Patent Application 20060002852 to Saltzman et al., (3) the lithographically constructed nanoparticles of Published US Patent Application 20090028910 to DeSimone et al., (4) the disclosure of WO 2009/051837 to von Andrian et
15 al., (5) the nanoparticles disclosed in Published US Patent Application 2008/0145441 to Penades et al., (6) the protein nanoparticles disclosed in Published US Patent Application 20090226525 to de los Rios et al., (7) the virus-like particles disclosed in published US Patent Application 20060222652 to Sebbel et al., (8) the nucleic acid coupled virus-like particles disclosed in published US Patent Application 20060251677 to Bachmann et al., (9)
20 the virus-like particles disclosed in WO2010047839A1 or WO2009106999A2, (10) the nanoprecipitated nanoparticles disclosed in P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" *Nanomedicine*. 5(6):843-853 (2010) or (11) apoptotic cells, apoptotic bodies or the synthetic or semisynthetic mimics disclosed in U.S. Publication 2002/0086049. In embodiments,
25 synthetic nanocarriers may possess an aspect ratio greater than 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

Synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface with hydroxyl groups that activate complement or alternatively comprise a surface
30 that consists essentially of moieties that are not hydroxyl groups that activate complement. In a preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that substantially activates complement or alternatively

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comprise a surface that consists essentially of moieties that do not substantially activate complement. In a more preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that activates complement or
5 alternatively comprise a surface that consists essentially of moieties that do not activate complement. In embodiments, synthetic nanocarriers exclude virus-like particles. In embodiments, when synthetic nanocarriers comprise virus-like particles, the virus-like particles comprise non-natural adjuvant (meaning that the VLPs comprise an adjuvant other than naturally occurring RNA generated during the production of the VLPs). In
10 embodiments, synthetic nanocarriers may possess an aspect ratio greater than 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

“T cell antigen” means any antigen that is recognized by and triggers an immune response in a T cell (e.g., an antigen that is specifically recognized by a T cell receptor on a T cell or an NKT cell via presentation of the antigen or portion thereof bound to a Class I or
15 Class II major histocompatibility complex molecule (MHC), or bound to a CD1 complex). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. T cell antigens generally are proteins or peptides. T cell antigens may be an antigen that stimulates a CD8+ T cell response, a CD4+ T cell response, or both. The nanocarriers, therefore, in some
20 embodiments can effectively stimulate both types of responses.

In some embodiments the T cell antigen is a T helper cell antigen (i.e. one that can generate an enhanced response to a B cell antigen, preferably an unrelated B cell antigen, through stimulation of T cell help). In embodiments, a T helper cell antigen may comprise one or more peptides obtained or derived from tetanus toxoid, Epstein-Barr virus, influenza
25 virus, respiratory syncytial virus, measles virus, mumps virus, rubella virus, cytomegalovirus, adenovirus, diphtheria toxoid, or a PADRE peptide (known from the work of Sette et al. US Patent 7,202,351). In other embodiments, a T helper cell antigen may comprise one or more lipids, or glycolipids, including but not limited to: α -galactosylceramide (α -GalCer), α -linked glycosphingolipids (from *Sphingomonas* spp.), galactosyl diacylglycerols (from
30 *Borrelia burgdorferi*), lipophosphoglycan (from *Leishmania donovani*), and phosphatidylinositol tetramannoside (PIM4) (from *Mycobacterium leprae*). For additional lipids and/or glycolipids useful as a T helper cell antigen, see V. Cerundolo et al., “Harnessing invariant NKT cells in vaccination strategies.” *Nature Rev Immun*, 9:28-38

(2009). In embodiments, CD4+ T-cell antigens may be derivatives of a CD4+ T-cell antigen that is obtained from a source, such as a natural source. In such embodiments, CD4+ T-cell antigen sequences, such as those peptides that bind to MHC II, may have at least 70%, 80%, 90%, or 95% identity to the antigen obtained from the source. In embodiments, the T cell antigen, preferably a T helper cell antigen, may be coupled to, or uncoupled from, a synthetic nanocarrier. In some embodiments, the T cell antigen is encapsulated in the synthetic nanocarriers of the compositions.

A “terminus of a polymer” is an end of a polymer chain or branch.

“Vaccine” means a composition of matter that improves the immune response to a particular pathogen or disease. A vaccine typically contains factors that stimulate a subject's immune system to recognize a specific antigen as foreign and eliminate it from the subject's body. A vaccine also establishes an immunologic ‘memory’ so the antigen will be quickly recognized and responded to if a person is re-challenged. Vaccines can be prophylactic (for example to prevent future infection by any pathogen), or therapeutic (for example a vaccine against a tumor specific antigen for the treatment of cancer). In embodiments, a vaccine may comprise dosage forms according to the invention.

“Weight”, as used herein, refers to mass unless otherwise noted. When a molecular weight of a polymer is measured, it can be measured as the weight average molecular weight or a number average molecular weight. “Weight average molecular weight” for the polymers of the compositions provided herein is calculated by the following formula:

$$\bar{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

Formula 1, where N_i is the number of molecules of molecular weight M_i . The weight average molecular weight can be determined by a variety of methods including light scattering, small angle neutron scattering (SANS), X-ray scattering, Nuclear Magnetic Resonance (NMR) and sedimentation velocity. An example of an alternative for weight average molecular weight is to perform gel permeation chromatography using suitable traceable-weight standards to establish a retention-time versus weight curve, and calculating the mean weight-averaged molecular weight of a sample polymer from the mean of the integrated sample peak as compared to the calibration curve. The “number average molecular weight” can be determined by NMR. For example, number average molecular weight can be determined by proton NMR wherein the ratio of the polymer repeating units to the end group is established and then multiplied by theoretical repeating unit molecular

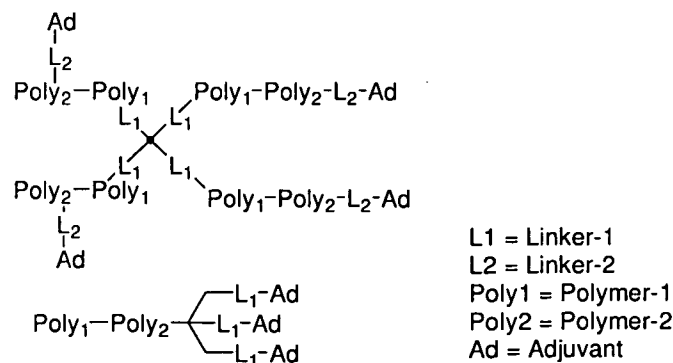
weight. Alternatively, in the case of a titratable (e.g., acid or base) end group polymer, a known weight concentration may be established and then titrated in the presence of an indicator dye with an appropriate neutralizing agent of known molar concentration to provide moles of end group per mass of polymer. Any of the weights of a polymer as provided herein can be a weight average molecular weight or a number average molecular weight.

C. INVENTIVE COMPOSITIONS

As generally described herein, the inventors have discovered that it is possible to couple multiple molecules of immunomodulatory agent to a polymer backbone, e.g., coupling two, three, four, five, six, seven, eight, nine, ten, or more moieties of immunomodulatory agent to the polymer. Such immunomodulatory agent moieties are, for example, coupled to the terminus (or end) of a polymer and/or to its backbone and/or are coupled to monomers and/or themselves are monomers used in the preparation of a polymer. The present invention, therefore, also provides synthetic nanocarriers comprising such polymers. Further provided are synthetic nanocarriers that further comprise other components coupled thereto, such as additional immunomodulatory agents, antigens, etc.

The immunomodulatory agent moieties can be coupled to or form the polymers by a variety of methods. For example, the immunomodulatory agent moieties (e.g., adjuvant moieties) can be coupled to the polymers at the end (or terminus) of a linear, branched or dendrimeric polymeric structure as shown below in Scheme 1 or can be coupled to a polymer along its backbone as shown below in Scheme 2.

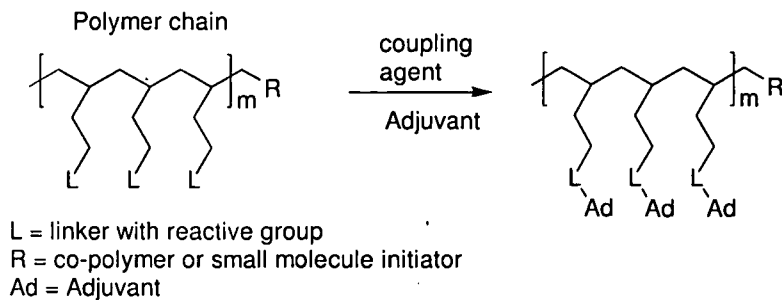
Scheme 1



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Scheme 2

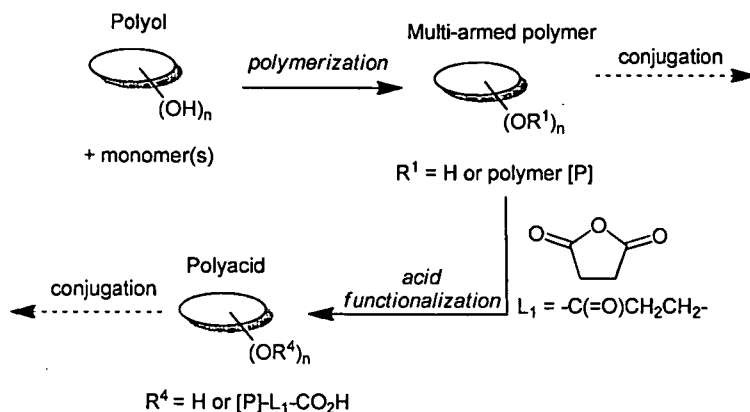
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More specifically, in certain embodiments, the polymer is a branched polymer prepared from the reaction of a polyol with one or more different types of monomers to provide a multi-armed polymer (see, e.g., Scheme 3). In the embodiments described in Scheme 3, the polyol, wherein n is an integer between 2 and 100, is reacted with lactide, glycolide, or caprolactam monomers, any of which may be functionalized with other groups, such as optionally substituted alkyl groups, to provide a multi-armed polymer. It is envisioned that not all of the hydroxyl groups of the polyol may react, or are desirable to react, under the polymerization conditions employed, thus, in certain embodiments, R^1 is selected from hydrogen and a polymer, wherein at least two R^1 groups comprise a polymer arm. The polymer arm of the multi-armed polymer may be further functionalized with other monomers, e.g., lactide, glycolide, or caprolactam monomers, to provide a block-co-polymer arm. The polymer arm may then be further functionalized with one or more groups useful for conjugation with an appropriately functionalized immunomodulatory agent moiety. In this way, a polymer is designed such that multiple immunomodulatory agent moieties may be installed along the branch points of the polymer.

For example, in Scheme 3, the polymer is treated with succinic anhydride to provide a polyacid, e.g., a polymer comprising two or more carboxylic acid groups. Scheme 3 depicts only one exemplary way of installing a carboxylic acid via a linking group L_1 , and many other ways are contemplated, e.g., wherein L_1 may be any group linking the polymer to one or more carboxylic acids. Exemplary L_1 groups include, but are not limited to, optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted carbocyclene, optionally substituted heterocyclene, optionally substituted arylene, and optionally substituted heteroarylene. In the case wherein the multi-armed polymer is treated with succinic anhydride, L_1 is a substituted alkylene group, *i.e.*, $-C(=O)CH_2CH_2-$, linking the polymer arm to a terminal carboxylic acid $-CO_2H$ to provide a polyacid.

Scheme 3

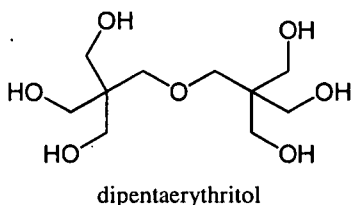
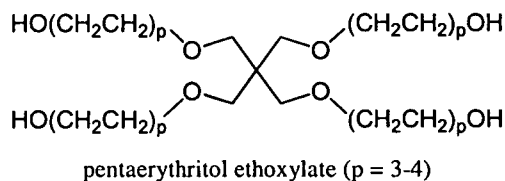
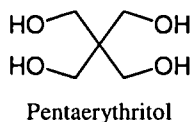


Monomer(s)	R ¹ polymer arm (R ³ = H)	R ⁴ acid functionalized arm
 lactide	polylactic acid [PLA] _m = R ² = -CH ₃ (PLA) or other group (functionalized PLA)	
 glycolide	polyglycolic acid [PGA] _m = 	
 glycolide + lactide lactide	poly(lactic-co-glycolic acid) [PGLA] _m = each R ² per unit m = -CH ₃ (PLA) or other group (functionalized PLA); or H (PGA)	
 caprolactone	polycaprolactone [PCL] _m = polycaprolactone (R ² = H) or functionalized polycaprolactone (at least one R ² is other group)	

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The polyacid may then be conjugated to an appropriately functionalized immunomodulatory agent moiety (e.g., comprising an amino group $-NH_2$), or the polyacid may be used as a handle to further functionalize the polymer arm prior to conjugation with an immunomodulatory agent moiety. Scheme 4 depicts these two possible situations. In each particular case, the polyacid is eventually conjugated via an amide linker to a group R5, wherein R5 comprises an immunomodulatory agent moiety, e.g., an adjuvant moiety such as a TLR agonist moiety.

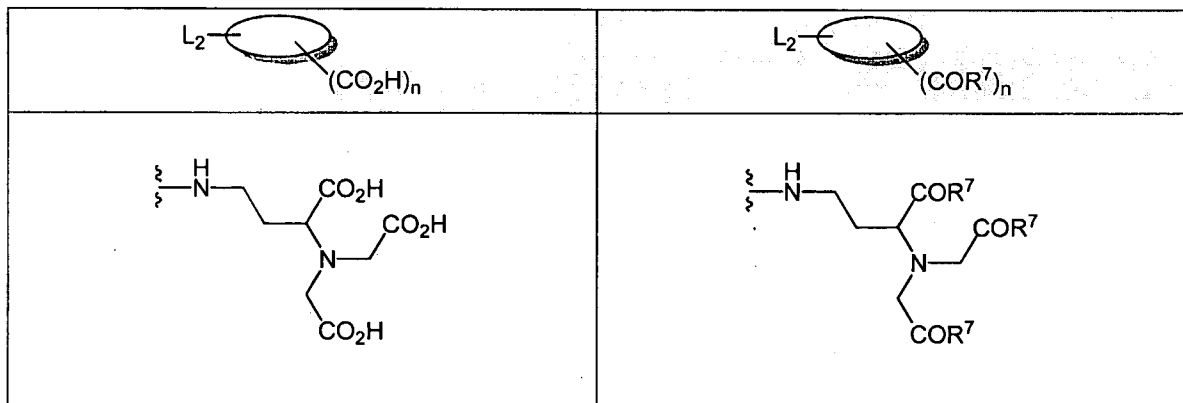
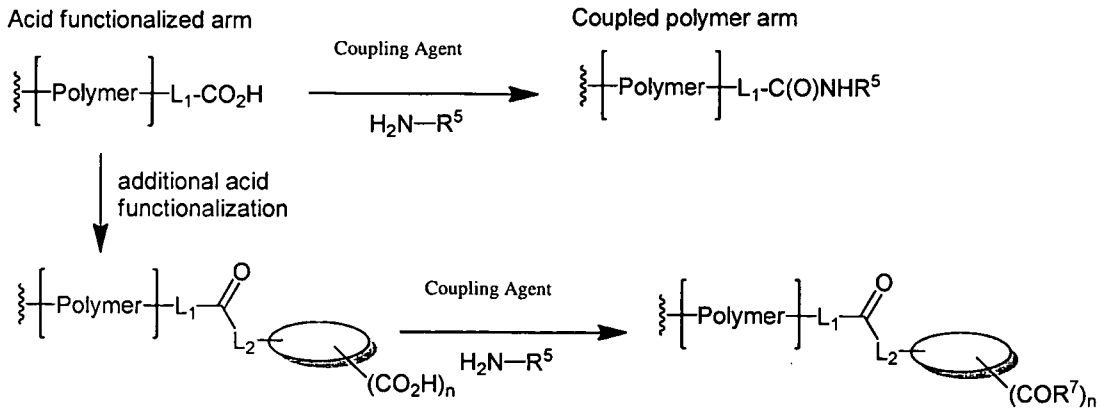
Exemplary polyols for use in the compositions and methods provided herein include the following:



Further exemplary polyols can be found in the literature (see, e.g., Chem Soc Rev 2011, 40, 1761-1776) and include the following: trimethylolpropane (3-arm), glycerol (3-arm), pentaerythritol (4-arm), erythritol (4-arm), xylitol (5-arm), di(trimethylolpropane) (4-arm), sorbitol (6-arm), inositol (6-arm) and tripentaerythritol (8-arm).

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Scheme 4



[Polymer] represents any polymer, e.g., PLA, PGA, PGLA, PCL, and block-co-polymers thereof.

5 $\text{R}^7 = -\text{OH}$ or $-\text{NHR}^5$, wherein at least two R^7 is $-\text{NHR}^5$

Other functionalized groups that can be used in the above schemes include any multiple acid groups, such as protected malic acid and citric acid with free hydroxyl groups. In addition, in some embodiment, R^5 may also be coupled to NH or OH as an alternative to NH_2 in the above scheme.

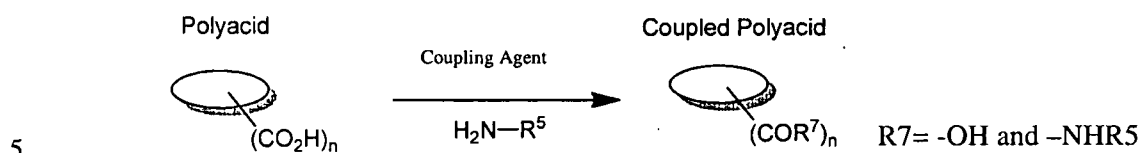
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Likewise, Scheme 5 depicts an embodiment where the polymer is a linear polymer functionalized along the polymeric backbone with carboxylic acid groups. In this particular embodiment, the polyacid may be conjugated to an appropriately functionalized immunomodulatory agent moiety (e.g., comprising an amino group $-\text{NH}_2$), or the polyacid may be used as a handle to further functionalize the polymer arm prior to conjugation with an immunomodulatory agent moiety. In each particular case, the polyacid is eventually conjugated via an amide linker to a group R^5 , wherein R^5 is an immunomodulatory agent moiety, e.g., a TLR agonist moiety of formulae (i), (ii), or (iii). In addition, in some

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embodiment, R^5 may also be coupled to NH or OH as an alternative to NH_2 in the below scheme.

Scheme 5



Acid functionalized polymer	Coupled polymer
Polyglutamic acid: $\left[\text{H}-\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})-\text{OH} \right]_m$	$\left[\text{H}-\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})-\text{R}^7 \right]_m$
Polymalic acid: $\left[\text{HO}-\text{C}(=\text{O})-\text{CH}_2-\text{CH}(\text{CO}_2\text{H})-\text{O} \right]_m$	$\left[\text{R}^7-\text{C}(=\text{O})-\text{CH}_2-\text{CH}(\text{COR}^7)-\text{O} \right]_m$

$R^7 = -OH \text{ and } -NHR^5$, wherein at least two R^7 is $-NHR^5$

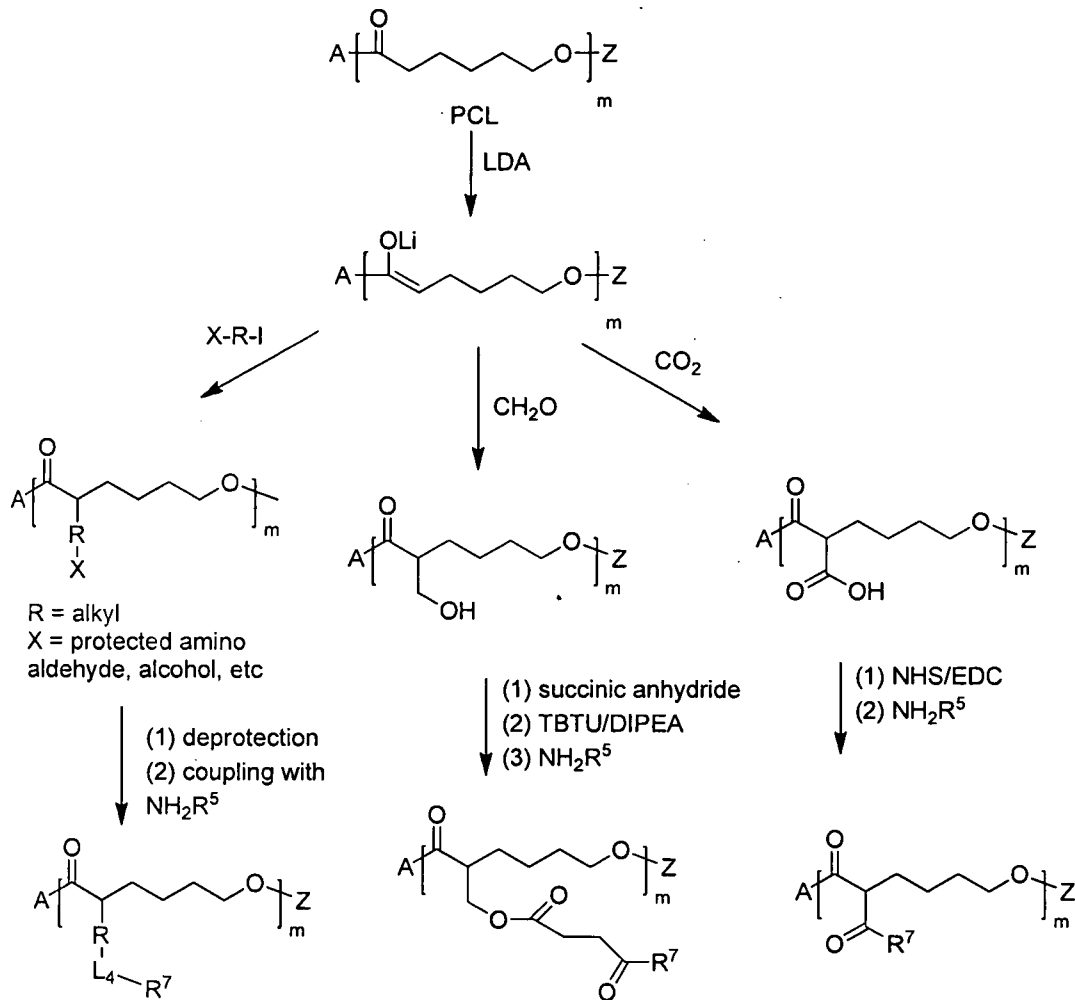
Other embodiments, such as depicted in Scheme 6, depict use of other functional groups useful in coupling the immunomodulatory agent moiety to the linear polymer backbone, e.g., as side chains. For example, in the embodiment depicted in Scheme 6, a polycaprolactone polymer is alkylated at the α -position via an enolate intermediate with various groups, e.g., $-CH_2OH$, $-CO_2H$, and/or other groups comprising nucleophilic or electrophilic moieties, which ultimately are used in the conjugation to an appropriately functionalized immunomodulatory agent moiety, e.g., NH_2R^5 , wherein R^5 is a group of the formulae (i), (ii), or (iii). In addition, in some embodiment, R^5 may also be coupled to NH or OH as an alternative to NH_2 in the above scheme.

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Scheme 6

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$\text{R}^7 = -\text{OH}$ or $-\text{NHR}^5$, wherein at least two R^7 is $-\text{NHR}^5$

- 5 In certain embodiments, the side chain chemical moiety is a carboxylic acid and the immunomodulatory agent moiety, comprising an amino group, is coupled to carboxylic acid group in the presence of a peptide coupling agent. In instances where the bond connecting the immunomodulatory agent moiety to the polymer is an amide bond, any coupling agent used for peptide synthesis may be used. These include, for example, EDC/NHS or
- 10 DCC/NHS; TBTU/base (DIPEA or Et3N), HBTU/base; PyBop/base, etc. These may also be used for making ester bond if excess base is present.

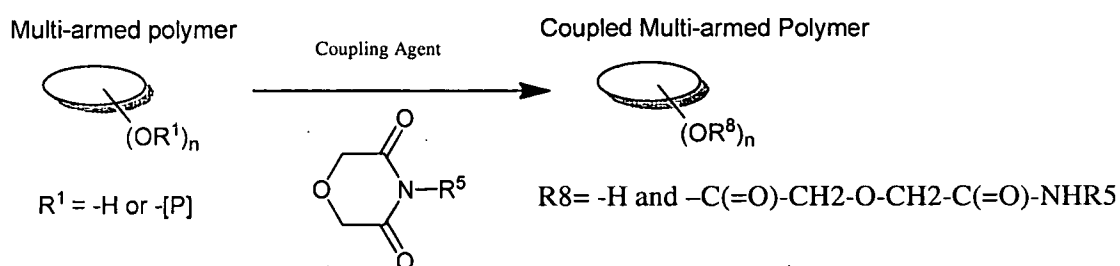
Scheme 7 depicts a particular embodiment wherein the polymer arm of the multi-armed polymer functionalized with a terminal hydroxyl group is conjugated to the

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immunomodulatory agent moiety via an amide linker through nucleophilic attack of a morpholine-3,5-dione present thereon. Other methods of coupling a terminal hydroxyl group present on the polymer to an electrophilic group present on the immunomodulatory agent moiety are further contemplated herein, e.g., nucleophilic attack of an α,β -unsaturated group or vinyl sulfone via Michael addition. Other electrophilic groups for functionalizing the immunomodulatory agent moiety contemplated herein include α -halo acetic acid ester or an amide derivative of the immunomodulatory agent moiety.

Scheme 7

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R^1 polymer arm	Coupled polymer arm

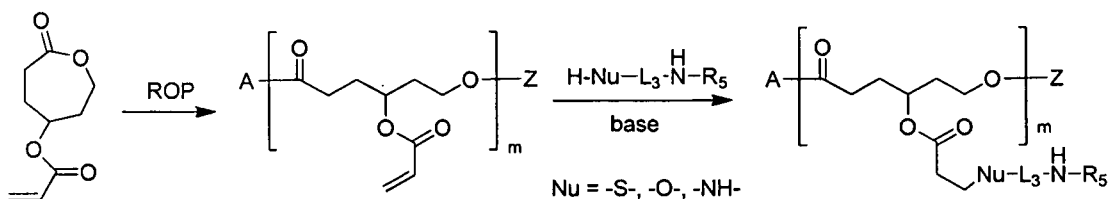
In certain embodiments, the polymer is prepared from a monomer which is functionalized with the immunomodulatory agent moiety, or is functionalized with a group suitable for conjugation to the immunomodulatory agent moiety after polymerization. Schemes 8 and 9 depict construction of a functionalized polycaprolactone and functionalized polylactide *via* such a monomer. For example, in the embodiment depicted in Scheme 8, a caprolactone monomer functionalized with an acrylate group is polymerized to provide a

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polycaprolactone polymer comprising an acrylate group, wherein A and Z are terminal groups, A is the initiator for the ring-opening polymerization (ROP) of the lactone, such as simple alcohol, MeO-PEG-OH; Z is Hydrogen in such case. The functionalized polycaprolactone polymer is then conjugated to the immunomodulatory agent via Michael addition.

As exemplified in Scheme 8, the immunomodulatory agent moiety may comprise a nucleophilic group, e.g., an -OH, -SH, or -NH₂ group, optionally tethered to the amino group, wherein L₃ is optionally substituted alkylene, optionally substituted alkenylene, optionally substituted alkynylene, optionally substituted carbocyclene, optionally substituted heterocyclene, optionally substituted arylene, or optionally substituted heteroarylene, to provide the conjugated product.

Scheme 8

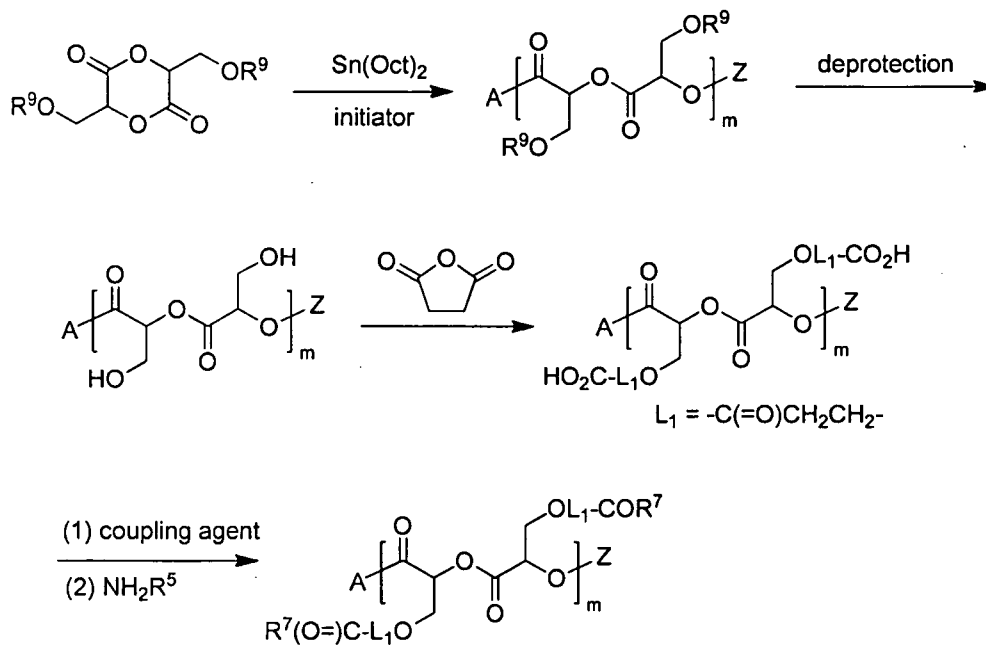


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In another embodiment, e.g., depicted in Scheme 9, the polylactide is constructed from a functionalized lactide monomer wherein R⁹ is an oxygen protecting group. Subsequent deprotection and treatment with succinic anhydride provides a polymer suitable for conjugation to an immunomodulatory agent moiety, e.g., NH₂R⁵, wherein R⁵ is a group of the formulae (i), (ii), or (iii). In some embodiment, R⁵ may also be coupled to NH or OH as an alternative to NH₂ as described above.

Scheme 9

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$\text{R}^7 = -\text{OH}$ and $-\text{NHR}^5$, wherein at least two R^7 is $-\text{NHR}^5$

5 Further examples for coupling polymers to immunomodulatory agent moieties are provided below in the **EXAMPLES**.

The synthetic nanocarriers provided herein comprise polymers to which immunomodulatory agent moieties are coupled. Such polymers can form part of or all of a synthetic nanocarrier. In some embodiments, such polymers form part of or all of a synthetic nanocarrier that is completely polymeric. In such embodiments, the polymeric synthetic nanocarriers may comprise other polymers to which immunomodulatory agents are not attached. These other polymers may be the same type or a different type of polymer as those that are coupled to the immunomodulatory agent moieties. In embodiments where the synthetic nanocarriers are not completely polymeric, the polymers that are coupled to the immunomodulatory agent moieties form part of the synthetic nanocarriers, and the synthetic nanocarriers are made up one or more different materials.

The polymers provided herein may be coupled to the immunomodulatory agent moieties and form at least part of the synthetic nanocarriers as provided or are not coupled to the immunomodulatory agent moieties but still form part of the synthetic nanocarriers. Such polymers preferably have a molecular weight of at least 2000 Da (as weight average or number average molecular weight). In some embodiments, the polymers have a molecular

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weight of at least 2500 Da, 3000 Da, 3500 Da, 4000 Da, 4500 Da, 5000 Da, 5500 Da, 6000 Da, 6500 Da, 7000 Da, 7500 Da, etc. In other embodiments, the polymers have a molecular weight of 2000 Da, 2500 Da, 3000 Da, 3500 Da, 4000 Da, 4500 Da, 5000 Da, 5500 Da, 6000 Da, 6500 Da, 7000 Da, 7500 Da, etc. The molecular weights are weight average molecular weights or number average molecular weights. In some embodiments, where the polymer comprises polyethylene glycol the molecular weight is a number average molecular weight. In other embodiments, where the polymer does not comprise polyethylene glycol the molecular weight is the weight average molecular weight. The polymers provided herein can comprise one or more types of polymers (e.g., a co-polymer and/or a block co-polymer).

10 Examples of polymers suitable for coupling to the immunomodulatory agent moieties or that are otherwise used to produce the synthetic nanocarriers of the present invention include, but are not limited to polyethylenes, polycarbonates (e.g. poly(1,3-dioxan-2one)), polyanhydrides (e.g. poly(sebacic anhydride)), polypropylfumerates, polyamides (e.g. polycaprolactam), polyacetals, polyethers, polyesters (e.g., polylactide, polyglycolide, poly(β-hydroxyalkanoate))), poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polyureas, polystyrenes, and polyamines, polylysine, polylysine-PEG copolymers, and poly(ethyleneimine), poly(ethylene imine)-PEG copolymers.

20 In some embodiments, polymers in accordance with the present invention include polymers which have been approved for use in humans by the U.S. Food and Drug Administration (FDA) under 21 C.F.R. § 177.2600, including but not limited to polyesters (e.g., polylactic acid, poly(lactic-co-glycolic acid), polycaprolactone, polyvalerolactone, poly(1,3-dioxan-2one)); polyanhydrides (e.g., poly(sebacic anhydride)); polyethers (e.g., polyethylene glycol); polyurethanes; polymethacrylates; polyacrylates; and polycyanoacrylates.

30 In some embodiments, polymers can be hydrophilic. For example, polymers may comprise anionic groups (e.g., phosphate group, sulphate group, carboxylate group); cationic groups (e.g., quaternary amine group); or polar groups (e.g., hydroxyl group, thiol group, amine group). In some embodiments, a synthetic nanocarrier comprising a hydrophilic polymeric matrix generates a hydrophilic environment within the synthetic nanocarrier. In some embodiments, polymers can be hydrophobic. In some embodiments, a synthetic nanocarrier comprising a hydrophobic polymeric matrix generates a hydrophobic

environment within the synthetic nanocarrier. Selection of the hydrophilicity or hydrophobicity of the polymer may have an impact on the nature of materials that are incorporated (e.g., coupled) within the synthetic nanocarrier.

5 In some embodiments, polymers may be modified with one or more moieties and/or functional groups. A variety of moieties or functional groups can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from polysaccharides (Papisov, 2001, ACS Symposium Series, 786:301). Certain embodiments may be made using the general teachings of US Patent No. 5543158 to Gref et al., or WO
10 publication WO2009/051837 by Von Andrian et al.

In some embodiments, polymers may be modified with a lipid or fatty acid group. In some embodiments, a fatty acid group may be one or more of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, or lignoceric acid. In some
15 embodiments, a fatty acid group may be one or more of palmitoleic, oleic, vaccenic, linoleic, alpha-linoleic, gamma-linoleic, arachidonic, gadoleic, arachidonic, eicosapentaenoic, docosahexaenoic, or erucic acid.

In some embodiments, polymers may be polyesters, including copolymers comprising lactic acid and glycolic acid units, such as poly(lactic acid-co-glycolic acid) and poly(lactide-co-glycolide), collectively referred to herein as "PLGA"; and homopolymers comprising
20 glycolic acid units, referred to herein as "PGA," and lactic acid units, such as poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, poly-L-lactide, poly-D-lactide, and poly-D,L-lactide, collectively referred to herein as "PLA." In some embodiments, exemplary polyesters include, for example, polyhydroxyacids; PEG copolymers and copolymers of lactide and glycolide (e.g., PLA-PEG copolymers, PGA-PEG copolymers, PLGA-PEG copolymers, and
25 derivatives thereof. In some embodiments, polyesters include, for example, poly(caprolactone), poly(caprolactone)-PEG copolymers, poly(L-lactide-co-L-lysine), poly(serine ester), poly(4-hydroxy-L-proline ester), poly[α -(4-aminobutyl)-L-glycolic acid], and derivatives thereof.

In some embodiments, a polymer may be PLGA. PLGA is a biocompatible and
30 biodegradable co-polymer of lactic acid and glycolic acid, and various forms of PLGA are characterized by the ratio of lactic acid:glycolic acid. Lactic acid can be L-lactic acid, D-lactic acid, or D,L-lactic acid. The degradation rate of PLGA can be adjusted by altering the lactic acid:glycolic acid ratio. In some embodiments, PLGA to be used in accordance with the

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present invention is characterized by a lactic acid:glycolic acid ratio of approximately 85:15, approximately 75:25, approximately 60:40, approximately 50:50, approximately 40:60, approximately 25:75, or approximately 15:85.

In some embodiments, polymers may be one or more acrylic polymers. In certain
5 embodiments, acrylic polymers include, for example, acrylic acid and methacrylic acid
copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl
methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid),
methacrylic acid alkylamide copolymer, poly(methyl methacrylate), poly(methacrylic acid
anhydride), methyl methacrylate, polymethacrylate, poly(methyl methacrylate) copolymer,
10 polyacrylamide, aminoalkyl methacrylate copolymer, glycidyl methacrylate copolymers,
polycyanoacrylates, and combinations comprising one or more of the foregoing polymers.
The acrylic polymer may comprise fully-polymerized copolymers of acrylic and methacrylic
acid esters with a low content of quaternary ammonium groups.

In some embodiments, polymers can be cationic polymers. In general, cationic
15 polymers are able to condense and/or protect negatively charged strands of nucleic acids (e.g.
DNA, or derivatives thereof). Amine-containing polymers such as poly(lysine) (Zauner et al.,
1998, *Adv. Drug Del. Rev.*, 30:97; and Kabanov et al., 1995, *Bioconjugate Chem.*, 6:7),
poly(ethylene imine) (PEI; Boussif et al., 1995, *Proc. Natl. Acad. Sci., USA*, 1995, 92:7297),
and poly(amidoamine) dendrimers (Kukowska-Latallo et al., 1996, *Proc. Natl. Acad. Sci.,*
20 USA, 93:4897; Tang et al., 1996, *Bioconjugate Chem.*, 7:703; and Haensler et al., 1993,
Bioconjugate Chem., 4:372) are positively-charged at physiological pH, form ion pairs with
nucleic acids, and mediate transfection in a variety of cell lines. In embodiments, the
synthetic nanocarriers may not comprise (or may exclude) cationic polymers.

In some embodiments, polymers can be degradable polyesters bearing cationic side
25 chains (Putnam et al., 1999, *Macromolecules*, 32:3658; Barrera et al., 1993, *J. Am. Chem.*
Soc., 115:11010; Kwon et al., 1989, *Macromolecules*, 22:3250; Lim et al., 1999, *J. Am.*
Chem. Soc., 121:5633; and Zhou et al., 1990, *Macromolecules*, 23:3399). Examples of these
polyesters include poly(L-lactide-co-L-lysine) (Barrera et al., 1993, *J. Am. Chem. Soc.*,
115:11010), poly(serine ester) (Zhou et al., 1990, *Macromolecules*, 23:3399), poly(4-
30 hydroxy-L-proline ester) (Putnam et al., 1999, *Macromolecules*, 32:3658; and Lim et al.,
1999, *J. Am. Chem. Soc.*, 121:5633), and poly(4-hydroxy-L-proline ester) (Putnam et al.,
1999, *Macromolecules*, 32:3658; and Lim et al., 1999, *J. Am. Chem. Soc.*, 121:5633).

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The properties of these and other polymers and methods for preparing them are well known in the art (see, for example, U.S. Patents 6,123,727; 5,804,178; 5,770,417; 5,736,372; 5,716,404; 6,095,148; 5,837,752; 5,902,599; 5,696,175; 5,514,378; 5,512,600; 5,399,665; 5,019,379; 5,010,167; 4,806,621; 4,638,045; and 4,946,929; Wang et al., 2001, J. Am. Chem. Soc., 123:9480; Lim et al., 2001, J. Am. Chem. Soc., 123:2460; Langer, 2000, Acc. Chem. Res., 33:94; Langer, 1999, J. Control. Release, 62:7; and Uhrich et al., 1999, Chem. Rev., 99:3181). More generally, a variety of methods for synthesizing certain suitable polymers are described in Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, Ed. by Goethals, Pergamon Press, 1980; Principles of Polymerization by Odian, John Wiley & Sons, Fourth Edition, 2004; Contemporary Polymer Chemistry by Allcock et al., Prentice-Hall, 1981; Deming et al., 1997, Nature, 390:386; and in U.S. Patents 6,506,577, 6,632,922, 6,686,446, and 6,818,732.

In some embodiments, polymers can be linear or branched polymers. In some embodiments, polymers can be dendrimers. In some embodiments, polymers can be substantially cross-linked to one another. In some embodiments, polymers can be substantially free of cross-links. In some embodiments, polymers can be used in accordance with the present invention without undergoing a cross-linking step. It is further to be understood that synthetic nanocarriers may comprise block copolymers, graft copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Those skilled in the art will recognize that the polymers listed herein represent an exemplary, not comprehensive, list of polymers that can be of use in accordance with the present invention.

In some embodiments, the polymers (coupled and/or not coupled to immunomodulatory agent moieties) can form a polymeric matrix. A wide variety of polymers and methods for forming polymeric matrices therefrom are known conventionally. In general, a polymeric matrix comprises one or more polymers. Polymers may be natural or unnatural (synthetic) polymers. Polymers may be homopolymers or copolymers comprising two or more monomers. In terms of sequence, copolymers may be random, block, or comprise a combination of random and block sequences. Typically, polymers in accordance with the present invention are organic polymers.

The polymers coupled to immunomodulatory agents can be combined with other materials to make up the synthetic nanocarriers. Accordingly, a wide variety of synthetic nanocarriers can be produced according to the invention. In some embodiments, synthetic nanocarriers are spheres or spheroids. In some embodiments, synthetic nanocarriers are flat

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or plate-shaped. In some embodiments, synthetic nanocarriers are cubes or cubic. In some embodiments, synthetic nanocarriers are ovals or ellipses. In some embodiments, synthetic nanocarriers are cylinders, cones, or pyramids.

In some embodiments, it is desirable to use a population of synthetic nanocarriers that is relatively uniform in terms of size, shape, and/or composition so that each synthetic nanocarrier has similar properties. For example, at least 80%, at least 90%, or at least 95% of the synthetic nanocarriers, based on the total number of synthetic nanocarriers, may have a minimum dimension or maximum dimension that falls within 5%, 10%, or 20% of the average diameter or average dimension of the synthetic nanocarriers. In some embodiments, a population of synthetic nanocarriers may be heterogeneous with respect to size, shape, and/or composition.

Synthetic nanocarriers can be solid or hollow and can comprise one or more layers. In some embodiments, each layer has a unique composition and unique properties relative to the other layer(s). To give but one example, synthetic nanocarriers may have a core/shell structure, wherein the core is one layer (e.g. a polymeric core) and the shell is a second layer (e.g. a lipid bilayer or monolayer). Synthetic nanocarriers may comprise a plurality of different layers.

In some embodiments, the polymers coupled to immunomodulatory agents can be combined with one or more lipids. In some embodiments, a synthetic nanocarrier may, therefore, comprise a liposome. In some embodiments, a synthetic nanocarrier may comprise a lipid bilayer. In some embodiments, a synthetic nanocarrier may comprise a lipid monolayer. In some embodiments, a synthetic nanocarrier may comprise a micelle. In some embodiments, a synthetic nanocarrier may comprise a non-polymeric core (e.g., metal particle, quantum dot, ceramic particle, bone particle, viral particle, proteins, nucleic acids, carbohydrates, etc.) surrounded by a polymeric layer.

In some embodiments, the polymers coupled to immunomodulatory agents can be combined with metal particles, quantum dots, ceramic particles, etc. In some embodiments, the polymers coupled to immunomodulatory agents can be combined with non-polymeric synthetic nanocarrier aggregates of non-polymeric components, such as an aggregate of metal atoms (e.g., gold atoms).

In some embodiments, the synthetic nanocarriers comprising the polymers coupled to immunomodulatory agents may optionally comprise one or more amphiphilic entities. In some embodiments, an amphiphilic entity can promote the production of synthetic

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nanocarriers with increased stability, improved uniformity, or increased viscosity. In some embodiments, amphiphilic entities can be associated with the interior surface of a lipid membrane (e.g., lipid bilayer, lipid monolayer, etc.). Many amphiphilic entities known in the art are suitable for use in making synthetic nanocarriers in accordance with the present invention. Such amphiphilic entities include, but are not limited to, phosphoglycerides; phosphatidylcholines; dipalmitoyl phosphatidylcholine (DPPC); dioleoylphosphatidyl ethanolamine (DOPE); dioleoyloxypropyltriethylammonium (DOTMA); dioleoylphosphatidylcholine; cholesterol; cholesterol ester; diacylglycerol; diacylglycerolsuccinate; diphosphatidyl glycerol (DPPG); hexanedecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; fatty acids; fatty acid monoglycerides; fatty acid diglycerides; fatty acid amides; sorbitan trioleate (Span®85) glycocholate; sorbitan monolaurate (Span®20); polysorbate 20 (Tween®20); polysorbate 60 (Tween®60); polysorbate 65 (Tween®65); polysorbate 80 (Tween®80); polysorbate 85 (Tween®85); polyoxyethylene monostearate; surfactin; a poloxomer; a sorbitan fatty acid ester such as sorbitan trioleate; lecithin; lysolecithin; phosphatidylserine; phosphatidylinositol; sphingomyelin; phosphatidylethanolamine (cephalin); cardiolipin; phosphatidic acid; cerebrosides; dicetylphosphate; dipalmitoylphosphatidylglycerol; stearylamine; dodecylamine; hexadecyl-amine; acetyl palmitate; glycerol ricinoleate; hexadecyl stearate; isopropyl myristate; tyloxapol; poly(ethylene glycol)5000-phosphatidylethanolamine; poly(ethylene glycol)400-monostearate; phospholipids; synthetic and/or natural detergents having high surfactant properties; deoxycholates; cyclodextrins; chaotropic salts; ion pairing agents; and combinations thereof. An amphiphilic entity component may be a mixture of different amphiphilic entities. Those skilled in the art will recognize that this is an exemplary, not comprehensive, list of substances with surfactant activity. Any amphiphilic entity may be used in the production of synthetic nanocarriers to be used in accordance with the present invention.

In some embodiments, the synthetic nanocarriers comprising the polymers coupled to immunomodulatory agents may optionally comprise one or more carbohydrates.

Carbohydrates may be natural or synthetic. A carbohydrate may be a derivatized natural carbohydrate. In certain embodiments, a carbohydrate comprises monosaccharide or disaccharide, including but not limited to glucose, fructose, galactose, ribose, lactose, sucrose, maltose, trehalose, cellbiose, mannose, xylose, arabinose, glucuronic acid,

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galacturonic acid, mannuronic acid, glucosamine, galatosamine, and neuramic acid. In certain embodiments, a carbohydrate is a polysaccharide, including but not limited to pullulan, cellulose, microcrystalline cellulose, hydroxypropyl methylcellulose (HPMC), hydroxycellulose (HC), methylcellulose (MC), dextran, cyclodextran, glycogen, 5 hydroxyethylstarch, carageenan, glycon, amylose, chitosan, N,O-carboxymethylchitosan, algin and alginic acid, starch, chitin, inulin, konjac, glucommannan, pustulan, heparin, hyaluronic acid, curdlan, and xanthan. In embodiments, the synthetic nanocarriers do not comprise (or specifically exclude) carbohydrates, such as a polysaccharide. In certain 10 embodiments, the carbohydrate may comprise a carbohydrate derivative such as a sugar alcohol, including but not limited to mannitol, sorbitol, xylitol, erythritol, maltitol, and lactitol.

Other elements, such as one or more antigens, can also be coupled to the synthetic nanocarriers, such as to polymers (that are coupled and/or not coupled to immunomodulatory agents) provided herein. Accordingly, the elements can be covalently associated with, for 15 example, a polymeric matrix. In some embodiments, covalent association is mediated by a linker. In some embodiments, a component can be noncovalently associated with a polymeric matrix. For example, in some embodiments, a component can be encapsulated within, surrounded by, and/or dispersed throughout a polymeric matrix. Alternatively or 20 additionally, a component can be associated with a polymeric matrix by hydrophobic interactions, charge interactions, van der Waals forces, etc.

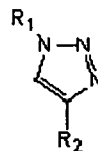
In certain embodiments, the coupling can be a covalent linker. In embodiments, components according to the invention can be covalently coupled to the external surface via a 1,2,3-triazole linker formed by the 1,3-dipolar cycloaddition reaction of azido groups on the surface of the nanocarrier with a component containing an alkyne group or by the 1,3-dipolar 25 cycloaddition reaction of alkynes on the surface of the nanocarrier with components containing an azido group. Such cycloaddition reactions are preferably performed in the presence of a Cu(I) catalyst along with a suitable Cu(I)-ligand and a reducing agent to reduce Cu(II) compound to catalytic active Cu(I) compound. This Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) can also be referred as the click reaction.

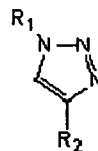
30 Additionally, the covalent coupling may comprise a covalent linker that comprises an amide linker, a disulfide linker, a thioether linker, a hydrazone linker, a hydrazide linker, an imine or oxime linker, an urea or thiourea linker, an amidine linker, an amine linker, and a sulfonamide linker.

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An amide linker is formed via an amide bond between an amine on one component with the carboxylic acid group of a second component such as the nanocarrier. The amide bond in the linker can be made using any of the conventional amide bond forming reactions with suitably protected amino acids and activated carboxylic acid such N-hydroxysuccinimide-activated ester.

A disulfide linker is made via the formation of a disulfide (S-S) bond between two sulfur atoms of the form, for instance, of R1-S-S-R2. A disulfide bond can be formed by thiol exchange of a component containing thiol/mercaptan group(-SH) with another activated thiol group on a polymer or nanocarrier or a nanocarrier containing thiol/mercaptan groups with a component containing activated thiol group.



A triazole linker, specifically a 1,2,3-triazole of the form , wherein R1 and R2 may be any chemical entities, is made by the 1,3-dipolar cycloaddition reaction of an azide attached to a first component such as the nanocarrier with a terminal alkyne attached to a second component. The 1,3-dipolar cycloaddition reaction is performed with or without a catalyst, preferably with Cu(I)-catalyst, which links the two components through a 1,2,3-triazole function. This chemistry is described in detail by Sharpless et al., *Angew. Chem. Int. Ed.* 41(14), 2596, (2002) and Meldal, et al, *Chem. Rev.*, 2008, 108(8), 2952-3015 and is often referred to as a “click” reaction or CuAAC.

In embodiments, a polymer containing an azide or alkyne group, terminal to the polymer chain is prepared. This polymer is then used to prepare a synthetic nanocarrier in such a manner that a plurality of the alkyne or azide groups are positioned on the surface of that nanocarrier. Alternatively, the synthetic nanocarrier can be prepared by another route, and subsequently functionalized with alkyne or azide groups. The component is prepared with the presence of either an alkyne (if the polymer contains an azide) or an azide (if the polymer contains an alkyne) group. The component is then allowed to react with the nanocarrier via the 1,3-dipolar cycloaddition reaction with or without a catalyst which covalently couples the component to the particle through the 1,4-disubstituted 1,2,3-triazole linker.

A thioether linker is made by the formation of a sulfur-carbon (thioether) bond in the form, for instance, of R1-S-R2. Thioether can be made by either alkylation of a

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thiol/mercaptan (-SH) group on one component with an alkylating group such as halide or epoxide on a second component such as the nanocarrier. Thioether linkers can also be formed by Michael addition of a thiol/mercaptan group on one component to an electron-deficient alkene group on a second component such as a polymer containing a maleimide group or vinyl sulfone group as the Michael acceptor. In another way, thioether linkers can be prepared by the radical thiol-ene reaction of a thiol/mercaptan group on one component with an alkene group on a second component such as a polymer or nanocarrier.

A hydrazone linker is made by the reaction of a hydrazide group on one component with an aldehyde/ketone group on the second component such as the nanocarrier.

A hydrazide linker is formed by the reaction of a hydrazine group on one component with a carboxylic acid group on the second component such as the nanocarrier. Such reaction is generally performed using chemistry similar to the formation of amide bond where the carboxylic acid is activated with an activating reagent.

An imine or oxime linker is formed by the reaction of an amine or N-alkoxyamine (or aminoxy) group on one component an aldehyde or ketone group on the second component such as the nanocarrier.

An urea or thiourea linker is prepared by the reaction of an amine group on one component with an isocyanate or thioisocyanate group on the second component such as the nanocarrier.

An amidine linker is prepared by the reaction of an amine group on one component with an imidoester group on the second component such as the nanocarrier.

An amine linker is made by the alkylation reaction of an amine group on one component with an alkylating group such as halide, epoxide, or sulfonate ester group on the second component such as the nanocarrier. Alternatively, an amine linker can also be made by reductive amination of an amine group on one component with an aldehyde or ketone group on the second component such as the nanocarrier with a suitable reducing reagent such as sodium cyanoborohydride or sodium triacetoxyborohydride.

A sulfonamide linker is made by the reaction of an amine group on one component with a sulfonyl halide (such as sulfonyl chloride) group on the second component such as the nanocarrier.

A sulfone linker is made by Michael addition of a nucleophile to a vinyl sulfone. Either the vinyl sulfone or the nucleophile may be on the surface of the nanocarrier or attached to the component.

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The components can also be conjugated to the nanocarrier via non-covalent conjugation methods. For examples, a negative charged component can be conjugated to a positive charged nanocarrier through electrostatic adsorption. A component containing a metal ligand can also be conjugated to a nanocarrier containing a metal complex via a metal-ligand complex.

In embodiments, the component can be attached to a polymer, for example polylactic acid-block-polyethylene glycol, prior to the assembly of the synthetic nanocarrier or the synthetic nanocarrier can be formed with reactive or activatable groups on its surface. In the latter case, the component may be prepared with a group which is compatible with the attachment chemistry that is presented by the synthetic nanocarriers' surface. In other embodiments, a component can be attached to VLPs or liposomes using a suitable linker. A linker is a compound or reagent that capable of coupling two molecules together. In an embodiment, the linker can be a homobifunctional or heterobifunctional reagent as described in Hermanson 2008. For example, an VLP or liposome synthetic nanocarrier containing a carboxylic group on the surface can be treated with a homobifunctional linker, adipic dihydrazide (ADH), in the presence of EDC to form the corresponding synthetic nanocarrier with the ADH linker. The resulting ADH linked synthetic nanocarrier is then conjugated with a peptide containing an acid group via the other end of the ADH linker on NC to produce the corresponding VLP or liposome peptide conjugate.

For detailed descriptions of available conjugation methods, see Hermanson G T "Bioconjugate Techniques", 2nd Edition Published by Academic Press, Inc., 2008. In addition to covalent attachment the component can be coupled by adsorption to a pre-formed synthetic nanocarrier or it can be coupled by encapsulation during the formation of the synthetic nanocarrier.

In some embodiments, a component, such as an antigen or immunomodulatory agent, may be isolated. Isolated refers to the element being separated from its native environment and present in sufficient quantities to permit its identification or use. This means, for example, the element may be (i) selectively produced by expression cloning or (ii) purified as by chromatography or electrophoresis. Isolated elements may be, but need not be, substantially pure. Because an isolated element may be admixed with a pharmaceutically acceptable excipient in a pharmaceutical preparation, the element may comprise only a small percentage by weight of the preparation. The element is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e.,

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isolated from other lipids or proteins. Any of the elements provided herein may be isolated. Any of the antigens provided herein can be included in the compositions in isolated form.

D. METHODS OF MAKING AND USING THE INVENTIVE COMPOSITIONS AND
5 RELATED METHODS

Synthetic nanocarriers may be prepared using a wide variety of methods known in the art. For example, synthetic nanocarriers can be formed by methods as nanoprecipitation, flow focusing using fluidic channels, spray drying, single and double emulsion solvent evaporation, solvent extraction, phase separation, milling, microemulsion procedures,
10 microfabrication, nanofabrication, sacrificial layers, simple and complex coacervation, and other methods well known to those of ordinary skill in the art. Alternatively or additionally, aqueous and organic solvent syntheses for monodisperse semiconductor, conductive, magnetic, organic, and other nanomaterials have been described (Pellegrino et al., 2005, Small, 1:48; Murray et al., 2000, Ann. Rev. Mat. Sci., 30:545; and Trindade et al., 2001,
15 Chem. Mat., 13:3843). Additional methods have been described in the literature (see, e.g., Doubrow, Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992; Mathiowitz et al., 1987, J. Control. Release, 5:13; Mathiowitz et al., 1987, Reactive Polymers, 6:275; and Mathiowitz et al., 1988, J. Appl. Polymer Sci., 35:755; US Patents 5578325 and 6007845; P. Paolicelli et al., "Surface-modified PLGA-based
20 Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010)).

Various materials may be encapsulated into synthetic nanocarriers as desirable using a variety of methods including but not limited to C. Astete et al., "Synthesis and characterization of PLGA nanoparticles" J. Biomater. Sci. Polymer Edn, Vol. 17, No. 3, pp.
25 247-289 (2006); K. Avgoustakis "Pegylated Poly(Lactide) and Poly(Lactide-Co-Glycolide) Nanoparticles: Preparation, Properties and Possible Applications in Drug Delivery" Current Drug Delivery 1:321-333 (2004); C. Reis et al., "Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles" Nanomedicine 2:8-21 (2006); P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate
30 and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010). Other methods suitable for encapsulating materials into synthetic nanocarriers may be used, including without limitation methods disclosed in United States Patent 6,632,671 to Unger October 14, 2003.

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In certain embodiments, synthetic nanocarriers are prepared by a nanoprecipitation process or spray drying. Conditions used in preparing synthetic nanocarriers may be altered to yield particles of a desired size or property (e.g., hydrophobicity, hydrophilicity, external morphology, "stickiness," shape, etc.). The method of preparing the synthetic nanocarriers and the conditions (e.g., solvent, temperature, concentration, air flow rate, etc.) used may depend on the materials to be coupled to the synthetic nanocarriers and/or the composition of the polymer matrix.

If particles prepared by any of the above methods have a size range outside of the desired range, particles can be sized, for example, using a sieve.

Elements (components) of the synthetic nanocarriers may be coupled to the overall synthetic nanocarrier, e.g., by one or more covalent bonds, or may be coupled by means of one or more linkers. Additional methods of functionalizing synthetic nanocarriers may be adapted from Published US Patent Application 2006/0002852 to Saltzman et al., Published US Patent Application 2009/0028910 to DeSimone et al., or Published International Patent Application WO/2008/127532 A1 to Murthy et al.

Alternatively or additionally, synthetic nanocarriers can be coupled to other elements directly or indirectly via non-covalent interactions. In non-covalent embodiments, the non-covalent coupling is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, π - π stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. Such couplings may be arranged to be on an external surface or an internal surface of a synthetic nanocarrier. In embodiments, encapsulation and/or absorption is a form of coupling.

In embodiments, the synthetic nanocarriers can be combined with other immunomodulatory agents or moieties thereof and/or one or more antigens by admixing in the same vehicle or delivery system. Such immunomodulatory agents or moieties thereof may include adjuvants that include, but are not limited to mineral salts, such as alum, alum combined with monophosphoryl lipid (MPL) A of Enterobacteria, such as Escherichia coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri or specifically with MPL® (AS04), MPL A of above-mentioned bacteria separately, saponins, such as QS-21, Quil-A, ISCOMs, ISCOMATRIX™, emulsions such as MF59™, Montanide® ISA 51 and ISA 720, AS02 (QS21+squalene+ MPL®), liposomes and liposomal formulations such

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as AS01, synthesized or specifically prepared microparticles and microcarriers such as bacteria-derived outer membrane vesicles (OMV) of *N. gonorrhoeae*, *Chlamydia trachomatis* and others, or chitosan particles, depot-forming agents, such as Pluronic® block co-polymers, specifically modified or prepared peptides, such as muramyl dipeptide, aminoalkyl
5 glucosaminide 4-phosphates, such as RC529, or proteins, such as bacterial toxoids or toxin fragments. The doses of such other immunomodulatory agents or moieies thereof can be determined using conventional dose ranging studies.

In embodiments, the synthetic nanocarriers can be combined with an antigen different, similar or identical to any coupled to a nanocarrier (with or without
10 immunomodulatory agent, utilizing or not utilizing another delivery vehicle) administered separately at a different time-point and/or at a different body location and/or by a different immunization route or with another antigen and/or immunomodulatory agent-carrying synthetic nanocarrier administered separately at a different time-point and/or at a different body location and/or by a different immunization route.

15 Compositions for use in the methods according to the invention comprise synthetic nanocarriers in combination with pharmaceutically acceptable excipients, such as preservatives, buffers, saline, or phosphate buffered saline. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. In an embodiment, synthetic nanocarriers are suspended in sterile saline
20 solution for injection together with a preservative.

In embodiments, when preparing synthetic nanocarriers as carriers for use in vaccines, methods for coupling to the synthetic nanocarriers may be useful. If the component is a small molecule it may be of advantage to attach the component to a polymer prior to the assembly of the synthetic nanocarriers. In embodiments, it may also be an advantage to prepare the
25 synthetic nanocarriers with surface groups that are used to couple the component to the synthetic nanocarrier through the use of these surface groups rather than attaching the component to a polymer and then using this polymer conjugate in the construction of synthetic nanocarriers.

Populations of synthetic nanocarriers may be combined to form pharmaceutical
30 dosage forms according to the present invention using traditional pharmaceutical mixing methods. These include liquid-liquid mixing in which two or more suspensions, each containing one or more subsets of nanocarriers, are directly combined or are brought together via one or more vessels containing diluent. As synthetic nanocarriers may also be produced

or stored in a powder form, dry powder-powder mixing could be performed as could the re-suspension of two or more powders in a common media. Depending on the properties of the nanocarriers and their interaction potentials, there may be advantages conferred to one or another route of mixing.

5 Typical compositions that comprise synthetic nanocarriers may comprise inorganic or organic buffers (e.g., sodium or potassium salts of phosphate, carbonate, acetate, or citrate) and pH adjustment agents (e.g., hydrochloric acid, sodium or potassium hydroxide, salts of citrate or acetate, amino acids and their salts) antioxidants (e.g., ascorbic acid, alpha-tocopherol), surfactants (e.g., polysorbate 20, polysorbate 80, polyoxyethylene9-10 nonyl
10 phenol, sodium desoxycholate), solution and/or cryo/lyo stabilizers (e.g., sucrose, lactose, mannitol, trehalose), osmotic adjustment agents (e.g., salts or sugars), antibacterial agents (e.g., benzoic acid, phenol, gentamicin), antifoaming agents (e.g., polydimethylsiloxane), preservatives (e.g., thimerosal, 2-phenoxyethanol, EDTA), polymeric stabilizers and viscosity-adjustment agents (e.g., polyvinylpyrrolidone, poloxamer 488,
15 carboxymethylcellulose) and co-solvents (e.g., glycerol, polyethylene glycol, ethanol).

 Compositions according to the invention comprise synthetic nanocarriers in combination with pharmaceutically acceptable excipients. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. Techniques suitable for use in practicing the present invention may be
20 found in Handbook of Industrial Mixing: Science and Practice, Edited by Edward L. Paul, Victor A. Atiemo-Obeng, and Suzanne M. Kresta, 2004 John Wiley & Sons, Inc.; and Pharmaceutics: The Science of Dosage Form Design, 2nd Ed. Edited by M. E. Auten, 2001, Churchill Livingstone. In an embodiment, synthetic nanocarriers are suspended in sterile saline solution for injection together with a preservative.

25 It is to be understood that the compositions of synthetic nanocarriers can be made in any suitable manner, and the invention is in no way limited to the use of compositions that can be produced using the methods described herein. Selection of an appropriate method may require attention to the properties of the particular moieties being associated.

 In some embodiments, synthetic nanocarriers are manufactured under sterile
30 conditions or are terminally sterilized. This can ensure that resulting compositions are sterile and non-infectious, thus improving safety when compared to non-sterile compositions. This provides a valuable safety measure, especially when subjects receiving synthetic nanocarriers have immune defects, are suffering from infection, and/or are susceptible to infection. In

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some embodiments, synthetic nanocarriers may be lyophilized and stored in suspension or as lyophilized powder depending on the formulation strategy for extended periods without losing activity.

The compositions of the invention can be administered by a variety of routes, including or not limited to subcutaneous, intranasal, oral, intravenous, intraperitoneal, intramuscular, transmucosal, transdermal, sublingual, rectal, ophthalmic, pulmonary, intradermal, transdermal, transcutaneous or intradermal or by a combination of these routes. Routes of administration also include administration by inhalation or pulmonary aerosol. Techniques for preparing aerosol delivery systems are well known to those of skill in the art (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp. 1694-1712; incorporated by reference).

Doses of dosage forms contain varying amounts of populations of synthetic nanocarriers and varying amounts of components, such as immunomodulatory agents and/or antigens, according to the invention. The amount of synthetic nanocarriers, immunomodulatory agents and/or antigens present in the dosage forms can be varied according to the nature of the immunomodulatory agents and/or antigens, the therapeutic benefit to be accomplished, and other such parameters. In embodiments, dose ranging studies can be conducted to establish optimal therapeutic amount of the population of synthetic nanocarriers and the amount of immunomodulatory agents and/or antigens to be present in the dosage form. In embodiments, the synthetic nanocarriers and the immunomodulatory agents and/or antigens are present in the dosage form in an amount effective to generate an immune response to the antigens upon administration to a subject. It may be possible to determine amounts of the immunomodulatory agents and/or antigens effective to generate an immune response using conventional dose ranging studies and techniques in subjects.

Dosage forms may be administered at a variety of frequencies. In a preferred embodiment, at least one administration of the dosage form is sufficient to generate a pharmacologically relevant response. In more preferred embodiment, at least two administrations, at least three administrations, or at least four administrations, of the dosage form are utilized to ensure a pharmacologically relevant response.

The compositions and methods described herein can be used to induce, enhance, suppress, modulate, direct, or redirect an immune response. The compositions and methods described herein can be used in the diagnosis, prophylaxis and/or treatment of conditions such as cancers, infectious diseases, metabolic diseases, degenerative diseases, non-

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autoimmune diseases or other disorders and/or conditions. The compositions and methods described herein can also be used for the prophylaxis or treatment of an addiction, such as an addiction to an illegal drug, an over-the-counter drug, a prescription drug. In some embodiments, the addiction is to cocaine, heroin, marijuana, methamphetamines, nicotine or a narcotic. The compositions and methods described herein can also be used for the prophylaxis and/or treatment of a condition resulting from the exposure to a toxin, hazardous substance, environmental toxin, or other harmful agent.

Examples of infectious disease include, but are not limited to, viral infectious diseases, such as AIDS, Chickenpox (Varicella), Common cold, Cytomegalovirus Infection, Colorado tick fever, Dengue fever, Ebola hemorrhagic fever, Hand, foot and mouth disease, Hepatitis, Herpes simplex, Herpes zoster, HPV, Influenza (Flu), Lassa fever, Measles, Marburg hemorrhagic fever, Infectious mononucleosis, Mumps, Norovirus, Poliomyelitis, Progressive multifocal leukoencephalopathy, Rabies, Rubella, SARS, Smallpox (Variola), Viral encephalitis, Viral gastroenteritis, Viral meningitis, Viral pneumonia, West Nile disease and Yellow fever; bacterial infectious diseases, such as Anthrax, Bacterial Meningitis, Botulism, Brucellosis, Campylobacteriosis, Cat Scratch Disease, Cholera, Diphtheria, Epidemic Typhus, Gonorrhea, Impetigo, Legionellosis, Leprosy (Hansen's Disease), Leptospirosis, Listeriosis, Lyme disease, Melioidosis, Rheumatic Fever, MRSA infection, Nocardiosis, Pertussis (Whooping Cough), Plague, Pneumococcal pneumonia, Psittacosis, Q fever, Rocky Mountain Spotted Fever (RMSF), Salmonellosis, Scarlet Fever, Shigellosis, Syphilis, Tetanus, Trachoma, Tuberculosis, Tularemia, Typhoid Fever, Typhus and Urinary Tract Infections; parasitic infectious diseases, such as African trypanosomiasis, Amebiasis, Ascariasis, Babesiosis, Chagas Disease, Clonorchiasis, Cryptosporidiosis, Cysticercosis, Diphyllbothriasis, Dracunculiasis, Echinococcosis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Free-living amebic infection, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Kala-azar, Leishmaniasis, Malaria, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Pinworm Infection, Scabies, Schistosomiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinellosis, Trichinosis, Trichuriasis, Trichomoniasis and Trypanosomiasis; fungal infectious disease, such as Aspergillosis, Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Tinea pedis (Athlete's Foot) and Tinea cruris; prion infectious diseases, such as Alpers' disease, Fatal Familial Insomnia, Gerstmann-Sträussler-Scheinker syndrome, Kuru and Variant Creutzfeldt-Jakob disease.

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Examples of cancers include, but are not limited to breast cancer; biliary tract cancer; bladder cancer; brain cancer including glioblastomas and medulloblastomas; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia, e.g., B
5 Cell CLL; T-cell acute lymphoblastic leukemia/lymphoma; hairy cell leukemia; chronic myelogenous leukemia, multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia/lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas including Hodgkin's disease and lymphocytic
10 lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Merkel cell carcinoma, Kaposi's sarcoma, basal cell carcinoma, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma
15 (teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor.

Examples of metabolic diseases include, but are not limited to, disorders of carbohydrate metabolism, amino acid metabolism, organic acid metabolism, fatty acid
20 oxidation and mitochondrial metabolism, porphyrin metabolism, purine or pyrimidine metabolism, steroid metabolism, lysosomal mitochondrial function, peroxisomal function, lysosomal storage, urea cycle disorders (e.g., N-acetyl glutamate synthetase deficiency, carbamylphosphate synthase deficiency, ornithine carbamyl transferase deficiency, arginosuccinic aciduria, citrullinaemia, arginase deficiency), amino acid disorders (e.g., Non-
25 ketotic hyperglycinaemia, tyrosinaemia (Type I), Maple syrup urine disease), organic acidemias (e.g. isovaleric acidemia, methylmalonic acidemia, propionic acidemia, glutaric aciduria type I, glutaric acidemia type I & II), mitochondrial disorders (e.g., carboxylase defects, mitochondrial myopathies, lactic acidosis (pyruvate dehydrogenase complex defects), congenital lactic acidosis, mitochondrial respiratory chain defects, cystinosis,
30 Gaucher's disease, Fabry's disease, Pompe's disease, mucopolysaccharoidosis I, mucopolysaccharoidosis II, mucopolysaccharoidosis VI).

Examples of degenerative diseases include, but are not limited to, mesenchyme/mesoderm degenerative disease, muscle degenerative disease, endothelial

degenerative disease, neurodegenerative disease, degenerative joint disease (e.g., osteoarthritis), major types of degenerative heart disease (e.g., coronary heart disease, congenital heart disease, rheumatic heart disease, angina pectoris), neurodegenerative disease (e.g., Alzheimer's disease, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, spinal muscular atrophy), neuromuscular disorders (e.g., muscular dystrophy, duchenne muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic muscular dystrophy, congenital myopathy, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease).

10 The compositions and methods described herein can be used in the diagnosis, prophylaxis and/or treatment of diseases, disorders or conditions in which immune suppression or tolerance would confer a treatment benefit. Such diseases, disorders or conditions include autoimmune diseases, inflammatory diseases, allergies or graft versus host disease. The compositions and methods described herein can also be used in subjects who
15 have undergone or will undergo transplantation. The compositions and methods described herein can also be used in subject who have undergone, are undergoing or will undergo treatment with a therapeutic agent against which agent an undesired immune response occurs or may occur.

Autoimmune disease include, but are not limited to, rheumatoid arthritis, multiple
20 sclerosis, immune-mediated or Type I diabetes mellitus, inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis), systemic lupus erythematosus, psoriasis, scleroderma, autoimmune thyroid disease, alopecia areata, Grave's disease, Guillain-Barré syndrome, celiac disease, Sjögren's syndrome, rheumatic fever, gastritis, autoimmune atrophic gastritis, autoimmune hepatitis, insulinitis, oophoritis, orchitis, uveitis, phacogenic uveitis, myasthenia
25 gravis, primary myxoedema, pernicious anemia, autoimmune haemolytic anemia, Addison's disease, scleroderma, Goodpasture's syndrome, nephritis, e.g., glomerulonephritis, psoriasis, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, idiopathic thrombocytopenic purpura, idiopathic feucopenia, Wegener's granulomatosis and poly/dermatomyositis.

Inflammatory diseases include, but are not limited to, Alzheimer's, Ankylosing
30 spondylitis, arthritis, asthma, atherosclerosis, Behcet's disease, chronic inflammatory demyelinating polyradiculoneuropathy, Crohn's disease, colitis, cystic fibrosis, dermatitis, diverticulitis, hepatitis, irritable bowel syndrome (IBS), lupus erythematosus, muscular dystrophy, nephritis, Parkinson's, shingles and ulcerative colitis. Inflammatory diseases also

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include, for example, cardiovascular disease, chronic obstructive pulmonary disease (COPD), bronchiectasis, chronic cholecystitis, tuberculosis, Hashimoto's thyroiditis, sepsis, sarcoidosis, silicosis and other pneumoconioses, and an implanted foreign body in a wound, but are not so limited. As used herein, the term "sepsis" refers to a well-recognized clinical
5 syndrome associated with a host's systemic inflammatory response to microbial invasion. The term "sepsis" as used herein refers to a condition that is typically signaled by fever or hypothermia, tachycardia, and tachypnea, and in severe instances can progress to hypotension, organ dysfunction, and even death.

In some embodiments, the inflammatory disease is non-autoimmune inflammatory
10 bowel disease, post-surgical adhesions, coronary artery disease, hepatic fibrosis, acute respiratory distress syndrome, acute inflammatory pancreatitis, endoscopic retrograde cholangiopancreatography-induced pancreatitis, burns, atherogenesis of coronary, cerebral and peripheral arteries, appendicitis, cholecystitis, diverticulitis, visceral fibrotic disorders, wound healing, skin scarring disorders (keloids, hidradenitis suppurativa), granulomatous
15 disorders (sarcoidosis, primary biliary cirrhosis), asthma, pyoderma gangrenosum, Sweet's syndrome, Behcet's disease, primary sclerosing cholangitis or an abscess. In some preferred embodiment the inflammatory disease is inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis).

In other embodiments, the inflammatory disease is an autoimmune disease. The
20 autoimmune disease in some embodiments is rheumatoid arthritis, rheumatic fever, ulcerative colitis, Crohn's disease, autoimmune inflammatory bowel disease, insulin-dependent diabetes mellitus, diabetes mellitus, juvenile diabetes, spontaneous autoimmune diabetes, gastritis, autoimmune atrophic gastritis, autoimmune hepatitis, thyroiditis, Hashimoto's thyroiditis, insulinitis, oophoritis, orchitis, uveitis, phacogenic uveitis, multiple sclerosis, myasthenia
25 gravis, primary myxoedema, thyrotoxicosis, pernicious anemia, autoimmune haemolytic anemia, Addison's disease, Ankylosing spondylitis, sarcoidosis, scleroderma, Goodpasture's syndrome, Guillain-Barre syndrome, Graves' disease, glomerulonephritis, psoriasis, pemphigus vulgaris, pemphigoid, excema, bulous pemphigous, sympathetic ophthalmia, idiopathic thrombocytopenic purpura, idiopathic feucopenia, Sjogren's syndrome, systemic
30 sclerosis, Wegener's granulomatosis, poly/dermatomyositis, primary biliary cirrhosis, primary sclerosing cholangitis, lupus or systemic lupus erythematosus.

In still other embodiments, the inflammatory disease is a condition in which a subject experiences pain due to inflammation. In some embodiments, the pain is injury-induced pain.

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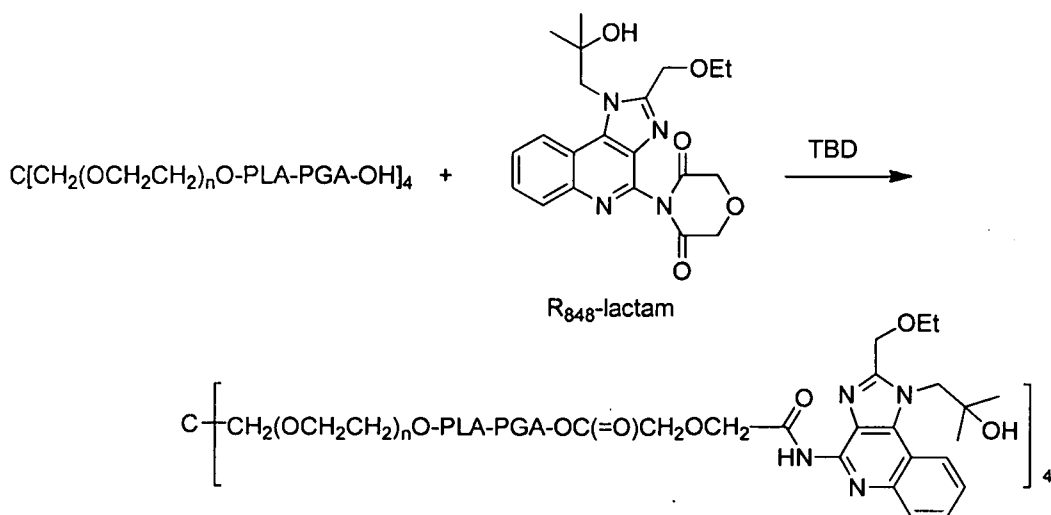
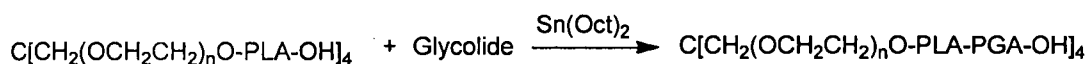
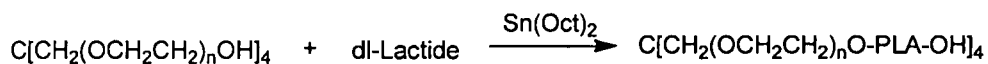
In other embodiments, the pain is cancer-induced. Accordingly, in some embodiments, a subject that is treated with a composition of the invention is one that has or has had an injury or cancer and has experienced, is experiencing or will experience pain that is or is thought to be associated with the injury or cancer.

5 Graft versus host disease (GVHD) is a complication that can occur after a pluripotent cell (e.g., stem cell) or bone marrow transplant in which the newly transplanted material results in an attack on the transplant recipient's body. In some instances, GVHD takes place after a blood transfusion. Graft-versus-host-disease can be divided into acute and chronic forms. The acute or fulminant form of the disease (aGVHD) is normally observed within the
10 first 100 days post-transplant, and is a major challenge to transplants owing to associated morbidity and mortality. The chronic form of graft-versus-host-disease (cGVHD) normally occurs after 100 days. The appearance of moderate to severe cases of cGVHD adversely influences long-term survival.

15

EXAMPLES

Example 1. Preparation of Dendrimeric Polymer PLGA-R848 Conjugate



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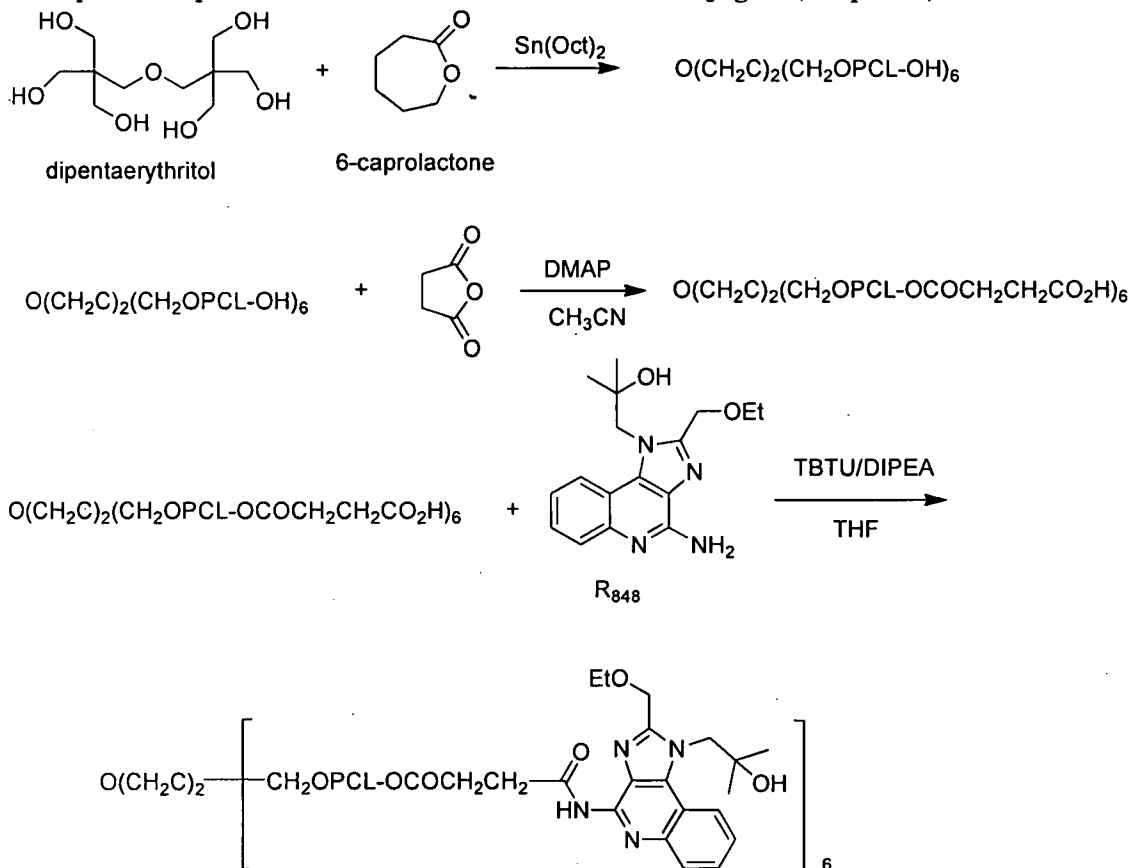
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Step-1: A mixture of pentaerythritol ethoxylate (1.35 g, 0.005 mol, $n = 3-4$), dl-lactide (20 g, 0.139 mol) and anhydrous sodium sulfate (10 g) in 250 mL dry toluene was heated to reflux while 50 mL of toluene was distilled out. $\text{Sn}(\text{Oct})_2$ (0.25 mL) was then added and the resulting mixture was heated at 120 °C under argon overnight. After cooling, the toluene solution was decanted from solid sodium sulfate and concentrated to dryness. The residue was then dissolved in 300 mL of dichloromethane (DCM) and the resulting solution was washed with 200 mL of water. After drying over MgSO_4 , the solution was filtered and concentrated to ca. 35 mL. This solution was then added to 300 mL of diethyl ether to precipitate out the polymer. The polymer was then washed once with 200 mL of ether and dried under vacuum give $\text{C}[\text{CH}_2(\text{OCH}_2\text{CH}_2)_n\text{O-PLA-OH}]_4$ ($n=3-4$) as a white powder (11.5 g, 53.9%, MW by GPC: 7400).

Step-2: The polymer from Step-1 (9 g) was combined with glycolide (2.43 g, 0.021 mol) and anhydrous sodium sulfate (10 g) in 200 mL of dry toluene. The mixture was heated to reflux while 30 mL of toluene was distilled out. $\text{Sn}(\text{Oct})_2$ (0.20 mL) was then added and the resulting mixture was heated at 120 °C under argon overnight. After cooling, the toluene solution was decanted from solid sodium sulfate and concentrated to dryness. The residue was then dissolved in 200 mL of dichloromethane (DCM) and the resulting solution was washed with 100 mL of water. After drying over MgSO_4 , the solution was filtered and concentrated to ca. 20 mL. This solution was then added to 300 mL of diethyl ether to precipitate out the polymer. The polymer was then washed once with 200 mL of ether and dried under vacuum give $\text{C}[\text{CH}_2(\text{OCH}_2\text{CH}_2)_n\text{O-PLA-PGA-OH}]_4$ ($n=3-4$) (6.4 g, 56%, ^1H NMR showed ratio of lactide to glycolide as 1:0.25 or lactide content at 80%).

Step-3. The polymer, $\text{C}[\text{CH}_2(\text{OCH}_2\text{CH}_2)_n\text{O-PLA-PGA-OH}]_4$ ($n=3-4$), from Step-2 (5.5 g) was dissolved in 50 mL of dry chloroform (CHCl_3) under argon. R848-lactam (1.04 g, 0.00251 mol) was added, followed by dropwise addition of a solution of TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) (0.222 g, 0.000159 mol) in 25 mL of CHCl_3 over 45 min. The resulting mixture was stirred at rt overnight. More TBD (30 mg) was added and the resulting clear solution was stirred for 1 h. The solution was diluted with 100 mL of CHCl_3 and washed with 10% tartaric acid (100 mL). After drying, the organic solution was filtered and concentrated under vacuum to ca. 25 mL. This solution was then added to 300 mL of diethyl ether to precipitate out the polymer. The polymer was then dried under high vacuum give the dendrimeric polymer-R848 conjugate as a white solid (4.2 g, 64%, ^1H NMR showed the R848 content at 18% wt).

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Example 2. Preparation of Dendrimeric PCL-R848 Conjugate (Prophetic)

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Step-1: A solution of dipentaerythritol (1.27 g, 5.0 mmol) and 6-caprolactone (25 g, 219 mmol) in 250 mL of dry toluene is heated to reflux while ca 50 mL of toluene is removed. Sn(Oct)₂ (0.25 mL) is then added. The resulting solution is refluxed under argon overnight. After cooling, the toluene solution is concentrated to ca. 50 mL in volume and added to 500 mL of 2-propanol (IPA) to precipitate out the polymer. The polymer is then washed with 100 mL of IPA followed by 100 mL of t-butylmethyl ether (MTBE). The polymer is then dried under high vacuum to give 6-arm dendrimeric polycaprolactone (PCL) (ca. 25 g as white solid).

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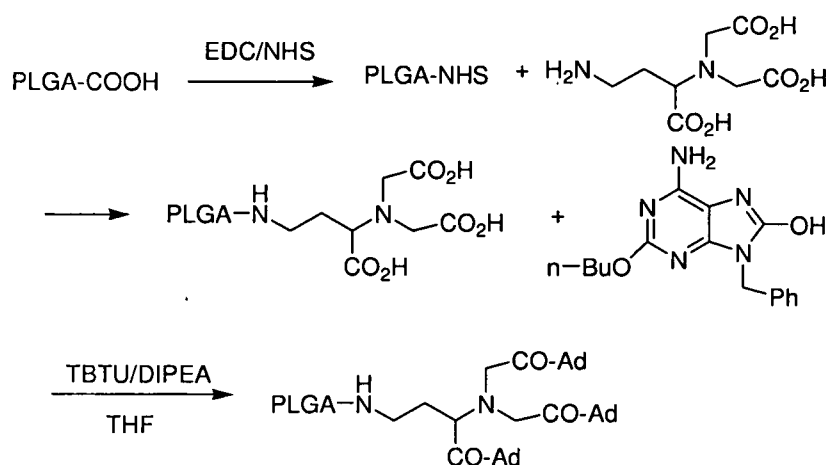
Step-2: The 6-arm PCL from Step-1 (25 g, ca. 5 mmol), succinic anhydride (12.0 g, 120 mmol) and 9.6 mL of pyridine (120 mmol) in 200 mL of chloroform are refluxed with argon overnight. After adding 100 mL of chloroform, the mixture is washed successively with 100 mL each of dilute HCl, saturated NaCl, and water. The organic layer is dried over

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magnesium sulfate, and then the volume of the mixture is reduced by half using rotary evaporation. After pouring the mixture into 800 mL of a 1:1 mixture of hexane and diethyl ether, the polymer is precipitated overnight at 4 °C. The polymer is collected and dried under vacuum to yield ca. 26 g of 6-arm PCL-succinic acid monoester.

- 5 Step-3: A mixture of the 6-arm PLC-succinic acid monoester from Step-2 (5.5 g, ca. 1.0 mmol), TBTU coupling agent (2.9 g, 9 mmol) in dry THF (100 mL) is stirred at rt under for 30 min. R848 (2.82 g, 9 mmol) is then added, followed by DIPEA (3.2 mL, 18 mmol). The resulting mixture is heated at ca. 50-60 C under argon overnight. The mixture is then diluted with EtOAc (300 mL) and washed with water, saturated NH₄Cl and NaCl solution.
- 10 After drying over Na₂SO₄, the solution is filtered and concentrated to ca 30 mL in volume. The solution is then added to 300 mL of IPA to precipitate out the polymer which is then washed with IPA and MTBE (100 mL each). The resulting polymer is then dried under high vacuum to give the 6-arm dendrimeric PCL-R848 polymer conjugate (ca. 5.5 g).

15 **Example 3. Preparation of PLGA-adjvant (Ad) Conjugate with Multiple Adjuvants at the End of the Polymer Chain (Prophetic)**



- 20 Step-1: PLGA-COOH (3.0 g, 0.17 mmol) in anhydrous methylene chloride (15 mL) is converted to PLGA-NHS with excess N-hydroxysuccinimide (NHS, 76 mg, 0.66 mmol, 4 equiv) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 140 mg, 0.72 mmol, 4.3 equiv) by magnetically stirring at room temperature for 12 h under nitrogen atmosphere. The PLGA NHS ester ("PLGA-NHS") is precipitated with cold diethyl ether

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(20 mL), filtered, repeatedly washed in a cold mixture of diethyl ether and methanol (few drops), and dried with nitrogen and under vacuum to remove solvent (yield: 97%).

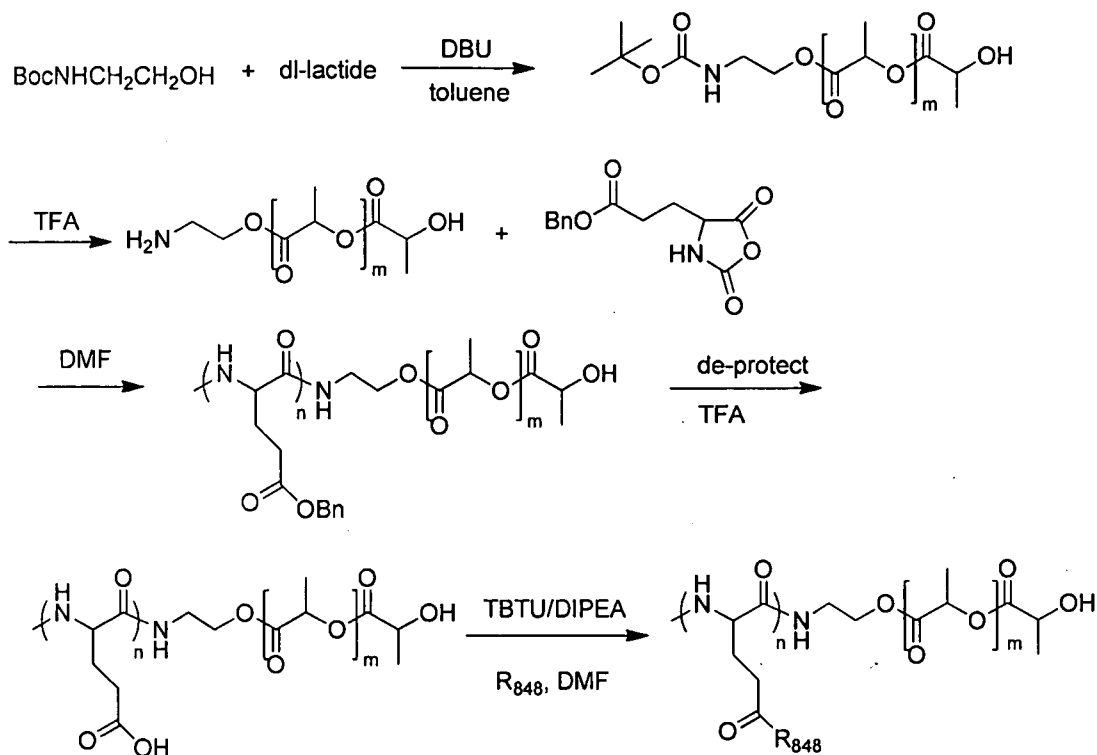
Step-2: PLGA-NHS (3.0 g, 0.17 mmol) is dissolved in anhydrous DMSO (10 mL) followed by addition of N,N-bis(carboxymethyl)-L-lysine hydrate (NTA-L-lysine) (60 mg, 0.20 mmol, 1.2 equiv) and N,N-diisopropylethylamine (DIPEA) (42 mg, 0.33 mmol, 3.8 equiv), and the reaction mixture is stirred at room temperature for 24 h. The PLGA-NTA-L-lysine is precipitated with cold diethyl ether and dried under vacuum as white powder (2.7 g, 91%).

Step-3: PLGA-NTA (2.5 g, 0.14 mmol) and TBTU coupling agent (0.21 g, 0.63 mmol) in dry THF (50 mL) is stirred at rt under for 30 min. The TLR7 agonist, 9-benzyl-2-butoxy-8-hydroxyadenine (0.20 g, 0.63 mmol) is then added, followed by DIPEA (0.21 mL, 1.2 mmol). The resulting mixture is heated at ca. 50-60 °C under argon overnight. The mixture is then diluted with EtOAc (200 mL) and washed with water, saturated NH₄Cl and NaCl solution. After drying over Na₂SO₄, the solution is filtered and concentrated to ca 10 mL in volume. The solution is then added to 200 mL of IPA to precipitate out the polymer which is then washed with IPA and MTBE (100 mL each). The conjugated polymer is then dried under high vacuum to give a light brown solid (2.5 g).

Example 4: Preparation of Poly(L-glutamic R848 amide)-block-Polylactide (Prophetic)

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- Poly(lactide) with amino end group (PLA-NH₂) is prepared by DBU (1,8-
- 5 diazabicyclo[5.4.0]undec-7-ene) catalyzed ring opening polymerization of dl-lactide with 2-BocNH-ethanol followed by deprotection with TFA in DCM. To synthesize γ -benzyl-L-glutamate-N-carboxylic anhydride (Bz-Glu-NCA), 10 g of triphosgene (33.7 mmol) in 20 mL dry ethyl acetate is gradually added through an addition funnel into a solution of 20 g of γ -benzyl-L-glutamate (76.0 mmol) in 600 mL of anhydrous ethyl acetate. The reaction mixture
- 10 is allowed to reflux under argon for 3 h. The resulting clear solution is cooled to -5°C and then washed with 200 mL of deionized water and 200 mL of 0.5% NaHCO₃ solution. The organic layer is dried with anhydrous MgSO₄. After filtration and evaporation of the solvent, the crude NCA product is recrystallized three times from THF/hexane solution to yield 7.2 g (81.1%) of Bz-Glu-NCA (m.p. 95-96°C).
- 15 Polymerization of Bz-Glu-NCA is performed by mixing a solution of 0.4 g of NH₂-PLA (0.0713 mmol) in 2 mL anhydrous dimethylformamide (DMF) with a solution of 0.66 g (0.0025 mol) freshly prepared Bz-Glu-NCA in 7.5 mL anhydrous DMF. The reaction mixture is stirred under argon at 40°C for 48 h. The reaction mixture is then poured into a

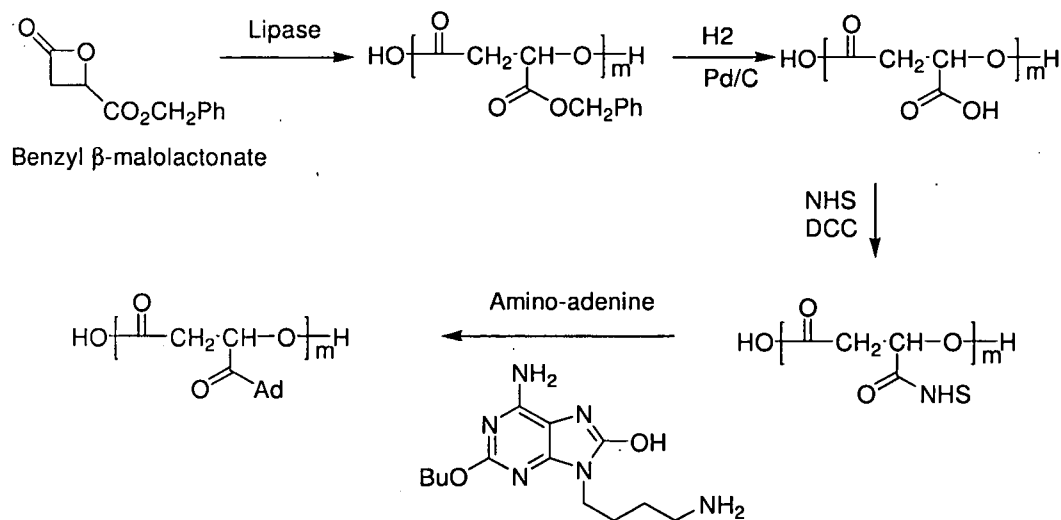
- 55 -

large excess of diethyl ether. The precipitate is collected by centrifugation, washed with diethyl ether, and dried in a vacuum to yield poly(benzyl-L-glutamate)-block-PLA (ca. 0.8 g).

To remove the benzyl protecting group, poly(benzyl-L-glutamate)-block-PLA (0.5 g) is dissolved in 6 mL trifluoroacetic acid (TFA) under argon at 0°C, followed by the addition of 0.6 mL of trifluoromethanesulfonic acid (TFMSA) and 0.7 mL of thioanisole. The reaction mixture is gently stirred under argon at 0°C for 1 h and then at room temperature for 30 min. The reaction mixture is poured into cooled diethyl ether. The resulting white precipitate is collected by filtration and dried in a vacuum to yield 0.4 g of Poly(L-glutamic acid)-block-PLA.

Poly(L-glutamic acid)-block-PLA is then conjugated with R848 in the presence of TBTU/DIPEA as described above to give Poly(L-glutamic R848 amide)-block-PLA after workup and precipitation from ether and drying under vacuum.

Example 5: Preparation of Polymalic Acid-adjvant (Ad) Conjugate (Prophetic)



Poly-L-malic acid is prepared by lipase catalyzed ring opening polymerization (ROP) of benzyl beta-malolactonate, followed by Pd-catalyzed debenzylation as described in Cameron et al., Chem. Soc. Rev. (2011) 40:1761–1776. See also Patil *et al.*, Pharm Res (2010) 27:2317–2329. N-Hydroxysuccinimide (NHS) (1 mmol) and N,N'-dicyclohexylcarbodiimide DCC (1 mmol) dissolved in 2 ml of DMF are added consecutively to the solution of Poly-L-malic acid (1 mmol with regard to maly units) dissolved in 1 ml of anhydrous acetone under vigorous stirring at room temperature (RT). After stirring at RT for 3 h to complete the activation of carboxyl groups, the TLR7 agonist

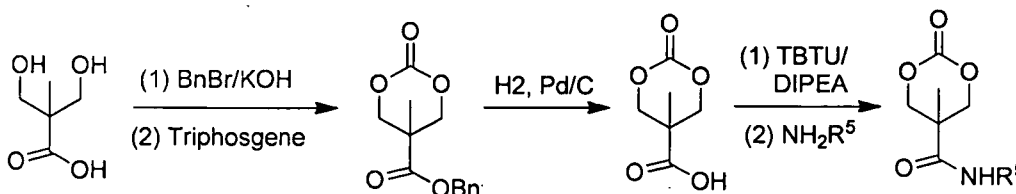
- 56 -

1.2 mmol of 9-aminobutyl-2-butoxy-8-hydroxyadenine in 0.5 ml of DMF is added followed by 2.4 mmol of triethylamine (TEA). After the reaction is completed, the reaction mixture is filtered, and most of the solvent is removed by rotary evaporation. The poly-L-malic acid-adenine conjugate is then precipitated from MTBE and dried under vacuum as a solid.

5

Example 6: Preparation of Polycarbonate-R848 Conjugate (Prophetic)

Step-1: Preparation of adjuvant-containing monomers

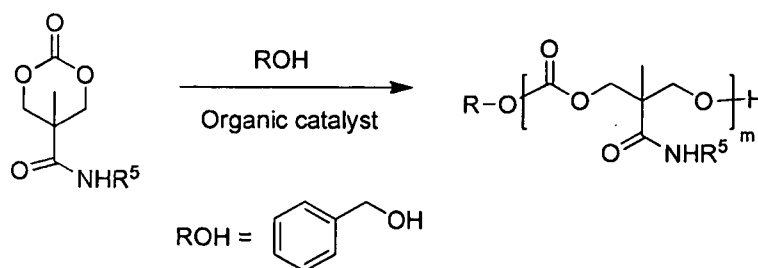


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Following literature procedure (see, e.g., Chem. Commun., 2008, 114–116), 2,2-Bis(methylol)propionic acid (bis-MPA) is converted to the corresponding benzyl ester with benzyl bromide in the presence of KOH. The benzyl ester is then treated with triphosgene to afford the benzyl ester cyclic carbonate. The benzyl group is removed by catalytic hydrogenation to provide the free acid which is then coupled with R848 in the presence of coupling agent, TBTU and base, DIPEA. After purification, the resulting R848 containing cyclic carbonate is then used in the next step.

15

Step-2: Preparation of polycarbonate-R848 conjugate by ROP of R848 containing cyclic carbonate monomer



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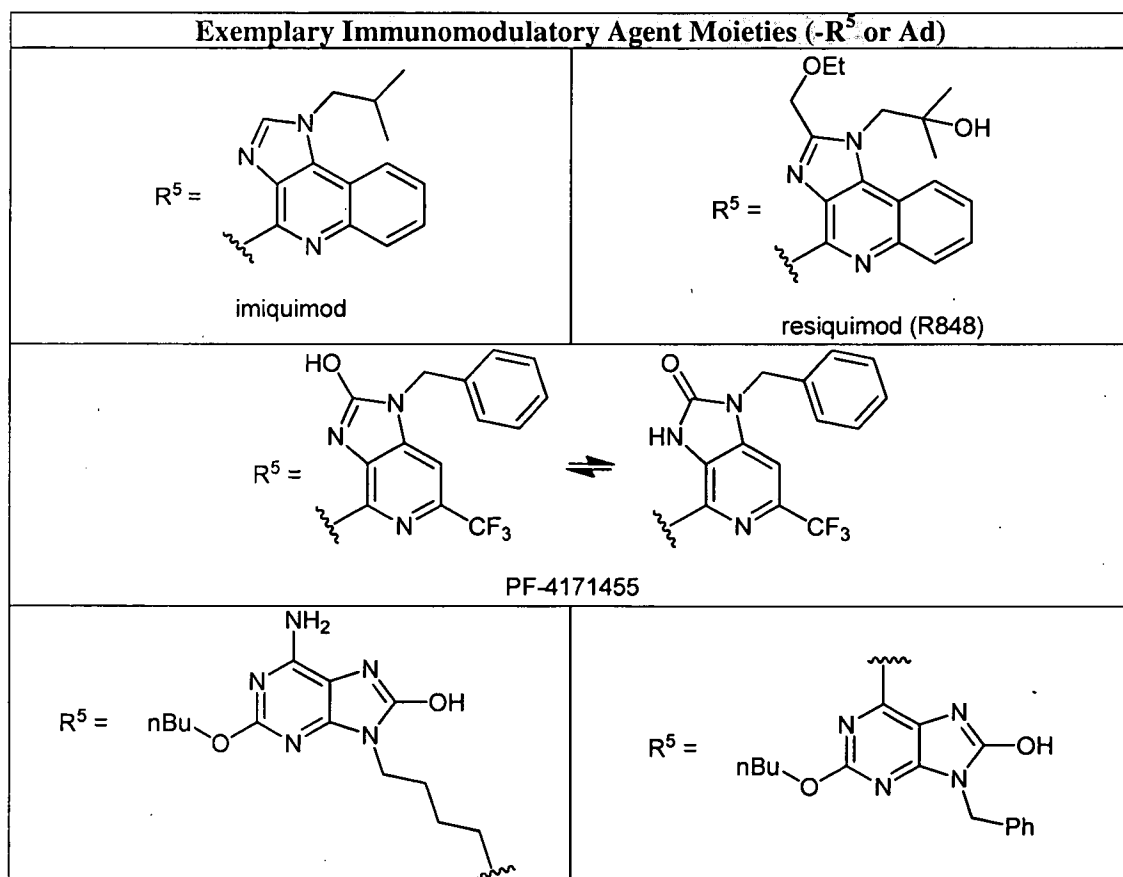
Following literature procedure (see, e.g., Chem. Commun., 2008, 114–116), ROP of cyclic carbonate can be performed in the presence of organic catalyst such as guanidines (TBD or MTBD), amidines (DBU), N-heterocyclic carbenes (NHCs), and bifunctional amino-thioureas. Thus, the cyclic carbonate from Step-1 is treated with an initiator such as an organic alcohol (e.g., wherein R is substituted or unsubstituted alkyl, such as benzyl alcohol) in the presence of TBD to give the polycarbonate-R848 conjugate. By controlling

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the ratio of alcohol to the cyclic carbonate and reaction time, polymer-R848 conjugates with varying molecular weight can then be obtained.

Other immunomodulatory agents comprising a free primary amine may be used or synthetically modified following any of the aforementioned Examples to provide compositions of the present invention. Examples of immunomodulatory agent moieties are provided below. Further examples of immunomodulatory agent moieties, such as adenine analogs, can also be found in the literature (see, e.g., J Med Chem. 2008 Nov 13;51(21):6621-6.)



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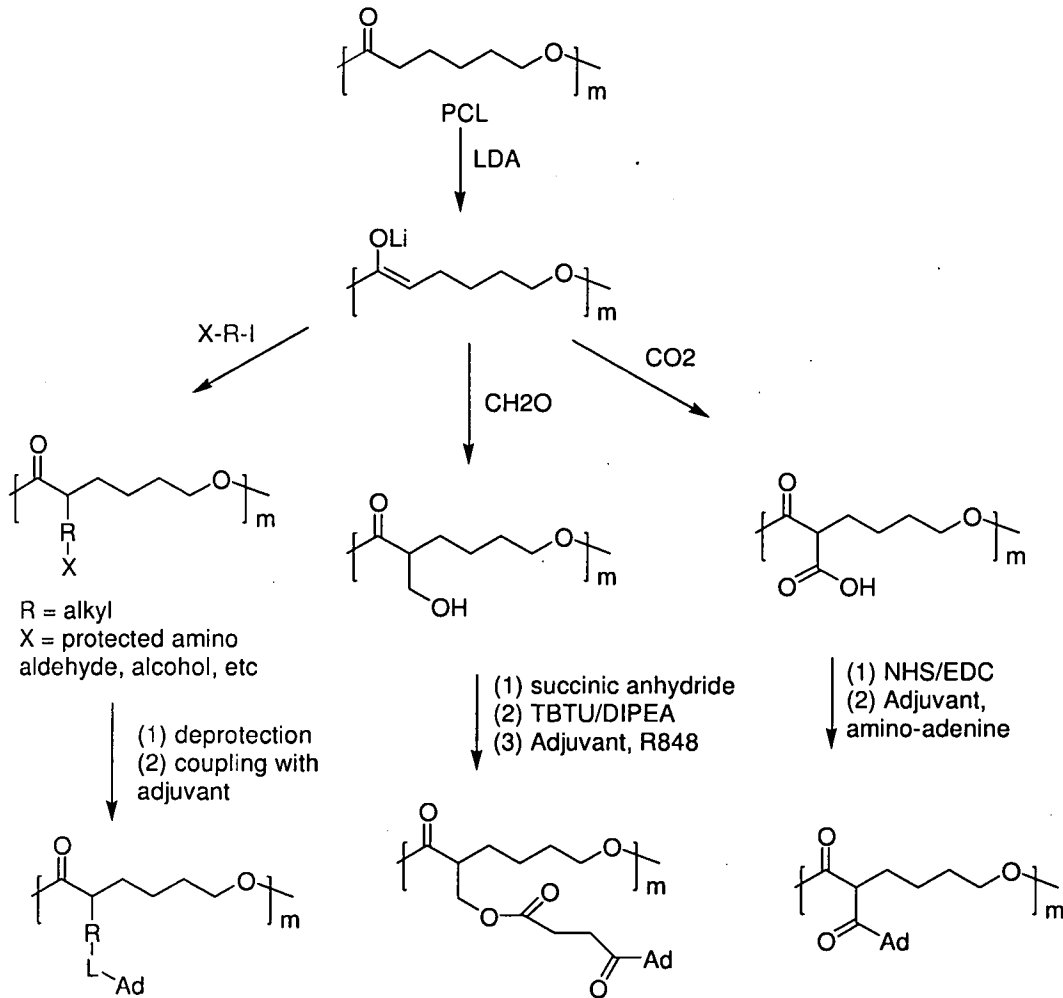
Example 7: Other General Methods for Making Polymer-immunomodulatory agent Conjugates (Prophetic)

Many functionalized polymers can be prepared and conjugated with the multiple immunomodulatory agents (e.g., adjuvants) to give the corresponding polymer-immunomodulatory agent conjugates. For example, polymers can be functionalized to

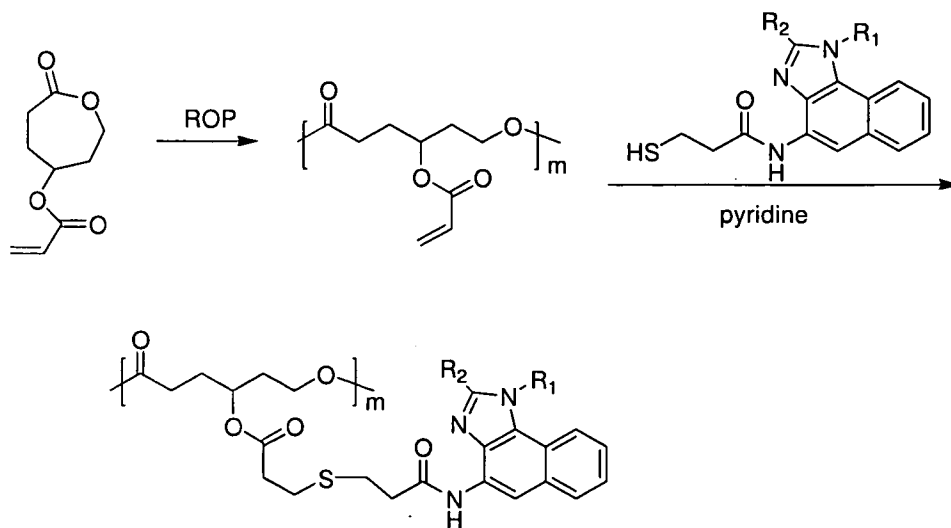
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provide reactive groups or linkers on the polymers for conjugation with suitably derivatized immunomodulatory agents. Examples of the reactive groups or linkers are carboxylic acid, aldehyde, ketone, alcohol, amine, alkene, azide and thiol groups. Additionally, functionalized monomers can be polymerized and then converted to polymers with reactive groups or linkers for conjugation with suitably derivatized immunomodulatory agents.

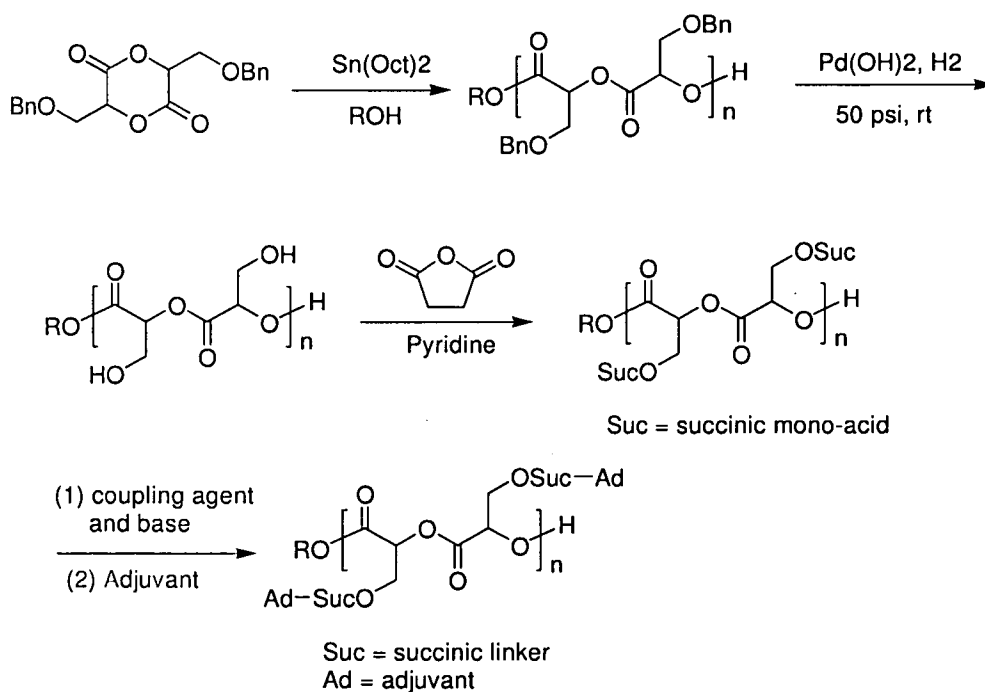
The following schemes exemplify these approaches.



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And



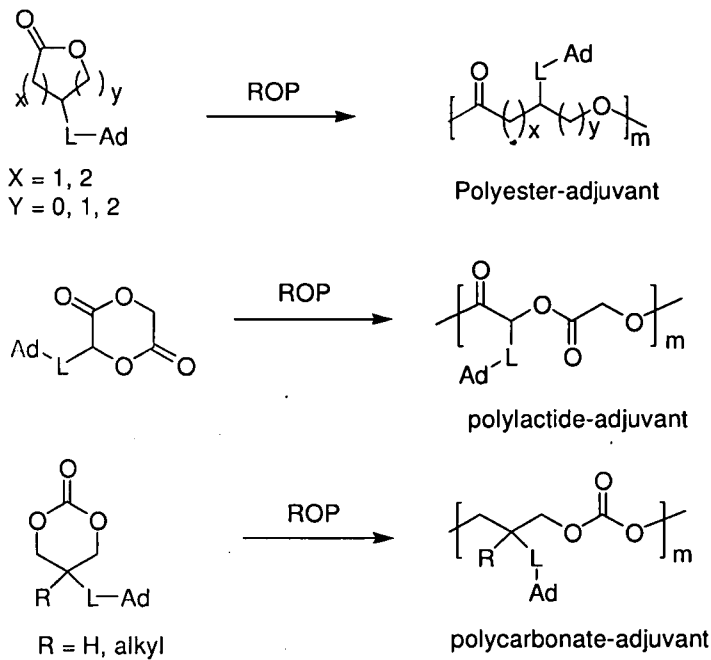
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Example 8: Preparation of Polymer-multi-immunomodulatory agent Conjugates from Polymerization of Adjuvant Containing Monomers (Prophetic)

Adjuvant containing monomers such as lactide, cyclic lactone and cyclic carbonate can be prepared by reaction of suitable immunomodulatory agents (e.g., adjuvants) such as imidazoquinolines or adenine derivatives with functionalized monomers. The resulting

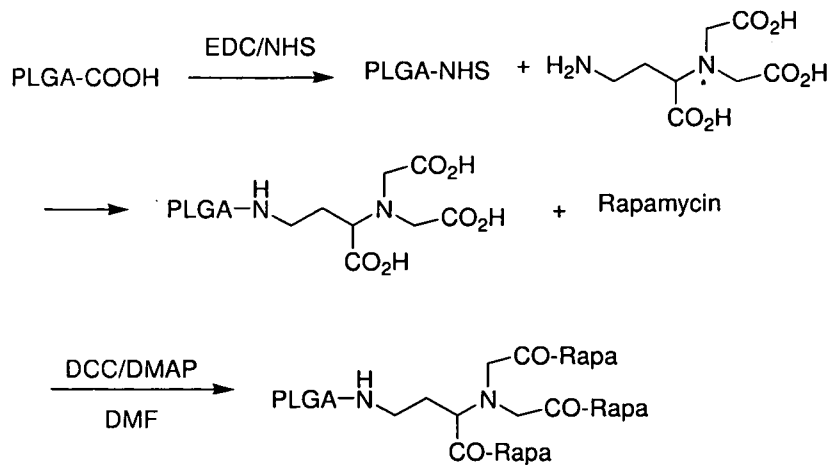
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monomers can then be polymerized under standard ring-opening polymerization (ROP) conditions to give polymer-multi-immunomodulatory agent conjugates. Some general schemes are shown as follows:



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Example 9: Preparation of PLGA-Rapamycin (Rapa) Conjugate with Multiple Rapamycin at the End of the Polymer Chain (Prophetic)



10

Step-1: PLGA-COOH (3.0 g, 0.17 mmol) in anhydrous methylene chloride (15 mL) is converted to PLGA-NHS with excess N-hydroxysuccinimide (NHS, 76 mg, 0.66 mmol, 4

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equiv) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 140 mg, 0.72 mmol, 4.3 equiv) by magnetically stirring at room temperature for 12 h under nitrogen atmosphere. The PLGA NHS ester ("PLGA-NHS") is precipitated with cold diethyl ether (20 mL), filtered, repeatedly washed in a cold mixture of diethyl ether and methanol (few drops), and dried with nitrogen and under vacuum to remove solvent (yield: 97%).

Step 2: PLGA-NHS (3.0 g, 0.17 mmol) is dissolved in anhydrous DMSO (10 mL) followed by addition of N,N-bis(carboxymethyl)-L-lysine hydrate (NTA-L-lysine) (60 mg, 0.20 mmol, 1.2 equiv) and N,N-diisopropylethylamine (DIPEA) (42 mg, 0.33 mmol, 3.8 equiv), and the reaction mixture is stirred at room temperature for 24 h. The PLGA-NTA-L-lysine is precipitated with cold diethyl ether and dried under vacuum as white powder (2.7 g, 91%).

Step 3: PLGA-NTA (2.5 g, 0.14 mmol) and DCC coupling agent (0.13 g, 0.63 mmol) in dry DMF (10 mL) is stirred at rt under for 30 min. Rapamycin (0.58 g, 0.63 mmol) is then added, followed by 4-dimethylaminopyridine (DMAP) (0.08 g, 0.63 mmol). The mixture is stirred at rt for 2 days. The mixture is then filtered to remove insoluble dicyclohexylurea. The filtrate is then added to 100 mL of isopropyl alcohol (IPA) to precipitate out the PLGA-(rapamycin)₃ conjugate. The IPA layer is removed and the polymer is then washed with 50 mL of IPA and 50 mL of methyl t-butyl ether (MTBE). The polymer is then dried under vacuum at 35 C for 2 days to give PLGA-(rapamycin)₃ conjugate (ca. 2.5 g).

Example 10: Preparation of Synthetic Nanocarriers (Prophetic)

Materials

Ovalbumin peptide 323-339 amide acetate salt, is purchased from Bachem Americas Inc. (3132 Kashiwa Street, Torrance CA 90505. Product code 4065609.) PLGA with 76% lactide and 24% glycolide content and an inherent viscosity of 0.49 dL/g is purchased from SurModics Pharmaceuticals (756 Tom Martin Drive, Birmingham, AL 35211. Product Code 7525 DLG 5A.) PLA-PEG-Nicotine, poly-D/L lactide-block-poly(ethylene glycol)-(±)-trans-3'-hydroxymethylnicotine ether with PEG block of approximately 5,000 Da and PLA block of approximately 21,000 Da is custom manufactured at Princeton Global Synthesis (300 George Patterson Drive #206, Bristol, PA 19007.) Dendrimeric (4-arm) PLGA-R848 conjugate of Example 1 is made having approximate molecular weight of 7,000 g/mol and 18% R848 loading on a weight/weight basis. Polyvinyl alcohol PhEur, USP (85-89%

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hydrolyzed, viscosity of 3.4-4.6 mPa.s) is purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027. Part Number 4-88).

Method

5 Solutions are prepared as follows:

Solution 1: Ovalbumin peptide 323 – 339 amide acetate salt at 20mg/mL is prepared by dissolution in 0.13N hydrochloric acid at room temperature.

10 Solution 2: PLGA-R848 at 50 mg/mL, PLGA at 25 mg/mL, and PLA-PEG-Nicotine at 25 mg/mL in dichloromethane is prepared by creating individual solutions of each polymer in dichloromethane at 100 mg/mL, and then combining portions of those solutions in a 2:1:1 volume ratio of the PLGA-R848 : PLGA : PLA-PEG-Nicotine.

Solution 3: Polyvinyl alcohol @ 50 mg/mL in 100 mM in 100mM phosphate buffer, pH 8.

Solution 4: 70 mM phosphate buffer, pH 8.

15 A primary (W1/O) emulsion is first created using Solution 1 & Solution 2. Solution 1 (0.2 mL) and Solution 2 (1.0 mL) are combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion is then formed by adding Solution 3 (3.0 mL) to the primary emulsion, vortexing to create a coarse dispersion, and then sonicating at 30% amplitude for 60 seconds
20 using the Branson Digital Sonifier 250. The secondary emulsion is added to an open 50 mL beaker containing 70 mM phosphate buffer solution (30 mL) and stirred at room temperature for 2 to 3 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers is washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 75,600 rcf for 60 minutes, removing the
25 supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure is repeated and then the pellet is re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension is stored frozen at -20°C until use. Nanocarrier size is determined by dynamic light scattering. The amounts of peptide and R848 in the nanocarrier are determined
30 by HPLC analysis. The total dry-nanocarrier mass per mL of suspension is determined by a gravimetric method.

Table 1

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Nanocarrier ID	Effective Diameter (nm)	R848 Content (% w/w)	Peptide Content (% w/w)
1	145	6.2	1.5

Example 11: Preparation of Synthetic Nanocarriers (Prophetic)

5

Materials

Ovalbumin protein, is purchased from Worthington Biochemical Corporation (730 Vassar Avenue, Lakewood, NJ 08701. Product Code 3048.) PLA with an inherent viscosity of 0.22 dL/g is purchased from SurModics Pharmaceuticals (756 Tom Martin Drive, Birmingham, AL 35211. Product Code 100 DL 2A.) PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and PLA block of approximately 19,000 Da is synthesized. Poly(L-glutamic R848 amide)-block-Polylactide of Example 4 is made having approximate PLA block molecular weight of 40,000 g/mol, and poly(L-glutamic R848 amide) block of approximately 18,000 g/mol, providing R848 loading of 22.5% on a weight/weight basis. Polyvinyl alcohol PhEur, USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa.s) is purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027. Part Number 4-88). Phosphate-buffered saline 1X (PBS 1X). From Mediatech Inc. (9345 Discovery Blvd. Manassas, VA 20109.) Product Code 21-040-CV.

20

Method

Solutions are prepared as follows:

Solution 1: Ovalbumin protein @ 40 mg/mL is prepared in PBS 1X at room temperature.

25

Solution 2: Poly(L-glutamic R848-amide)-block-polylactide at 20 mg/mL, PLA at 55 mg/mL, and PLA-PEG-OMe at 25 mg/mL in dichloromethane is prepared by creating individual solutions of each polymer in dichloromethane at 100 mg/mL, and then combining portions of those solutions in a 0.2:0.55:0.25 volume ratio, respectively.

30

Solution 3: Polyvinyl alcohol @ 100 mg/mL in 100 mM phosphate buffer, pH 8.

Solution 4: 70 mM phosphate buffer, pH 8.

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A primary (W1/O) emulsion is first created using Solution 1 & Solution 2. Solution 1 (0.2 mL) and Solution 2 (1.0 mL) are combined in a small glass pressure tube and sonicated at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary (W1/O/W2) emulsion is then formed by adding Solution 3 (3.0 mL) to the primary emulsion, vortexing to create a coarse dispersion, and then sonicating at 30% amplitude for 60 seconds using the Branson Digital Sonifier 250. The secondary emulsion is added to an open 50 mL beaker containing 70 mM phosphate buffer solution (30 mL) and stirred at room temperature for 2 to 3 hours to allow the dichloromethane to evaporate and the nanocarriers to form in suspension. A portion of the suspended nanocarriers is washed by transferring the nanocarrier suspension to a centrifuge tube, spinning at 75,600 rcf for 60 minutes, removing the supernatant, and re-suspending the pellet in phosphate buffered saline. This washing procedure is repeated and then the pellet is re-suspended in phosphate buffered saline to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The suspension is stored frozen at -20°C until use. Nanocarrier size is determined by dynamic light scattering. The amount of R848 in the nanocarrier are determined by HPLC analysis. Ovalbumin protein content is established by PAGE. The total dry-nanocarrier mass per mL of suspension is determined by a gravimetric method.

Table 2

Nanocarrier ID	Effective Diameter (nm)	R848 Content (% w/w)	Protein Content (% w/w)
2	135	3.7	6.1

20

What is claimed is:

CLAIMS

- 5 1. A composition comprising:
synthetic nanocarriers comprising a first type of polymer that comprises at least two
immunomodulatory agent moieties.
2. The composition of claim 1, wherein the immunomodulatory agent moieties are
10 between 2 and 100% of the polymer weight.
3. The composition of claim 2, wherein the immunomodulatory agent moieties are at
least 4% of the polymer weight.
- 15 4. The composition of claim 3, wherein the immunomodulatory agent moieties are at
least 8% of the polymer weight.
5. The composition of any of claims 1-4, wherein at least a portion of the
immunomodulatory agent moieties are not present at the surface of the synthetic nanocarriers.
20
6. The composition of any of claims 1-5, wherein at least a portion of the
immunomodulatory agent moieties are not at a terminus of a polymer.
7. The composition of any of claims 1-6, wherein the first type of polymer comprises at
25 least three, four, five, six, seven, eight, nine or ten immunomodulatory agent moieties.
8. The composition of any of claims 1-7, wherein the synthetic nanocarriers comprise at
least a second type of polymer.
- 30 9. The composition of any of claims 1-8, wherein the at least two immunomodulatory
agent moieties are at at least one terminus of the first type of polymer.

10. The composition of any of claims 1-8, wherein the at least two immunomodulatory agent moieties are along the backbone of the first type of polymer.
11. The composition of claim any of claims 1-8, wherein the at least two
5 immunomodulatory agent moieties are themselves polymerized and form the backbone of the first type of polymer.
12. The composition of any of claims 1-11, wherein the synthetic nanocarriers further comprise another material in addition to the immunomodulatory agent moieties.
10
13. The composition of claim 12, wherein the other material is another type of polymer.
14. The composition of any of claims 11-13, wherein when the immunomodulatory agent moieties form at least part of a polymer backbone, the polymer backbone comprises at least
15 one type of monomeric residue that is not the immunomodulatory agent moiety.
15. The composition of any of claims 1-14, wherein the first type of polymer and/or the second type of polymer is a linear polymer.
- 20 16. The composition of any of claims 1-14, wherein the first type of polymer and/or the second type of polymer is a branched polymer.
17. The composition of any of claims 1-14, wherein the first type of polymer and/or the second type of polymer is part of or forms a dendrimer.
25
18. The composition of any of claims 1-14, wherein the first type of polymer and/or the second type of polymer is part of or forms a polymeric matrix.
19. The composition of any of claims 1-18, wherein the immunomodulatory agent
30 moieties are the same type of immunomodulatory agent moiety.
20. The composition of any of claims 1-19, wherein the immunomodulatory agent moieties comprise a toll-like receptor (TLR) agonist.

21. The composition of claim 20, wherein the TLR agonist is a TLR-7 and/or TLR-8 agonist.
- 5 22. The composition of claim 20, wherein the TLR agonist comprises an aminodiazepine, adenine derivative or an imidazoquinoline.
23. The composition of claim 22, wherein the imidazoquinoline comprises resiquimod or imiquimod.
- 10 24. The composition of claim 22, wherein the adenine derivative comprises PF-4171455 or SM-276001.
- 15 25. The composition of any of claims 1-24, wherein the composition further comprises an antigen.
26. The composition of claim 25, wherein the antigen comprises a B cell or T cell antigen.
- 20 27. The composition of claim 26, wherein the T cell antigen is a T helper cell antigen.
28. The composition of any of claims 1-27, wherein the first type of polymer and/or the second type of polymer comprises a polyester, polyether, polycarbonate or polyamino acid.
- 25 29. The composition of claim 28, wherein the polyester comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone.
- 30 30. The composition of claim 28 or 29, wherein the polyester is coupled to a hydrophilic polymer.
31. The composition of claim 30, wherein the hydrophilic polymer comprises a polyether.
32. The composition of claim 31, wherein the polyether comprises polyethylene glycol.

33. The composition of claim 28, wherein the polyamino acid comprises polyglutamic acid.
- 5 34. The composition of any of claims 1-33, wherein the first type of polymer and/or the second type of polymer has a weight average or number average molecular weight of at least 2000 Da, at least 2500 Da, at least 3000 Da, at least 3500 Da, at least 4000 Da, at least 4500 Da or at least 5000 Da.
- 10 35. The composition of any of claims 1-34, wherein the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 500 nm.
36. The composition of claim 35, wherein the mean of a particle size distribution obtained
15 using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 400 nm.
37. The composition of claim 36, wherein the mean of a particle size distribution obtained
20 using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 300 nm.
38. The composition of claim 37, wherein the mean of a particle size distribution obtained
25 using dynamic light scattering of the synthetic nanocarriers is a maximum dimension of from 20 nm to 250 nm.
39. The composition of any of claims 1-38, wherein the composition further comprises a pharmaceutically acceptable excipient.
40. The composition of any of claims 1-39, wherein the composition is sterile.
- 30 41. The composition of any of claims 1-40, wherein the composition is in lyophilized form.

42. A dosage form comprising the composition of any of claims 1-41.
43. A vaccine comprising the dosage form of claim 42.
- 5 44. A method comprising:
administering the composition of any of claims 1-41, the dosage form of claim 42 or
the vaccine of claim 43 to a subject.
45. The method of claim 44, wherein the subject is a human.
- 10 46. The method of claim 44 or 45, wherein the method further comprises administering an
antigen.
47. The method of claim 46, wherein the antigen comprises a B cell or T cell antigen.
- 15 48. The method of claim 47, wherein the T cell antigen is T helper cell antigen.
49. The method of any of claims 44-48, wherein the subject has or is at risk of having
cancer.
- 20 50. The method of any of claims 44-48, wherein the subject has or is at risk of having an
infection or infectious disease.
51. The method of any of claims 44-48, wherein the subject has or is at risk of having an
25 autoimmune disease, an inflammatory disease, an allergy or graft versus host disease.
52. The method of any of claims 44-48, wherein the subject has undergone or will
undergo transplantation.
- 30 53. A method of producing a polymer comprising at least two immunomodulatory agent
moieties at a terminus of a polymer, comprising:
preparing a ring-opened polyester polymer with polyalcohol,
contacting the ring-opened polyester polymer with succinic anhydride, and

reacting the polyester polymer with immunomodulatory agent moieties in the presence of a coupling agent and a base.

54. The method of claim 53, wherein the polyester comprises a poly(lactic acid),
5 poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone.
55. The method of claim 53 or 54, wherein the coupling agent comprises TBTU, HBTU, EDC, DCC or PyBop.
- 10 56. The method of any of claims 53-55, wherein the base comprises DIPEA, DMAP or Et3N.
57. A method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising:
15 preparing a polyamino acid polymer with a free side chain acid group, and
coupling the polymer with immunomodulatory agent moieties in the presence of a coupling agent and a base.
58. The method of claim 57, wherein the polyamino acid polymer comprises
20 polyglutamic acid.
59. The method of claim 57 or 58, wherein the coupling agent comprises TBTU, HBTU, EDC, DCC or PyBop.
- 25 60. The method of any of claims 57-59, wherein the base comprises DIPEA, DMAP or Et3N.
61. A method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, the method comprising:
30 polymerizing a monomer in the present of a polyol to provide a multi-armed polymer,
functionalizing the multi-armed polymer with one or more carboxylic acid groups,
and

coupling the immunomodulatory agent moieties comprising an amino group with the multi-armed polymer in the presence of a coupling agent.

62. The method of claim 61, wherein multi-armed polymer comprises a poly(lactic acid),
5 poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone.
63. The method of claim 61 or 62, wherein the coupling agent comprises TBTU, HBTU,
EDC, DCC or PyBop.
- 10 64. The method of any of claims 61-63, wherein the coupling is performed also in the
presence of a base.
65. The method of claim 64, wherein the base is DIPEA, DMAP or Et3N.
- 15 66. A method of producing a polymer comprising at least two immunomodulatory agent
moieties, the method comprising:
providing a linear polymer comprising two or more side chain groups comprising an
electrophilic or nucleophilic chemical moiety attached thereto, and
coupling the immunomodulatory agent moieties to the side chain group.
- 20 67. The method of claim 66, wherein the side chain chemical moiety is a carboxylic acid
and the immunomodulatory agent moieties, comprising an amino group, are coupled to
carboxylic acid group in the presence of a coupling agent.
- 25 68. The method of claim 67, wherein the coupling agent is TBTU, HBTU, EDC, DCC or
PyBop.
69. The method of any of claims 66-68, wherein the coupling is performed also in the
presence of a base.
- 30 70. The method of claim 69, wherein the base is DIPEA, DMAP or Et3N.

71. A method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising:
functionalizing monomers of a polymer,
coupling the functionalized monomers with immunomodulatory agent moieties, and
5 polymerizing the coupled monomers.
72. A method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, the method comprising:
providing a monomer functionalized with immunomodulatory agent moieties, and
10 polymerizing the monomer.
73. The method of claim 71 or 72, wherein the monomer comprises lactide, glycolide or caprolactone monomer.
- 15 74. A method of producing a polymer comprising at least two immunomodulatory agent moieties along the polymer backbone, comprising:
producing or obtaining reactive bifunctional immunomodulatory agent moieties, and
reacting the bifunctional immunomodulatory agent moieties such that a polymer is formed.
- 20 75. The method of any of claims 53-74, wherein the immunomodulatory agent moieties comprise a TLR agonist.
76. The method of claim 75, wherein the TLR agonist is a TLR-7 and/or TLR-8 agonist.
- 25 77. The method of claim 75, wherein the TLR agonist comprises an aminodiazepine, adenine derivative or an imidazoquinoline.
78. The method of claim 77, wherein the imidazoquinoline comprise resiquimod or imiquimod.
30
79. The method of claim 77, wherein the adenine derivative comprise PF-4171455 or SM-276001.

80. The method of any of claims 53-79, wherein the method further comprises producing a synthetic nanocarrier with the polymer.
81. A polymer, synthetic nanocarrier or vaccine obtainable by a process comprising the
5 method steps as defined in any one of claims 53-80.
82. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in therapy or prophylaxis.
10
83. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in a method as defined in any of claims 44-52.
- 15 84. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in a method of treating or preventing cancer.
- 20 85. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in a method of treating or preventing infection or infectious disease.
- 25 86. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in a method of treating or preventing an autoimmune disease, an inflammatory disease, an allergy or graft versus host disease.
- 30 87. The composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for use in a method of treating a subject that has undergone or will undergo transplantation.

88. Use of the composition of any of claims 1-41, the dosage form of claim 42, the vaccine of claim 43 or the polymer, synthetic nanocarrier or vaccine of claim 81 for the manufacture of a medicament for use in a method as defined in any of claims 44-52.