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(54) Titre : CONJUGUES ET COMPOSITIONS POUR ADMINISTRATION CELLULAIRE
(54) Title: CONJUGATES AND COMPOSITIONS FOR CELLULAR DELIVERY

(57) Abrégé/Abstract:
This invention features conjugates, degradable linkers, compositions, methods of synthesis, and applications thereof, including folate, galactose, galactosamine, N-acetyl galactosamine, PEG, phospholipid, peptide and human serum albumin (HAS) derived conjugates of biologically active compounds, including antibodies, antivirals, chemotherapeutics, peptides, proteins, hormones nucleosides, nucleotides, non-nucleosides, and nucleic acids including enzymatic nucleic acids, DNAzymes, allozymes, antisense, dsRNA, siRNA, triplex oligonucleotides, 2,5-A chimeras, decoys and aptamers.

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(54) Title: CONJUGATES AND COMPOSITIONS FOR CELLULAR DELIVERY

(57) Abstract: This invention features conjugates, degradable linkers, compositions, methods of synthesis, and applications thereof, including folate, galactose, galactosamine, N-acetyl galactosamine, PEG, phospholipid, peptide and human serum albumin (HAS) derived conjugates of biologically active compounds, including antibodies, antivirals, chemotherapeutics, peptides, proteins, hormones nucleosides, nucleotides, non-nucleosides, and nucleic acids including enzymatic nucleic acids, DNAzymes, allozymes, antisense, dsRNA, siRNA, triplex oligonucleotides, 2,5-A chimeras, decoys and aptamers.

CONJUGATES AND COMPOSITIONS FOR CELLULAR DELIVERY

Background of the Invention

This patent application claims priority from Adamic *et al.,* USSN (60/292,217), filed May 18, 2001, and Adamic *et al.,* USSN (60/362,016) filed March 6, 2002 both entitled 'CONJUGATES AND COMPOSITIONS FOR CELLULAR DELIVERY'. This patent application also claims priority from Vargeese *et al.,* USSN (60/306,883), filed July 20, 2001 entitled "CONJUGATES AND COMPOSITIONS FOR TRANSPORT ACROSS CELLULAR MEMBRANES" and Vargeese *et al.,* USSN (60/311,865), filed August 13, 2001, entitled "CONJUGATES AND COMPOSITIONS FOR CELLULAR DELIVERY". These applications are hereby incorporated by reference herein in their entirety including the drawings.

The present invention relates to conjugates, compositions, methods of synthesis, and applications thereof. The discussion is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

The cellular delivery of various therapeutic compounds, such as antiviral and chemotherapeutic agents, is usually compromised by two limitations. First the selectivity of chemotherapeutic agents is often low, resulting in high toxicity to normal tissues. Secondly, the trafficking of many compounds into living cells is highly restricted by the complex membrane systems of the cell. Specific transporters allow the selective entry of nutrients or regulatory molecules, while excluding most exogenous molecules such as nucleic acids and proteins. Various strategies can be used to improve transport of compounds into cells, including the use of lipid carriers and various conjugate systems. Conjugates are often selected based on the ability of certain molecules to be selectively transported into specific cells, for example via receptor mediated endocytosis. By attaching a compound of interest to molecules that are actively transported across the cellular membranes, the effective transfer of that compound into cells or specific cellular organelles can be realized. Alternately, molecules that are able to penetrate cellular membranes without active transport mechanisms, for example, various lipophilic molecules, can be used to deliver compounds of interest. Examples of molecules that can

be utilized as conjugates include but are not limited to peptides, hormones, fatty acids, vitamins, flavonoids, sugars, reporter molecules, reporter enzymes, chelators, porphyrins, intercalcators, and other molecules that are capable of penetrating cellular membranes, either by active transport or passive transport.

The delivery of compounds to specific cell types, for example, cancer cells, can be accomplished by utilizing receptors associated with specific cell types. Particular receptors are overexpressed in certain cancerous cells, including the high affinity folic acid receptor. For example, the high affinity folate receptor is a tumor marker that is overexpressed in a variety of neoplastic tissues, including breast, ovarian, cervical, colorectal, renal, and nasoparyngeal tumors, but is expressed to a very limited extent in normal tissues. The use of folic acid based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to the treatment and diagnosis of disease and can provide a reduction in the required dose of therapeutic compounds. Furthermore, therapeutic bioavialability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of bioconjugates, including folate bioconjugates. Godwin *et al.,* 1972, *J. Biol. Chem.,* 247, 2266-2271, report the synthesis of biologically active pteroyloligo-L-glutamates. Habus *et al.,* 1998, *Bioconjugate Chem.,* 9, 283-291, describe a method for the solid phase synthesis of certain oligonucleotide-folate conjugates. Cook, US Patent No. 6,721,208, describes certain oligonucleotides modified with specific conjugate groups. The use of biotin and folate conjugates to enhance transmembrane transport of exogenous molecules, including specific oligonucleotides has been reported by Low *et al.,* US Patent Nos. 5,416,016, 5,108,921, and International PCT publication No. WO 90/12096. Manoharan *et al.,* International PCT publication No. WO 99/66063 describe certain folate conjugates, including specific nucleic acid folate conjugates with a phosphoramidite moiety attached to the nucleic acid component of the conjugate, and methods for the synthesis of these folate conjugates. Nomura *et al.,* 2000, *J. Org. Chem.,* 65, 5016-5021, describe the synthesis of an intermediate, alpha-[2-(trimethylsilyl)ethoxycarbonl]folic acid, useful in the synthesis of ceratin types of folate-nucleoside conjugates. Guzaev *et al.,* US 6,335,434, describes the synthesis of certain folate oligonucleotide conjugates.

The delivery of compounds to other cell types can be accomplished by utilizing receptors associated with a certain type of cell, such as hepatocytes. For example, drug

delivery systems utilizing receptor-mediated endocytosis have been employed to achieve drug targeting as well as drug-uptake enhancement. The asialoglycoprotein receptor (ASGPr) (see for example Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). Binding of such glycoproteins or synthetic glycoconjugates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly *et al.,* 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.,* 1981, *J. Med. Chem.,* 24, 1388-1395). The use of galactose and galactosamine based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to the treatment of liver disease such as HBV and HCV infection or hepatocellular carcinoma. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavialability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of bioconjugates.

A number of peptide based cellular transporters have been developed by several research groups. These peptides are capable of crossing cellular membranes *in vitro* and *in vivo* with high efficiency. Examples of such fusogenic peptides include a 16-amino acid fragment of the homeodomain of ANTENNAPEDIA, a Drosophila transcription factor (Wang *et al.,* 1995, *PNAS USA.,* 92, 3318-3322); a 17-mer fragment representing the hydrophobic region of the signal sequence of Kaposi fibroblast growth factor with or without NLS domain (Antopolsky *et al.,* 1999, *Bioconj. Chem.,* 10, 598-606); a 17-mer signal peptide sequence of caiman crocodylus Ig(5) light chain (Chaloin *et al.,* 1997, *Biochem. Biophys. Res. Comm.,* 243, 601-608); a 17-amino acid fusion sequence of HIV envelope glycoprotein gp4114, (Morris *et al.,*1997, *Nucleic Acids Res.,* 25, 2730-2736); the HIV-1 Tat49-57 fragment (Schwarze *et al.,* 1999, *Science,* 285, 1569-1572); a transportan A - achimeric 27-mer consisting of N-terminal fragment of neuropeptide

galanine and membrane interacting wasp venom peptide mastoporan (Lindgren *et al.*, 2000, *Bioconjugate Chem.*, 11, 619-626); and a 24-mer derived from influenza virus hemagglutinin envelop glycoprotein (Bongartz *et al.*, 1994, *Nucleic Acids Res.*, 22, 4681-4688).

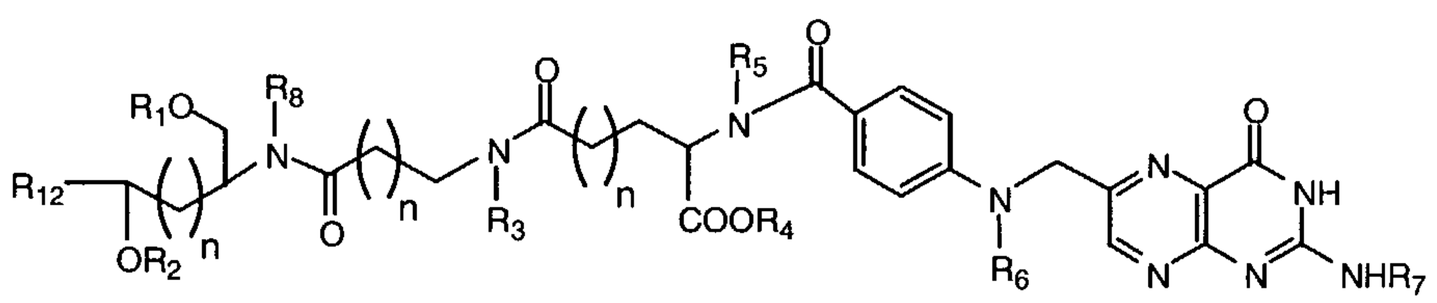
These peptides were successfully used as part of an antisense oligonucleotide-peptide conjugate for cell culture transfection without lipids. In a number of cases, such conjugates demonstrated better cell culture efficacy then parent oligonucleotides transfected using lipid delivery. In addition, use of phage display techniques has identified several organ targeting and tumor targeting peptides *in vivo* (Ruoslahti, 1996, *Ann. Rev. Cell Dev. Biol.*, 12, 697-715). Conjugation of tumor targeting peptides to doxorubicin has been shown to significantly improve the toxicity profile and has demonstrated enhanced efficacy of doxorubicin in the *in vivo* murine cancer model MDA-MB-435 breast carcinoma (Arap *et al.*, 1998, *Science*, 279, 377-380).

Hudson *et al.*, 1999, *Int. J. Pharm.*, 182, 49-58, describes the cellular delivery of specific hammerhead ribozymes conjugated to a transferrin receptor antibody. Janjic *et al.*, US 6,168,778, describes specific VEGF nucleic acid ligand complexes for targeted drug delivery. Bonora *et al.*, 1999, *Nucleosides Nucleotides*, 18, 1723-1725, describes the biological properties of specific antisense oligonucleotides conjugated to certain polyethylene glycols. Davis and Bishop, International PCT publication No. WO 99/17120 and Jaeschke *et al.*, 1993, *Tetrahedron Lett.*, 34, 301-4 describe specific methods of preparing polyethylene glycol conjugates. Tullis, International PCT Publication No. WO 88/09810; Jaschke, 1997, *ACS Sympl Ser.*, 680, 265-283; Jaschke *et al.*, 1994, *Nucleic Acids Res.*, 22, 4810-17; Efimov *et al.*, 1993, *Bioorg. Khim.*, 19, 800-4; and Bonora *et al.*, 1997, *Bioconjugate Chem.*, 8, 793-797, describe specific oligonucleotide polyethylene glycol conjugates. Manoharan, International PCT Publication No. WO 00/76554, describes the preparation of specific ligand-conjugated oligodeoxyribonucleotides with certain cellular, serum, or vascular proteins. Defrancq and Lhomme, 2001, *Bioorg Med Chem Lett.*, 11, 931-933; Cebon *et al.*, 2000, Aust. J. Chem., 53, 333-339; and Salo *et al.*, 1999, *Bioconjugate Chem.*, 10, 815-823 describe specific aminooxy peptide oligonucleotide conjugates.

Summary of the Invention

The present invention features compositions and conjugates to facilitate delivery of molecules into a biological system, such as cells. The conjugates provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes. The present invention encompasses the design and synthesis of novel agents for the delivery of molecules, including but not limited to small molecules, lipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. The compounds of the invention generally shown in the Formulae below are expected to improve delivery of molecules into a number of cell types originating from different tissues, in the presence or absence of serum.

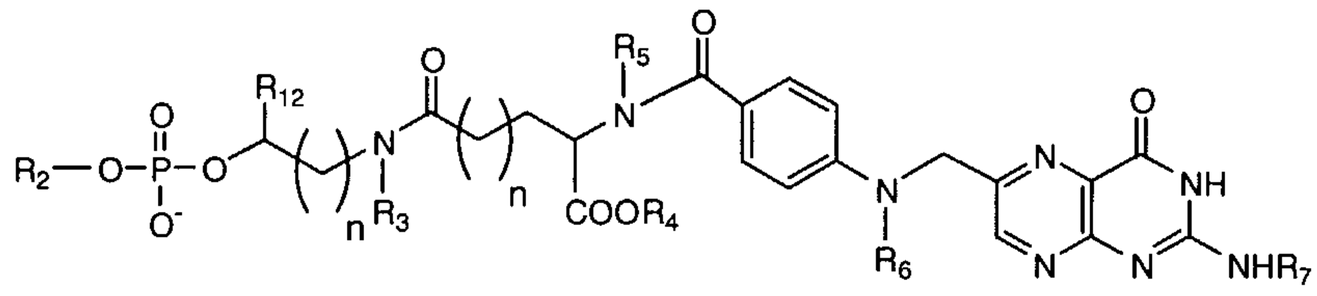
The present invention features a compound having the Formula 1:



1

wherein each R_1, R_3, R_4 ,R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

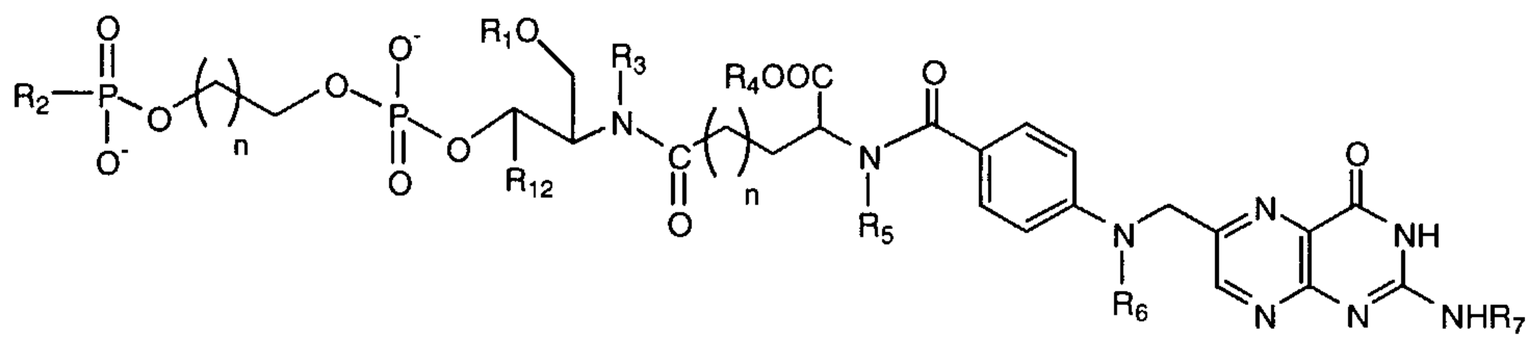
The present invention features a compound having the Formula 2:



2

wherein each R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

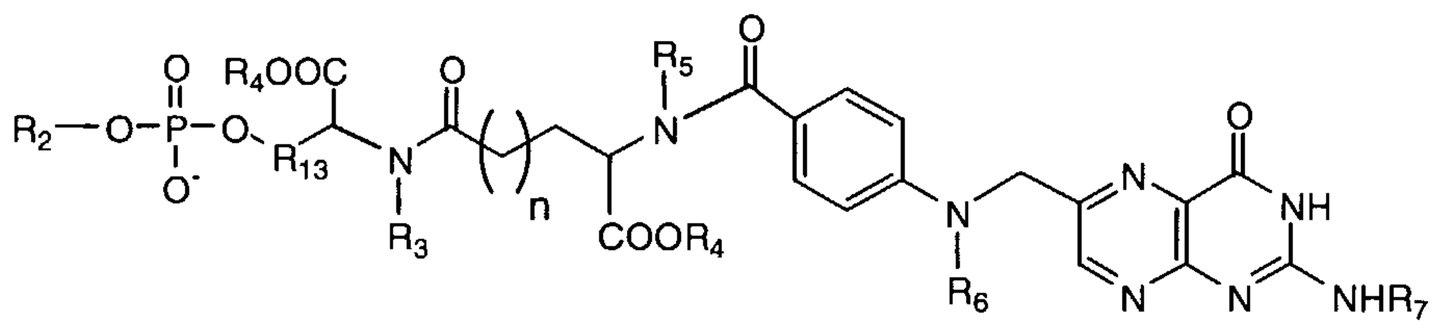
The present invention features a compound having the Formula 3:



3

wherein each R_1, R_3, R_4 ,R_5 R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, or nucleic acid.

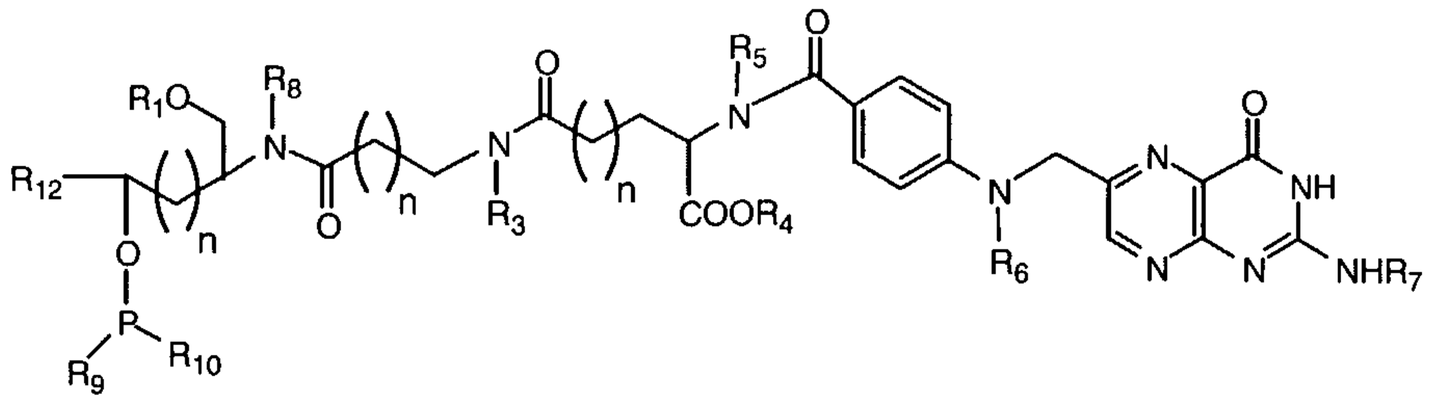
The present invention features a compound having the Formula 4:



4

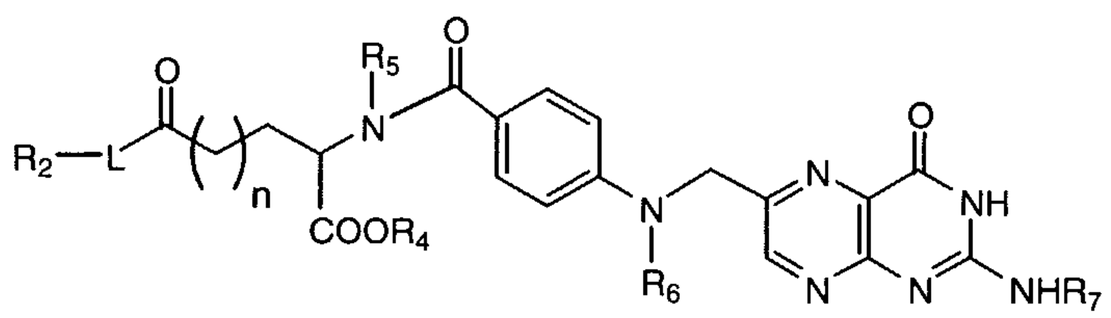
wherein each R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, and R_{13} is an amino acid side chain.

The present invention features a compound having the Formula 5:



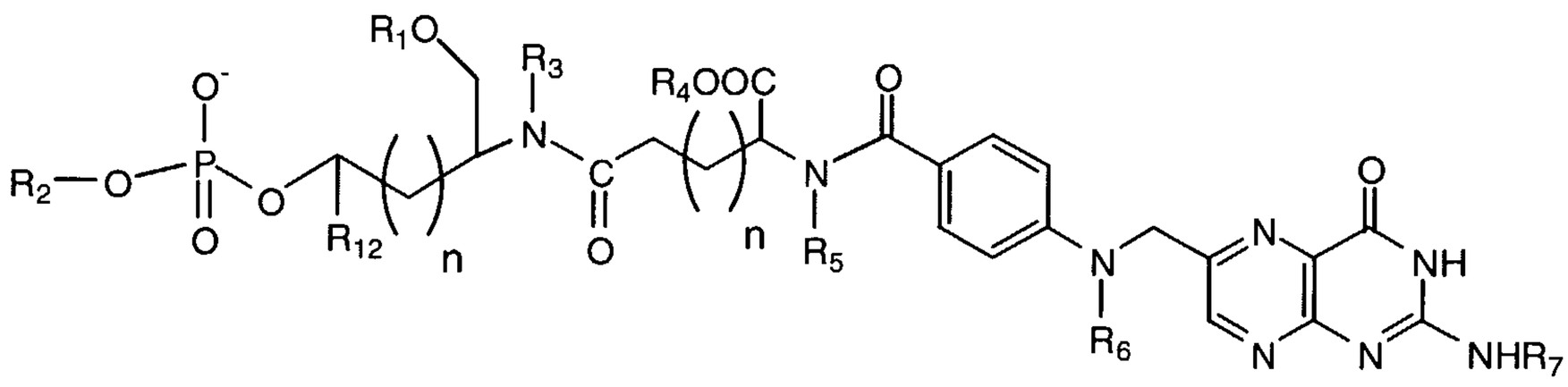
wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

The present invention features a compound having the Formula 6:



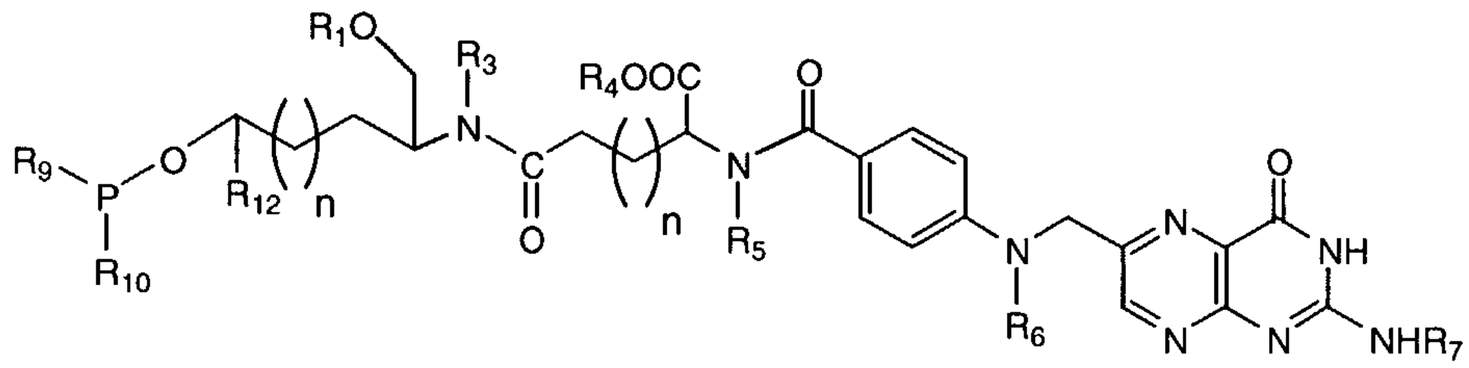
wherein each R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, each "n" is independently an integer from 0 to about 200, and L is a degradable linker.

The present invention features a compound having the Formula 7:



wherein each R_1, R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

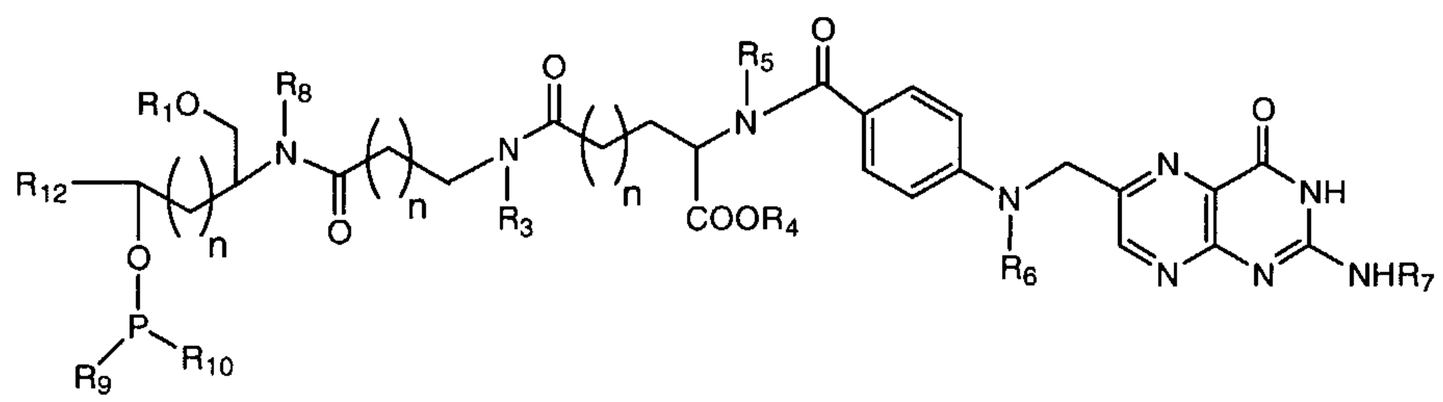
The present invention features a compound having the Formula 8:



8

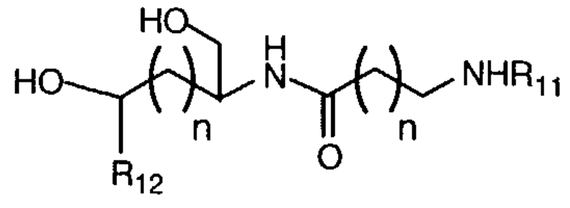
wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

The present invention features a method for synthesizing a compound having Formula 5:



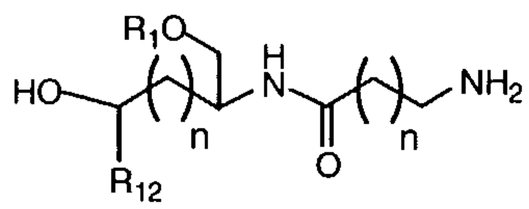
5

wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, comprising: coupling a bis-hydroxy aminoalkyl derivative, for example D-threoninol, with a N-protected aminoalkanoic acid to yield a compound of Formula 9;



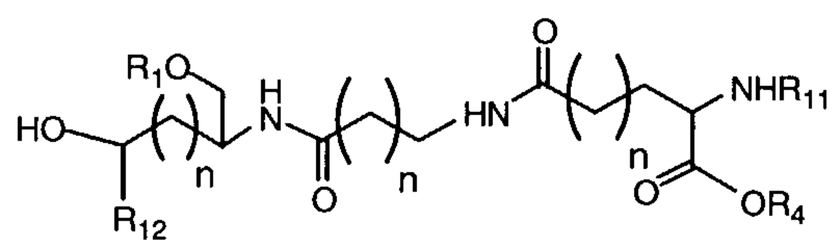
9

wherein R_{11} is an amino protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; introducing primary hydroxy protection R_1 followed by amino deprotection of R_{11} to yield a compound of Formula 10;



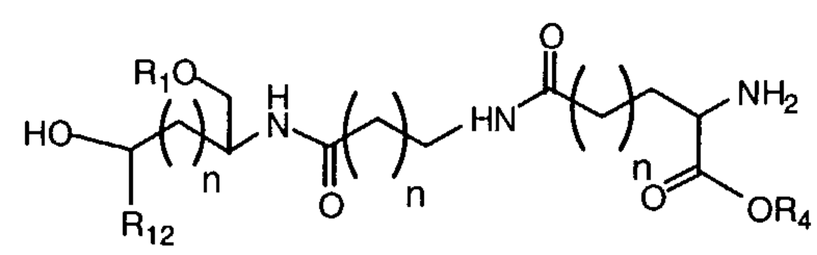
10

wherein R_1 is a protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; coupling the deprotected amine of Formula 10 with a protected amino acid, for example glutamic acid, to yield a compound of Formula 11;



11

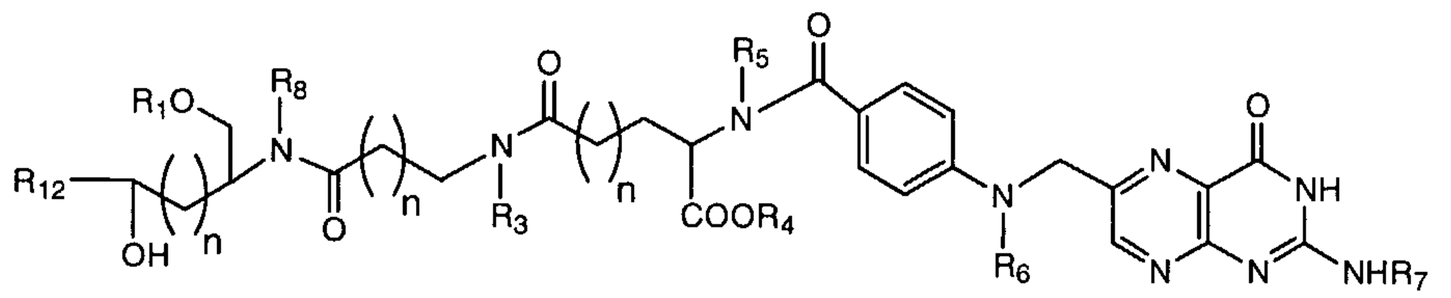
wherein each R_1 and R_4 is independently a protecting group or hydrogen, each "n" is independently an integer from 0 to about 200, R_{11} is an amino protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; deprotecting the amine R_{11} of the conjugated glutamic acid of Formula XI to yield a compound of Formula 12;



12

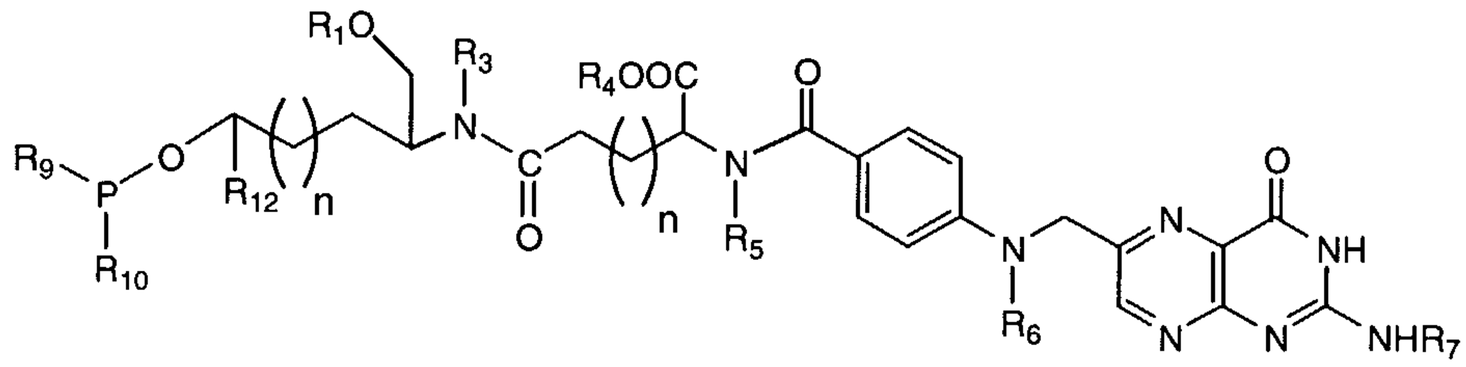
wherein each R_1 and R_4 is independently a protecting group or hydrogen, each "n" is independently an integer from 0 to about 200, R_{11} is an amino protecting group, and

R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; coupling the deprotected amine of Formula 12 with an amino protected pteroic acid to yield a compound of Formula 13;



wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and introducing a phosphorus containing 10 group at the secondary hydroxyl of Formula 13 to yield a compound of Formula 5.

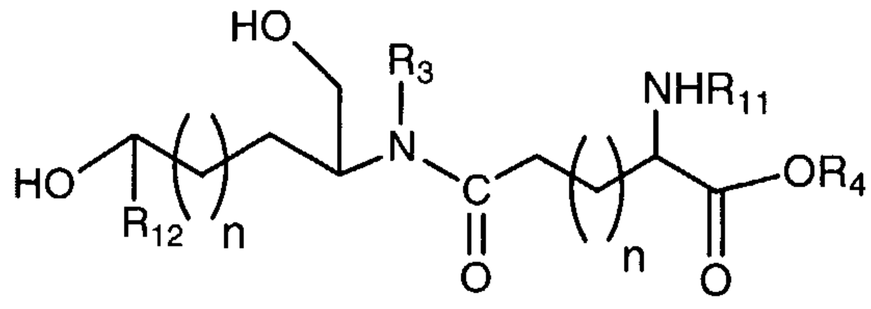
The present invention features a method for synthesizing a compound having Formula 8:



8

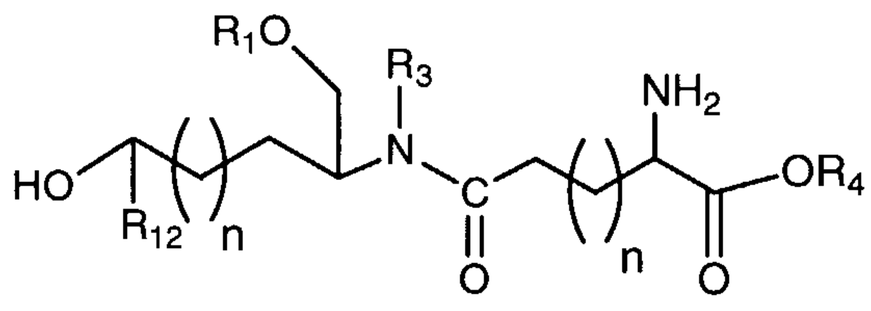
15 wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, comprising; 20 coupling a bis-hydroxy aminoalkyl derivative, for example D-threoninol, with a protected amino acid, for example glutamic acid, to yield a compound of Formula 14;

11



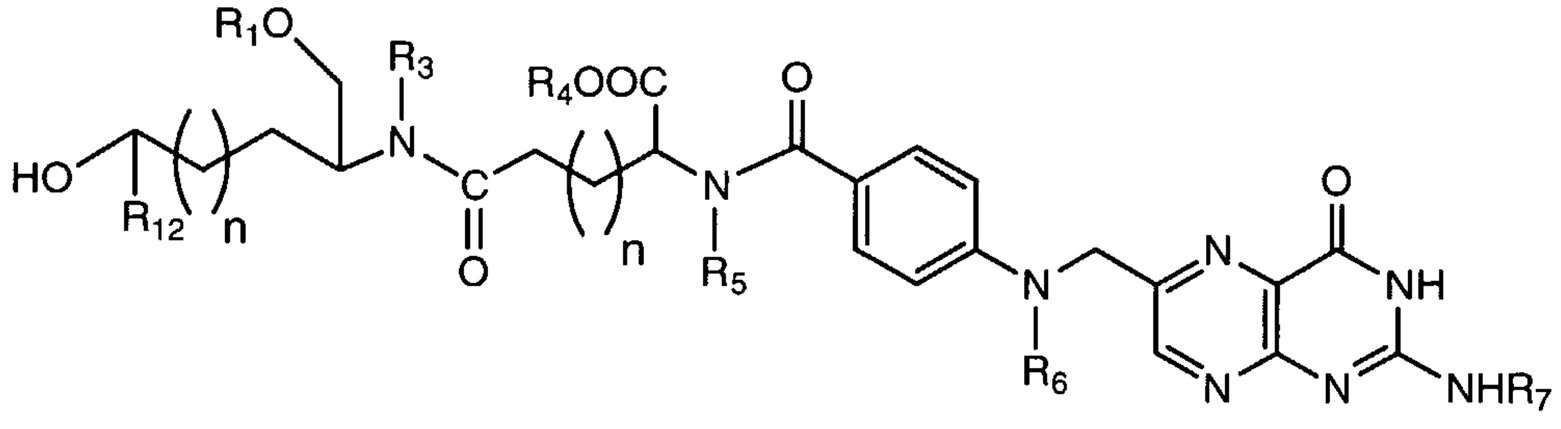
14

wherein R_{11} is an amino protecting group, each "n" is independently an integer from 0 to about 200, R_4 is independently a protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; introducing primary hydroxy protection R_1 followed by amino deprotection of R_{11} of Formula 14 to yield a compound of Formula 15;



15

wherein each R_1 and R_4 is independently a protecting group or hydrogen, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; coupling the deprotected amine of Formula 15 with an amino protected pteroic acid to yield a compound of Formula 16;



16

wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and introducing a phosphorus containing group at the secondary hydroxyl of Formula 16 to yield a compound of Formula 8.

In one embodiment, R_2 of a compound of the invention comprises a phosphorus containing group.

In another embodiment, R_2 of a compound of the invention comprises a nucleoside, for example, a nucleoside with beneficial activity such as anticancer or antiviral activity.

In yet another embodiment, R_2 of a compound of the invention comprises a nucleotide, for example, a nucleotide with beneficial activity such as anticancer or antiviral activity.

In a further embodiment, R_2 of a compound of the invention comprises a small molecule, for example, a small molecule with beneficial activity such as anticancer or antiviral activity.

In another embodiment, R_2 of a compound of the invention comprises a nucleic acid, for example, a nucleic acid with beneficial activity such as anticancer or antiviral activity..

In one embodiment, R_2 of a compound of the invention comprises a solid support comprising a linker.

In another embodiment, a nucleoside (R_2) of the invention comprises a nucleoside with anticancer activity.

In another embodiment, a nucleoside (R_2) of the invention comprises a nucleoside with antiviral activity.

In another embodiment, the nucleoside (R_2) of the invention comprises fludarabine, lamivudine (3TC), 5-fluro uridine, AZT, ara-adenosine, ara-adenosine monophosphate, a dideoxy nucleoside analog, carbodeoxyguanosine, ribavirin, fialuridine, lobucavir, a pyrophosphate nucleoside analog, an acyclic nucleoside analog, acyclovir, gangciclovir, penciclovir, famciclovir, an L-nucleoside analog, FTC, L-FMAU, L-ddC, L-FddC, L-d4C, L-Fd4C, an L-dideoxypurine nucleoside analog, cytallene, bis-POM PMEA (GS-840), BMS-200,475, carbovir or abacavir.

In one embodiment, R_{13} of a compound of the invention comprises an alkylamino or an alkoxy group, for example, $-CH_2O-$ or $-CH(CH_2)CH_2O-$.

In another embodiment, R_{12} of a compound of the invention is an alkylhyrdroxyl, for example, $-(CH_2)_nOH$, where n comprises an integer from about 1to about 10.

In another embodiment, L of Formula 6 of the invention comprises serine, threonine, or a photolabile linkage.

In one embodiment, R_9 of a compound of the invention comprises a phosphorus protecting group, for example -OCH_2CH_2CN (oxyethylcyano).

In one embodiment, R_{10} of a compound of the invention comprises a nitrogen containing group, for example, –$N(R_{14})$ wherein R_{14} is a straight or branched chain alkyl having from about 1 to about 10 carbons.

In another embodiment, R_{10} of a compound of the invention comprises a heterocycloalkyl or heterocycloalkenyl ring containing from about 4 to about 7 atoms, and having from about 1 to about 3 heteroatoms comprising oxygen, nitrogen, or sulfur.

In another embodiment, R_1 of a compound of the invention comprises an acid labile protecting group, such as a trityl or substituted trityl group, for example, a dimethoxytrityl or mono-methoxytrityl group.

In another embodiment, R_4 of a compound of the invention comprises a *tert*-butyl, Fm (fluorenyl-methoxy), or allyl group.

In one embodiment, R_6 of a compound of the invention comprises a TFA (trifluoracetyl) group.

In another embodiment, R_3, R_5 R_7 and R_8 of a compound of the invention are independently hydrogen.

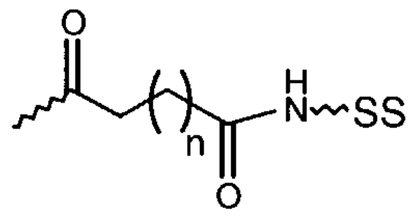
In one embodiment, R_7 of a compound of the invention is independently isobutyryl, dimethylformamide, or hydrogen.

In another embodiment, R_{12} of a compound of the invention comprises a methyl group or ethyl group.

In one embodiment, a nucleic acid of the invention comprises an enzymatic nucleic acid, for example a hammerhead, Inozyme, DNAzyme, G-cleaver, Zinzyme, Amberzyme, or allozyme.

In another embodiment, a nucleic acid of the invention comprises an antisense nucleic acid, 2-5A nucleic acid chimera, or decoy nucleic acid.

In another embodiment, the solid support having a linker of the invention comprises a structure of Formula 17:



17

wherein SS is a solid support, and each "n" is independently an integer from about 1 to about 200.

In another embodiment, the solid support of the instant invention is controlled pore glass (CPG) or polystyrene, and can be used in the synthesis of a nucleic acid.

In one embodiment, the invention features a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

In another embodiment, the invention features a method of treating a cancer patient, comprising contacting cells of the patient with a pharmaceutical composition of the invention under conditions suitable for the treatment. This treatment can comprise the use of one or more other drug therapies under conditions suitable for the treatment. The cancers contemplated by the instant invention include but are not limited to breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancers.

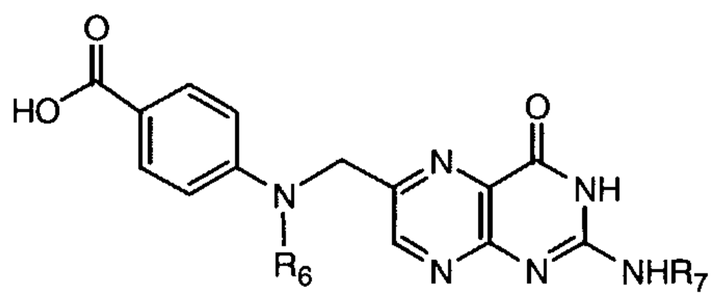
In one embodiment, the invention features a method of treating a patient infected with a virus, comprising contacting cells of the patient with a pharmaceutical composition of the invention, under conditions suitable for the treatment. This treatment can comprise the use of one or more other drug therapies under conditions suitable for the treatment. The viruses contemplated by the instant invention include but are not limited to HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, west nile virus, Ebola virus, foot and mouth virus, and papilloma virus.

In one embodiment, the invention features a kit for detecting the presence of a nucleic acid molecule or other target molecule in a sample, for example, a gene in a cancer cell, comprising a compound of the instant invention.

In one embodiment, the invention features a kit for detecting the presence of a nucleic acid molecule, or other target molecule in a sample, for example, a gene in a virus-infected cell, comprising a compound of the instant invention.

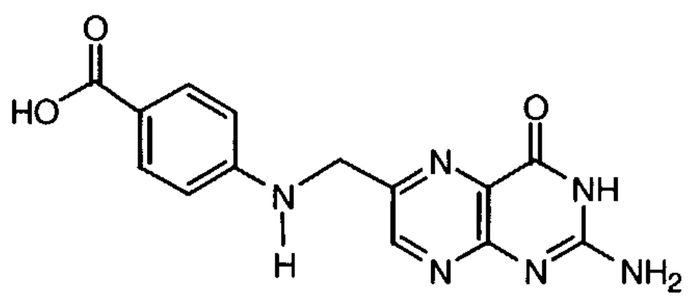
In another embodiment, the invention features a compound of the instant invention comprising a modified phosphate group, for example, a phosphoramidite, phosphodiester, phosphoramidate, phosphorothioate, phosphorodithioate, alkylphosphonate, arylphosphonate, monophosphate, diphosphate, triphosphate, or pyrophosphate.

In one embodiment, the invention features a method for synthesizing a compound having Formula 18:



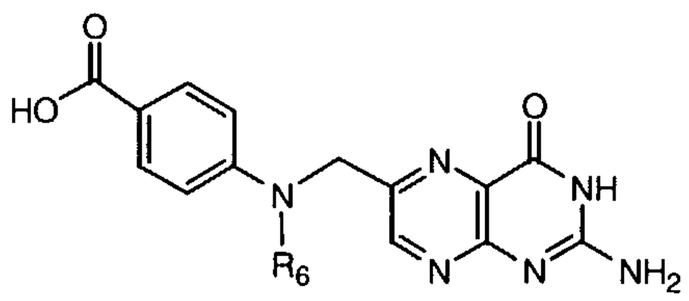
18

wherein each R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, comprising: reacting folic acid with a carboxypeptidase to yield a compound of Formula 19;



19

introducing a protecting group R_6 on the secondary amine of Formula 19 to yield a compound of Formula 20;

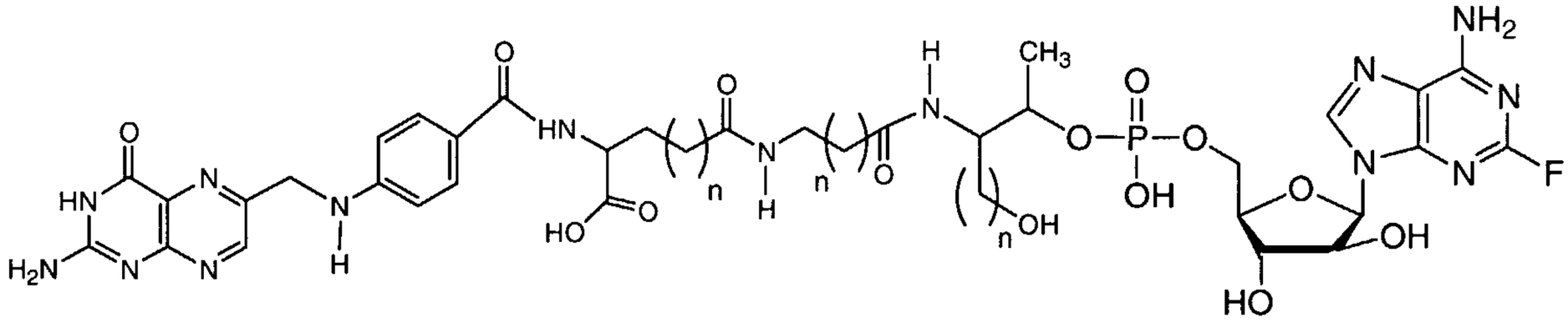


20

wherein R_6 is a nitrogen protecting group; and introducing a protecting group R_7 on the primary amine of Formula 20 to yield a compound of Formula 18.

In another embodiment, the amino protected pteroic acid of the invention is a compound of Formula 18.

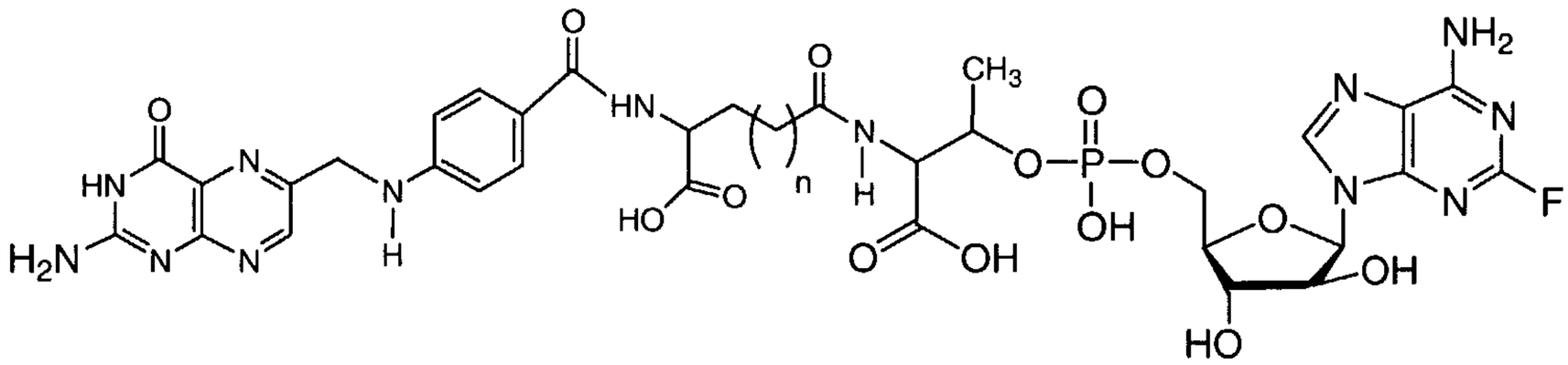
In one embodiment, the invention encompasses a compound of Formula 1 having Formula 21:



21

wherein each "n" is independently an integer from 0 to about 200.

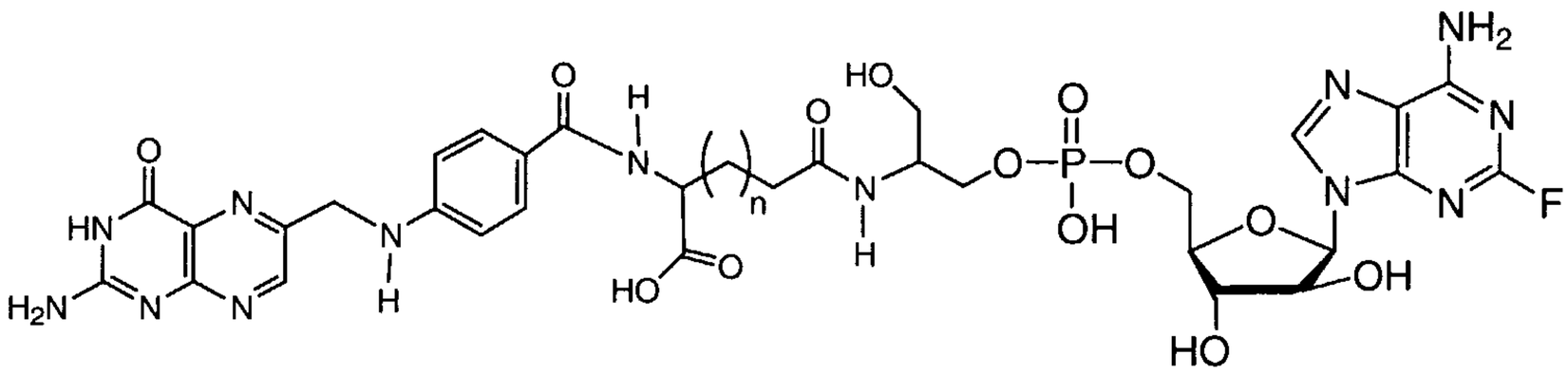
In another embodiment, the invention encompasses a compound of Formula 7 having Formula 22:



22

wherein each "n" is independently an integer from 0 to about 200.

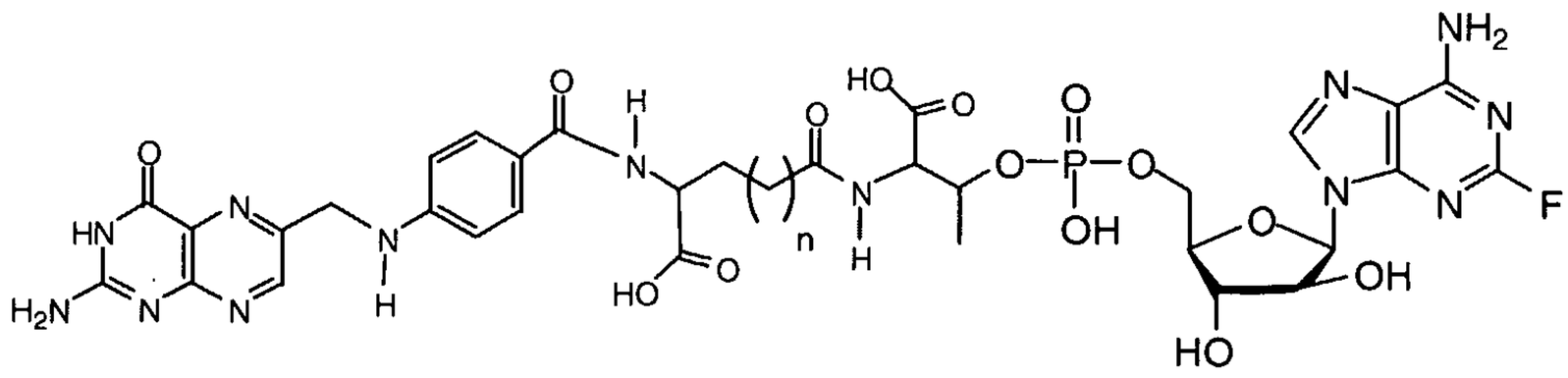
In another embodiment, the invention encompasses a compound of Formula 4 having Formula 23:



23

wherein "n" is an integer from 0 to about 200.

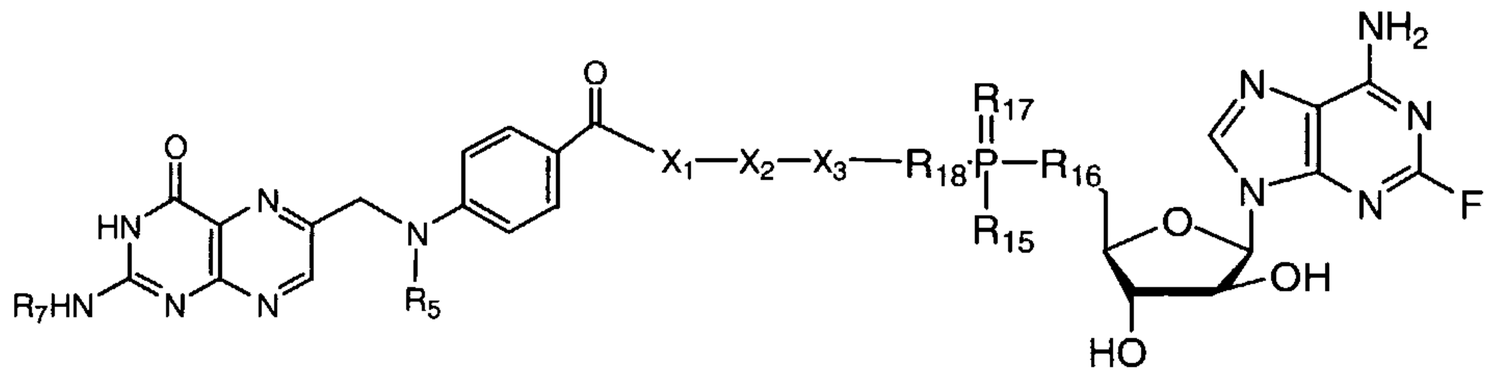
In another embodiment, the invention encompasses a compound of Formula 4 having Formula 24:



24

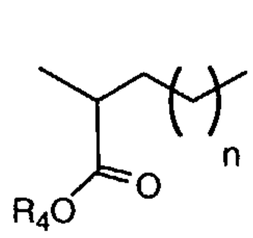
wherein "n" is an integer from 0 to about 200.

In another embodiment, the invention features a compound having Formula 25:



25

wherein each R_5 and R_7 is independently hydrogen, alkyl or a nitrogen protecting group, each R_{15}, R_{16}, R_{17}, and R_{18} is independently O, S, alkyl, substituted alkyl, aryl, substituted aryl, or halogen, X_1 is $-CH(X_{1'})$ or a group of Formula 38:

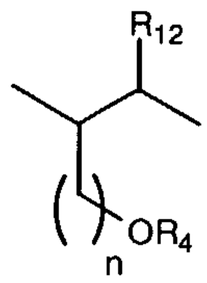


38

wherein R_4 is a protecting group and "n" is an integer from 0 to about 200;

$X_{1'}$ is the protected or unprotected side chain of a naturally occurring or non-naturally-occurring amino acid, X_2 is amide, alkyl, or carbonyl containing linker or a bond, and X_3 is a degradable linker which is optionally absent.

In another embodiment, the X_3 group of Formula 25 comprises a group of Formula 26:

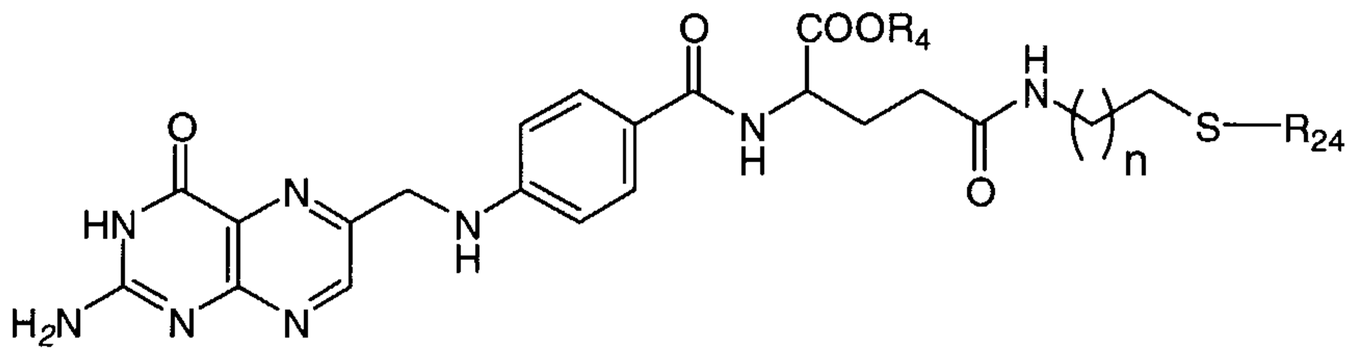


26

wherein R_4 is hydrogen or a protecting group, "n" is an integer from 0 to about 200 and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl.

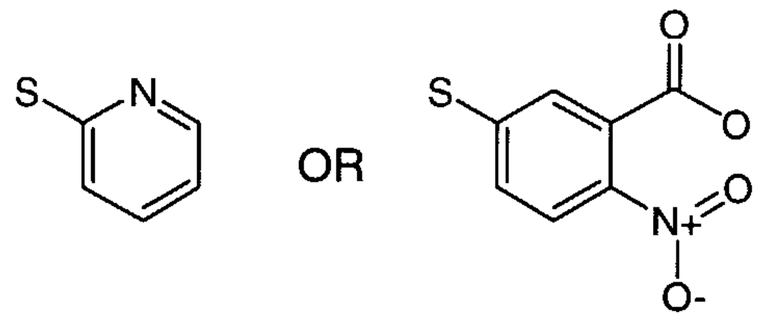
In yet another embodiment, R_4 of Formula 26 is hydrogen and R_{12} is methyl or hyrdogen.

In still another embodiment, the invention features a compound having Formula 27:



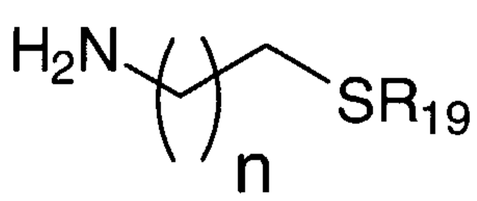
27

wherein "n" is an integer from about 0 to about 20, R_4 is H or a cationic salt, and R_{24} is a sulfur containing leaving group, for example a group comprising:



In another embodiment, the invention features a method for synthesizing a compound having Formula 27 comprising:

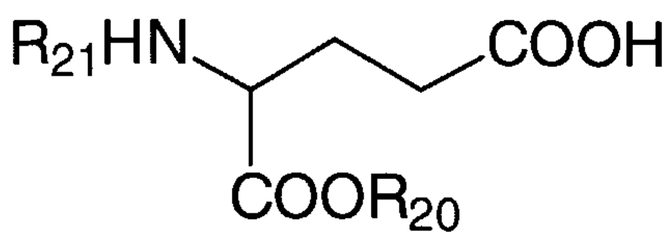
(a) selective tritylation of the thiol of cysteamine under conditions suitable to yield a compound having Formula 28:



28

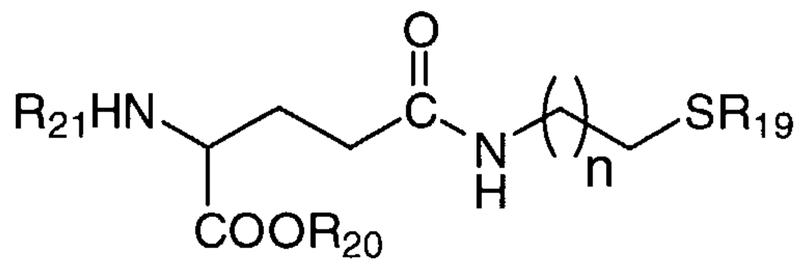
wherein "n" is an integer from about 0 to about 20 and R_{19} is a thiol protecting group;

(b) peptide coupling of the product of (a) with a compound having Formula 29:



29

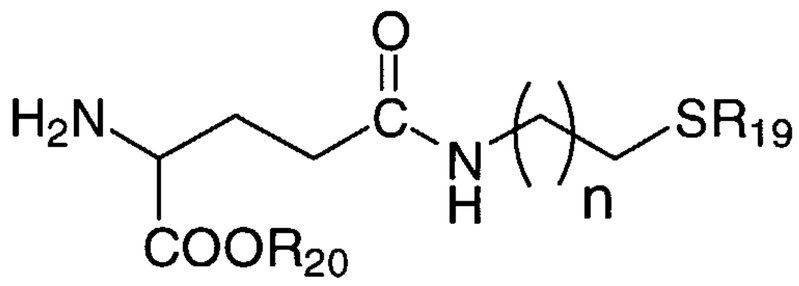
wherein R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group, under conditions suitable to yield a compound having Formula 30:



30

wherein "n" is an integer from about 0 to about 20, R_{19} is a thiol protecting group, R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group;

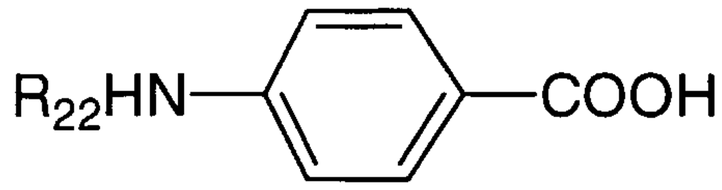
(c) removing the amino protecting group R_{21} of the product of (b) under conditions suitable to yield a compound having Formula 31:



31

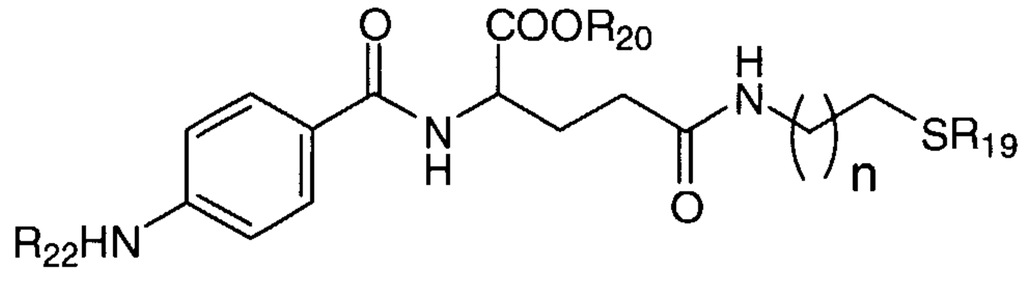
wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b);

(d) condensation of the product of (c) with a compound having Formula 32:



32

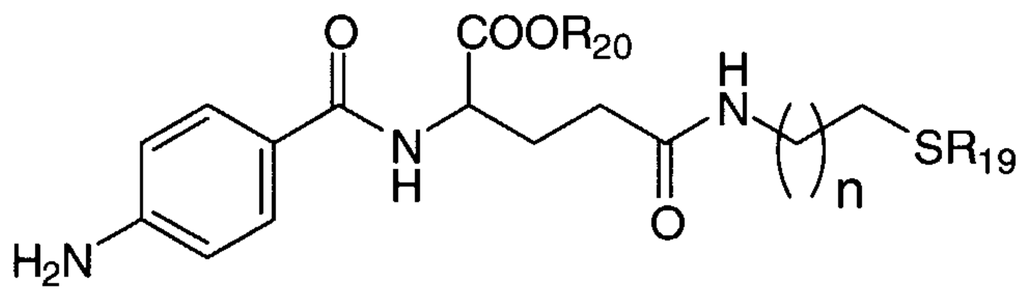
wherein R_{22} is an amino protecting group, under conditions suitable to yield a compound having Formula 33:



33

wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b) and R_{22} is as described in (d);

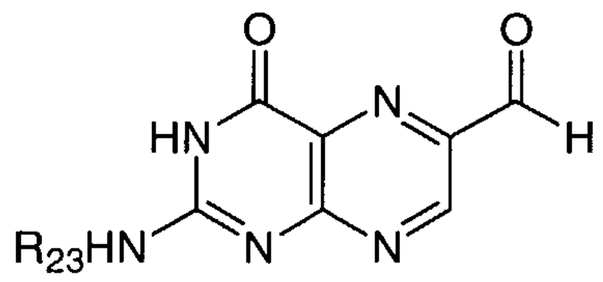
(e) selective cleavage of R_{22} from the product of (d) under conditions suitable to yield a compound having Formula 34:



34

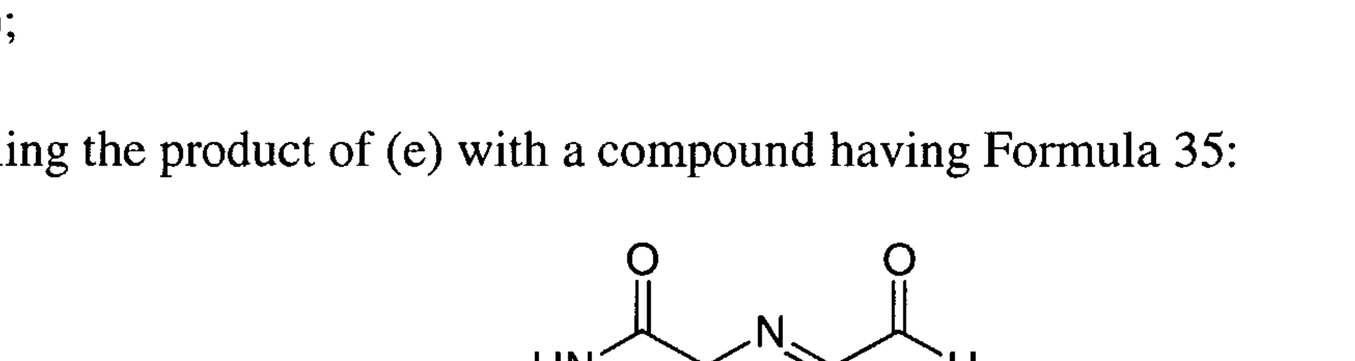
wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b);

(f) coupling the product of (e) with a compound having Formula 35:



35

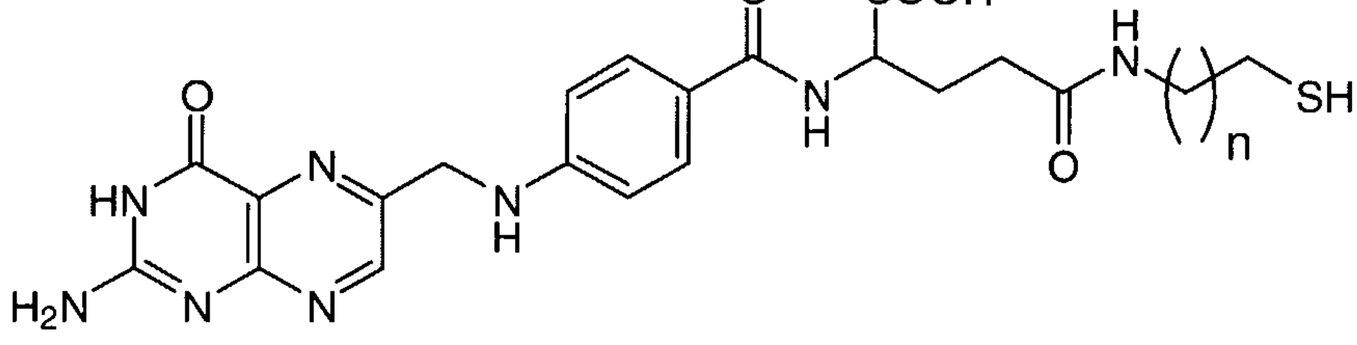
wherein R_{23} is an amino protecting group under conditions suitable to yield a compound having Formula 36:



36

wherein R_{23} is an amino protecting group, "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b);

(g) deprotecting the product of (f) under conditions suitable to yield a compound having Formula 37.

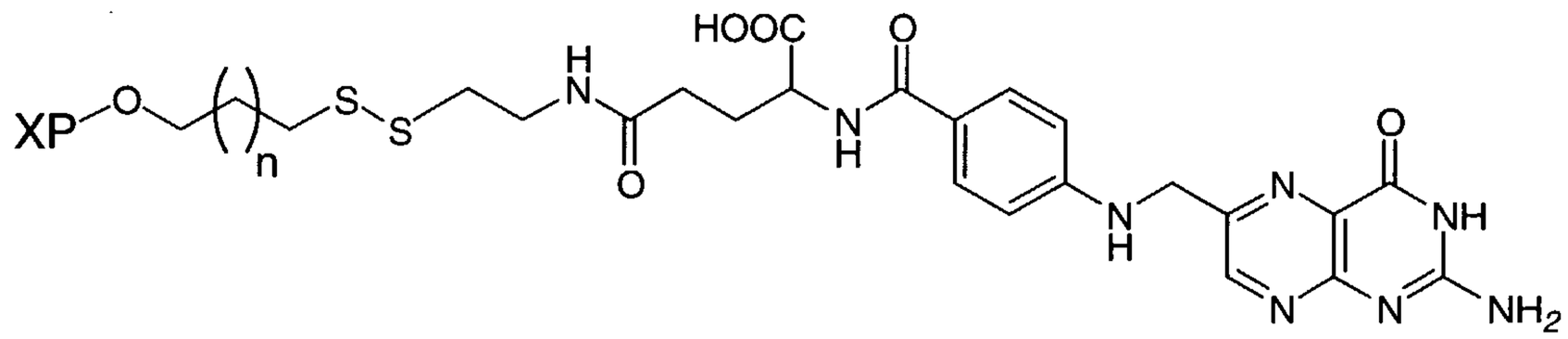


37

wherein "n" is an integer from about 0 to about 20; and

(h) introducing a disulphide-based leaving group to the product of (g) under conditions suitable to yield a compound having Formula 27.

In one embodiment, the invention features a compound having Formula 39:

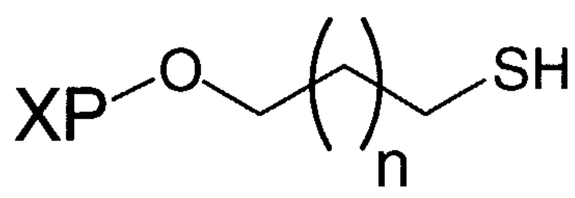


39

wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group.

In another embodiment, the invention features a method for synthesizing a compound having Formula 39, comprising:

(a) Coupling a thiol containing linker to a nucleic acid, polynucleotide or oligonucleotide under conditions suitable to yield a compound having Formula 40:

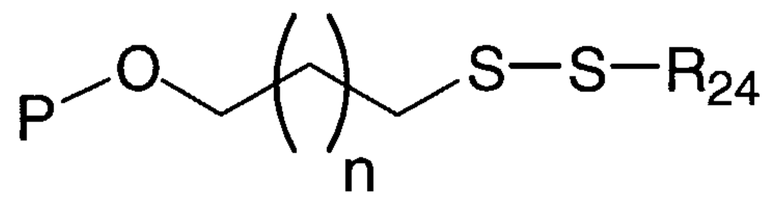


40

wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group; and

(b) coupling the product of (a) with a compound having Formula 37 under conditions suitable to yield a compound having Formula 39.

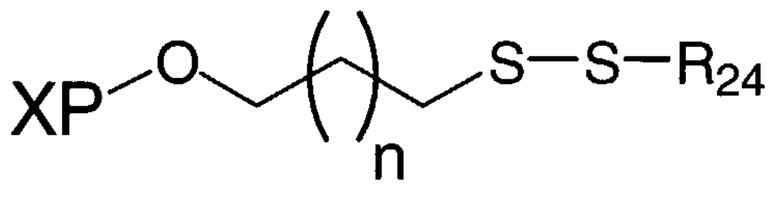
In another embodiment, the thiol containing linker of the invention is a compound having Formula 41:



41

wherein "n" is an integer from about 0 to about 20, P is a phosphorus containing group, for example a phosphine, phosphite, or phosphate, and R_{24} is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

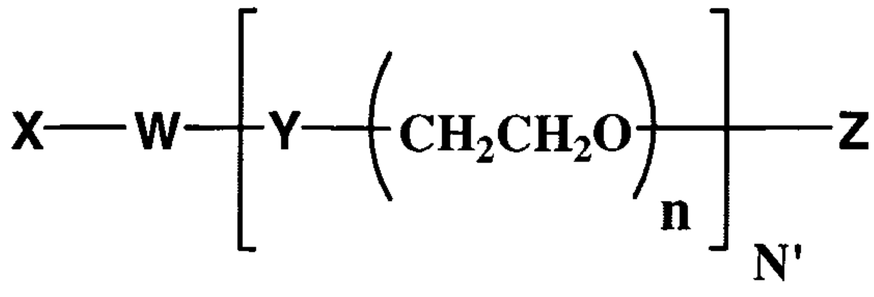
In another embodiment, the conditions suitable to yield a compound having Formula 40 comprises reduction, for example using dithiothreitol (DTT) or any equivalent disulphide reducing agent, of the disulfide bond of a compound having Formula 42:



42

wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, P is a phosphorus containing group, and R_{24} is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

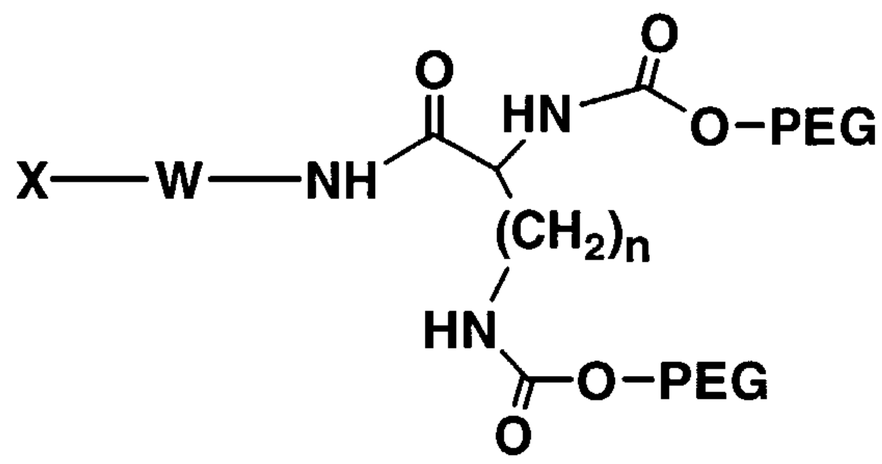
In one embodiment, the invention features a compound having Formula 43:



43

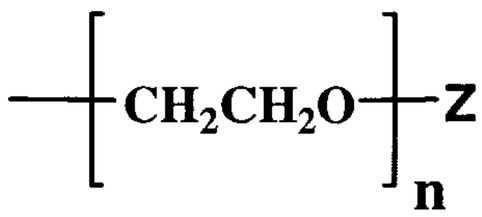
wherein X comprises a biologically active molecule; W comprises a degradable nucleic acid linker; Y comprises a linker molecule or amino acid that can be present or absent; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; n is an integer from about 1 to about 100; and N' is an integer from about 1 to about 20.

In another embodiment, the invention features a compound having Formula 44:



44

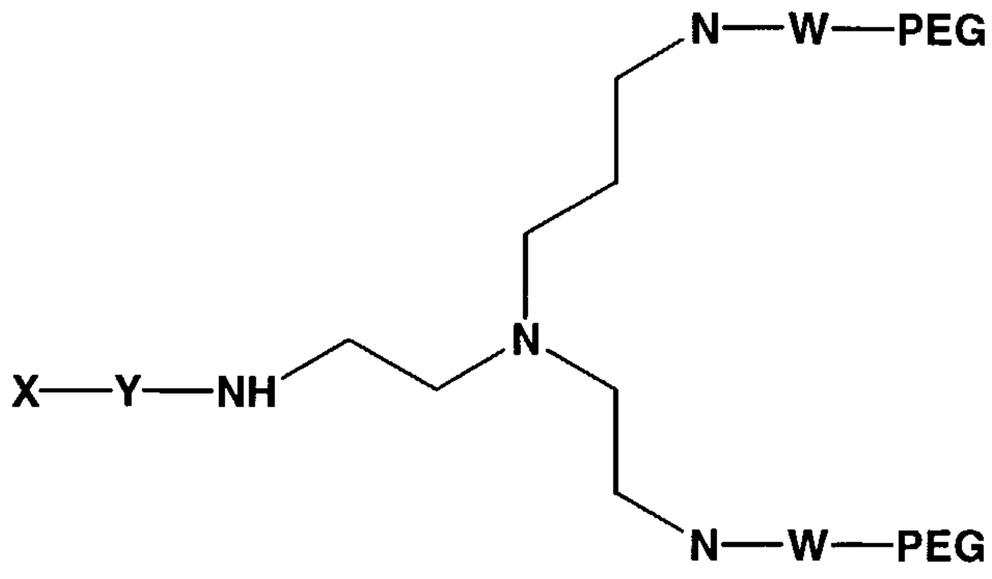
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; n is an integer from about 1 to about 50, and PEG represents a compound having Formula 45:



45

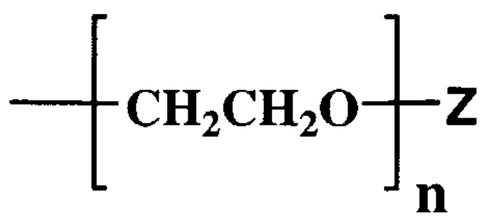
wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

In another embodiment, the invention features a compound having Formula 46:



46

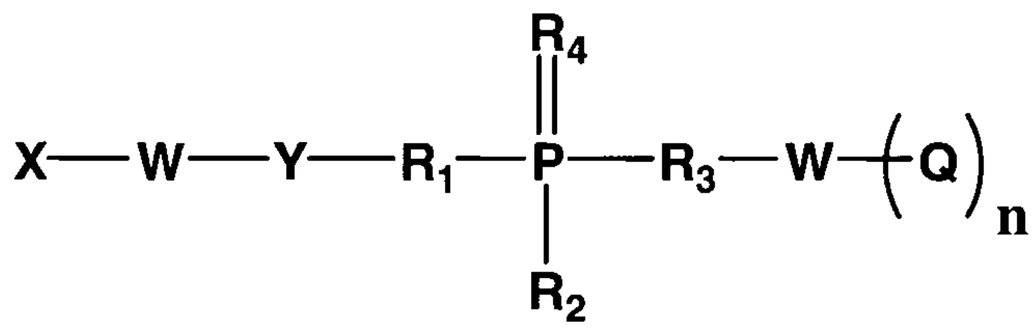
wherein X comprises a biologically active molecule; each W independently comprises linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule or chemical linkage that can be present or absent; and PEG represents a compound having Formula 45:



45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

In one embodiment, the invention features a compound having Formula 47:

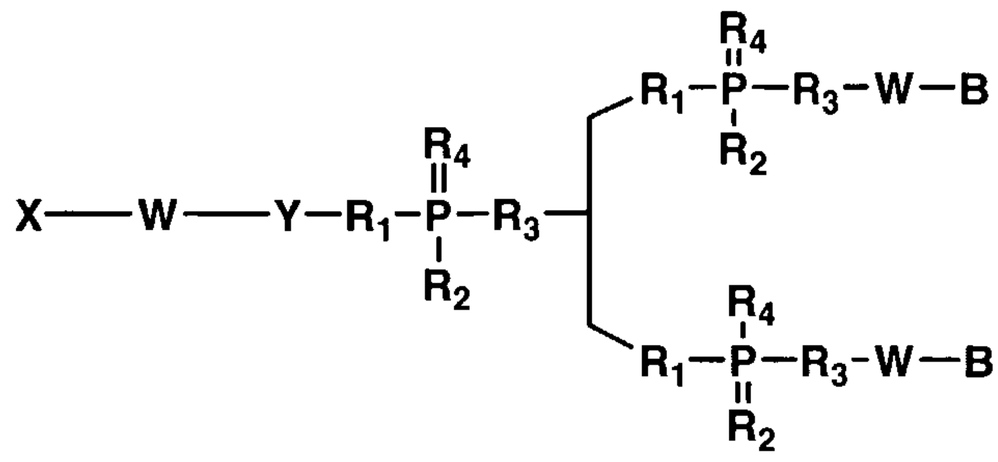


47

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be the same or different and can be present or absent, Y comprises a linker molecule that can be present or absent; each Q independently comprises a hydrophobic group or phospholipid; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-

alkyl, S-alkylcyano, N or substituted N, and n is an integer from about 1 to about 10.

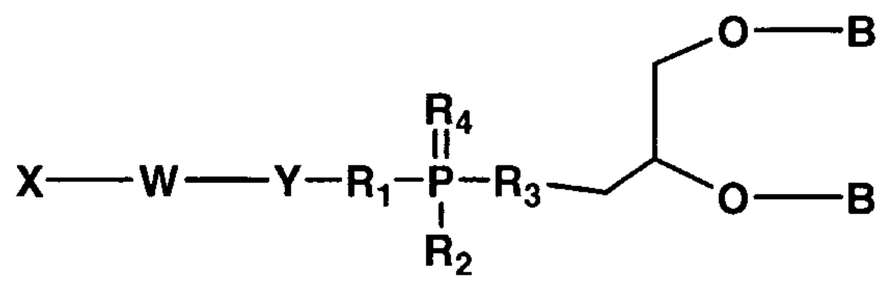
In another embodiment, the invention features a compound having Formula 48:



48

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and B represents a lipophilic group, for example a saturated or unsaturated linear, branched, or cyclic alkyl group.

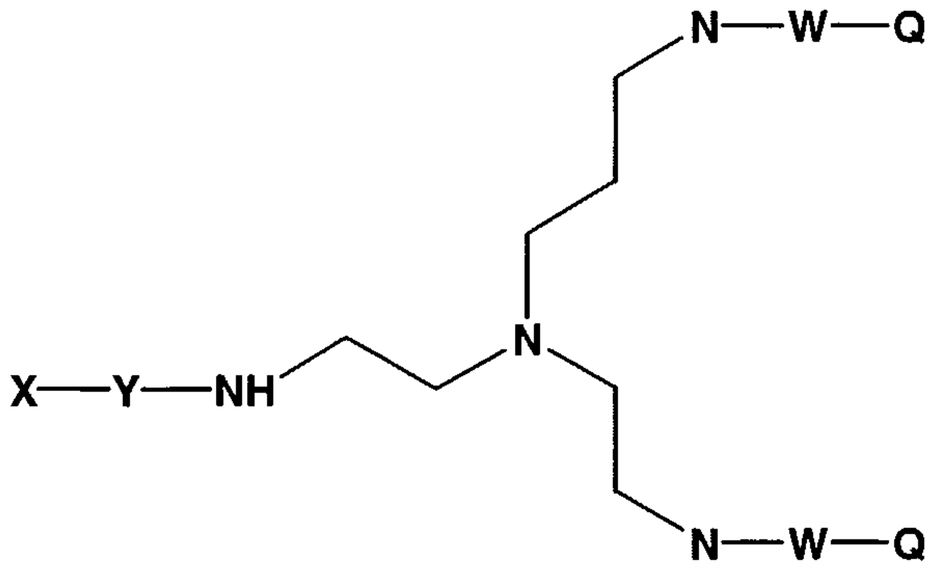
In another embodiment, the invention features a compound having Formula 49:



49

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and B represents a lipophilic group, for example a saturated or unsaturated linear, branched, or cyclic alkyl group..

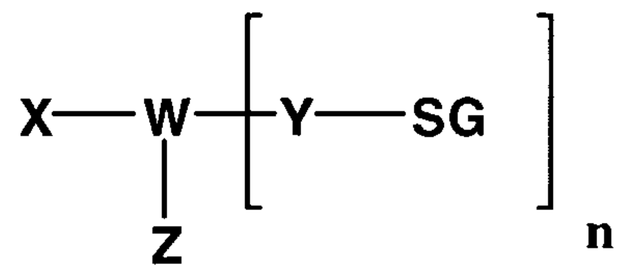
In another embodiment, the invention features a compound having Formula 50:



50

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule or chemical linkage that can be present or absent; and each Q independently comprises a hydrophobic group or phospholipid.

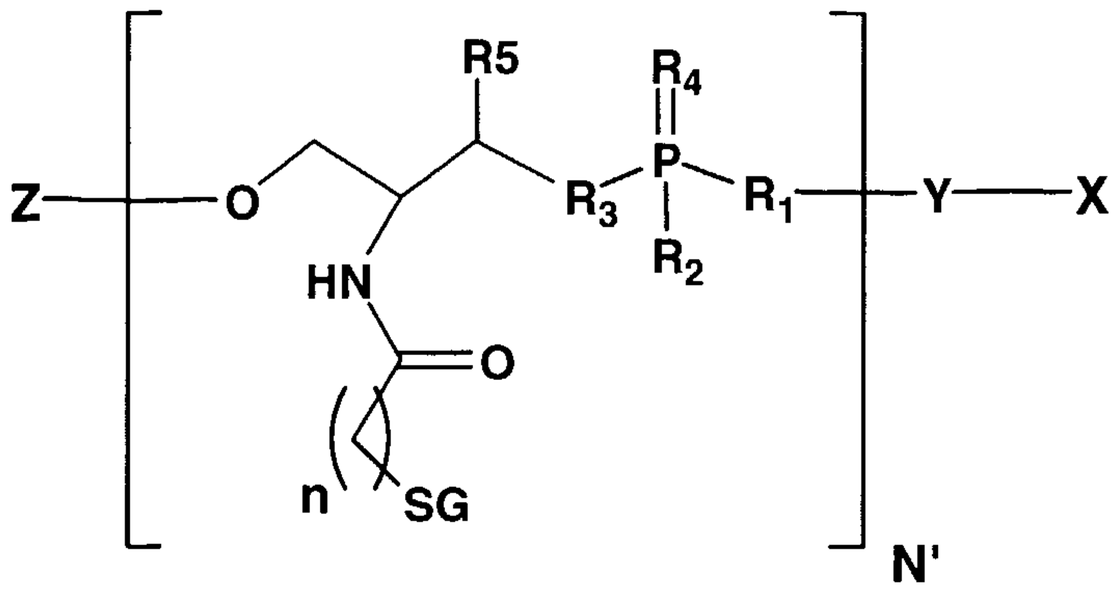
In one embodiment, the invention features a compound having Formula 51:



51

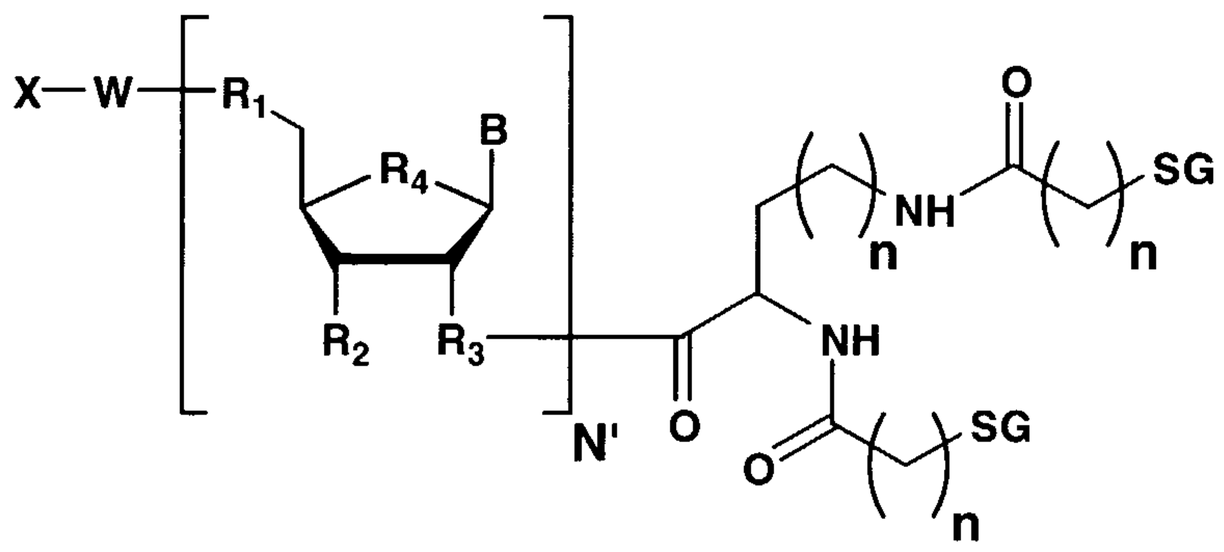
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; Y comprises a linker molecule or amino acid that can be present or absent; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and n is an integer from about 1 to about 20.

In another embodiment, the invention features a compound having Formula 52:



wherein X comprises a biologically active molecule; Y comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, R3, R4, and R5 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, n is an integer from about 1 to about 20; and N' is an integer from about 1 to about 20..

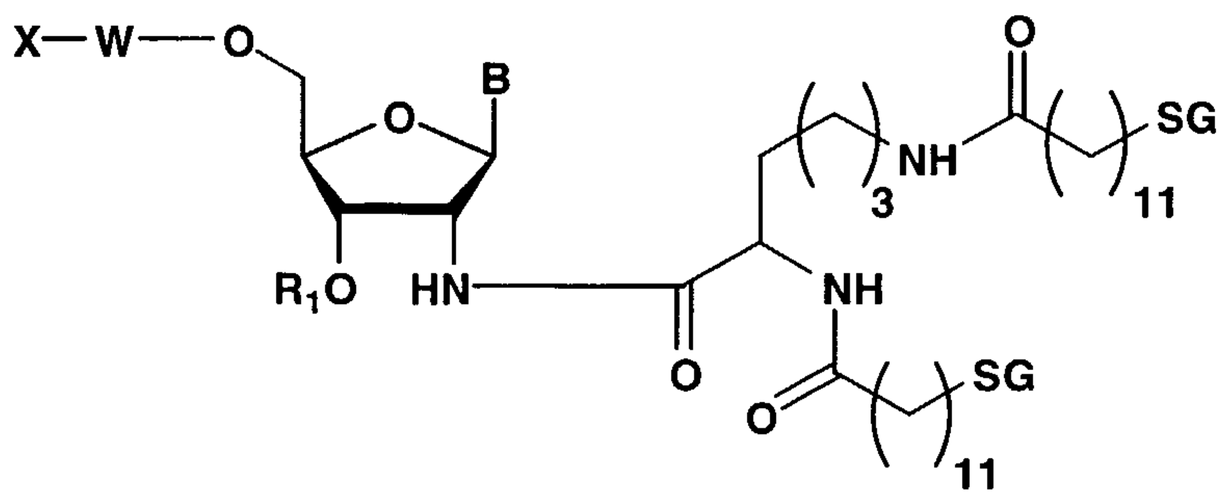
In another embodiment, the invention features a compound having Formula 53:



wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises N or O-N, each R4 independently comprises O, CH2, S, sulfone, or sulfoxy; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic

acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers,, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 10.

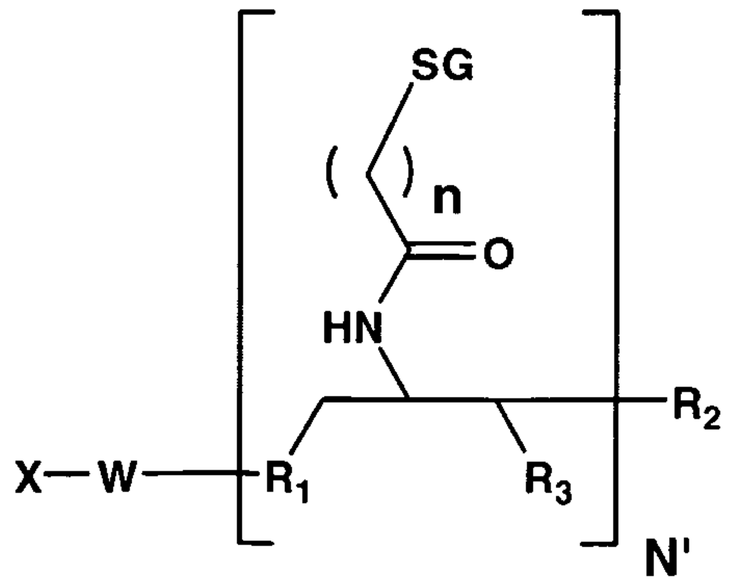
In another embodiment, the invention features a compound having Formula 54:



54

wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; and SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers.

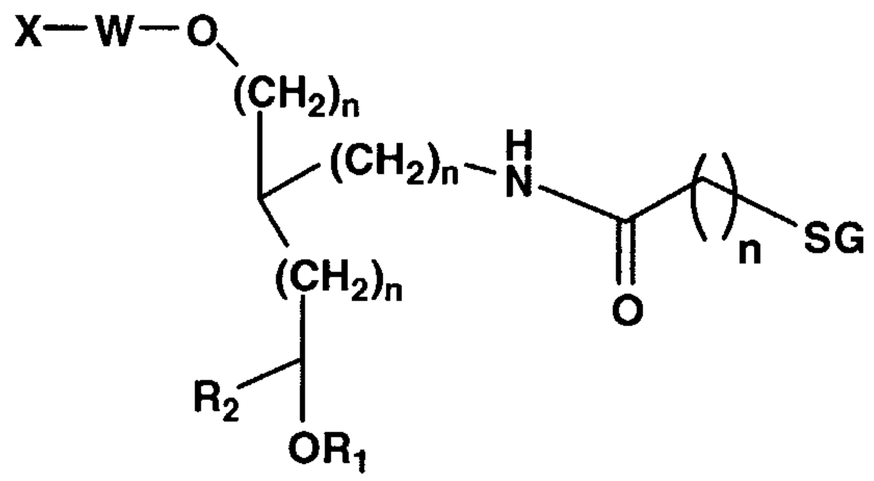
In one embodiment, the invention features a compound having Formula 55:



55

wherein each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100.

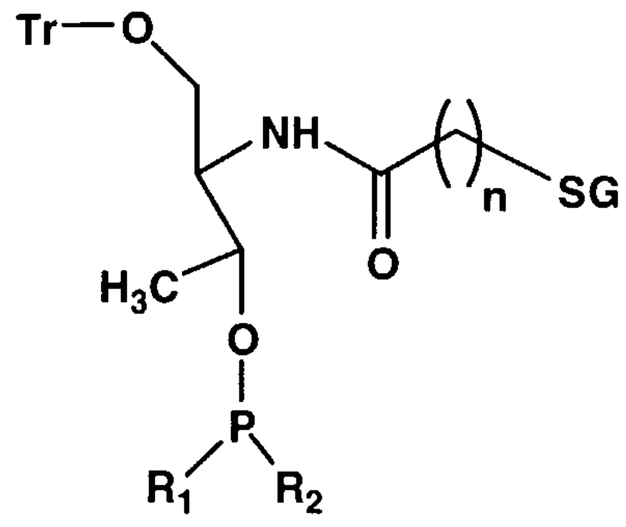
In another embodiment, the invention features a compound having Formula 56:



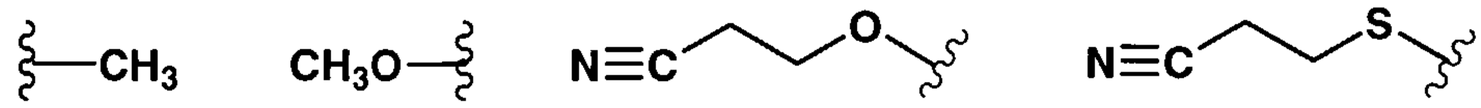
56

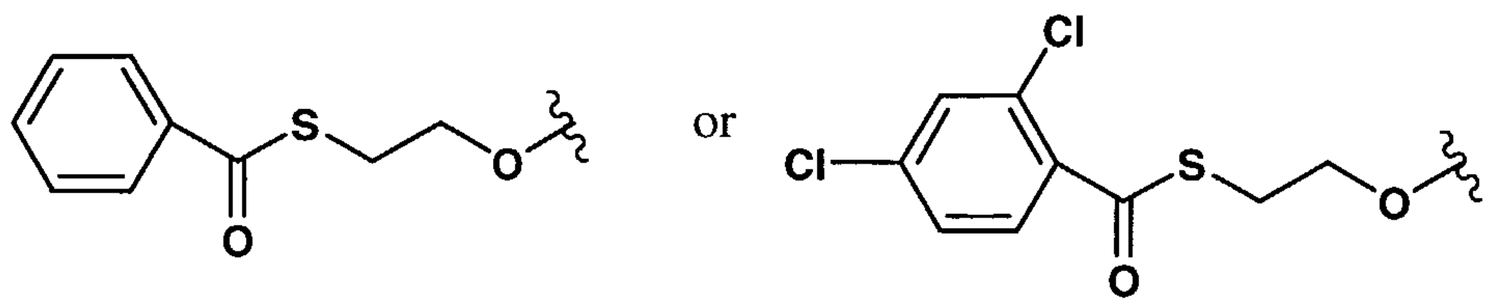
wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and each n is independently an integer from about 0 to about 20.

In another embodiment, the invention features a compound having Formula 57:

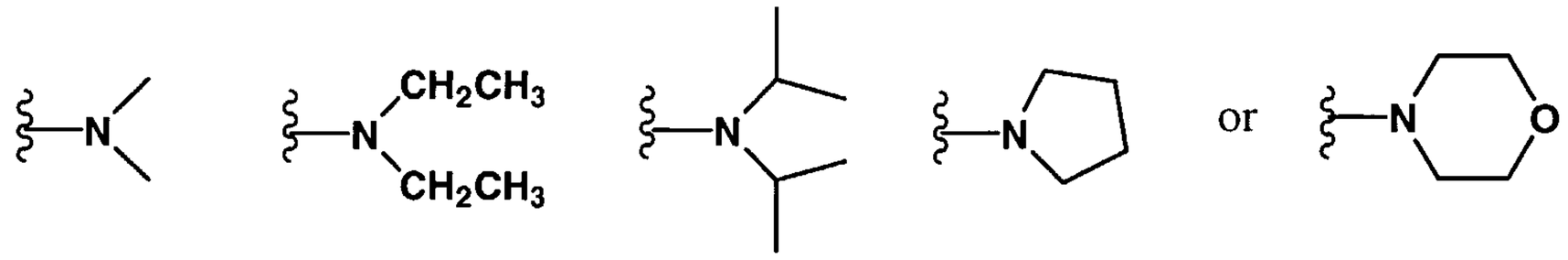


wherein R1 can include the groups:





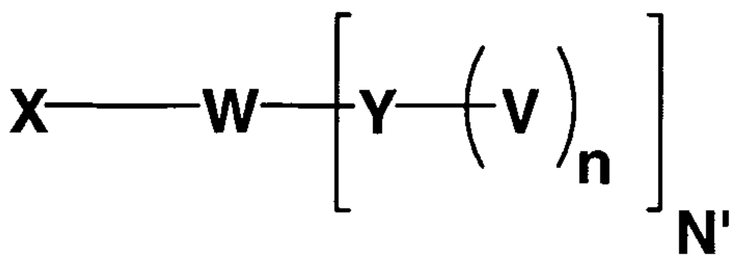
and wherein R2 can include the groups:



and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and n is an integer from about 1 to about 20.

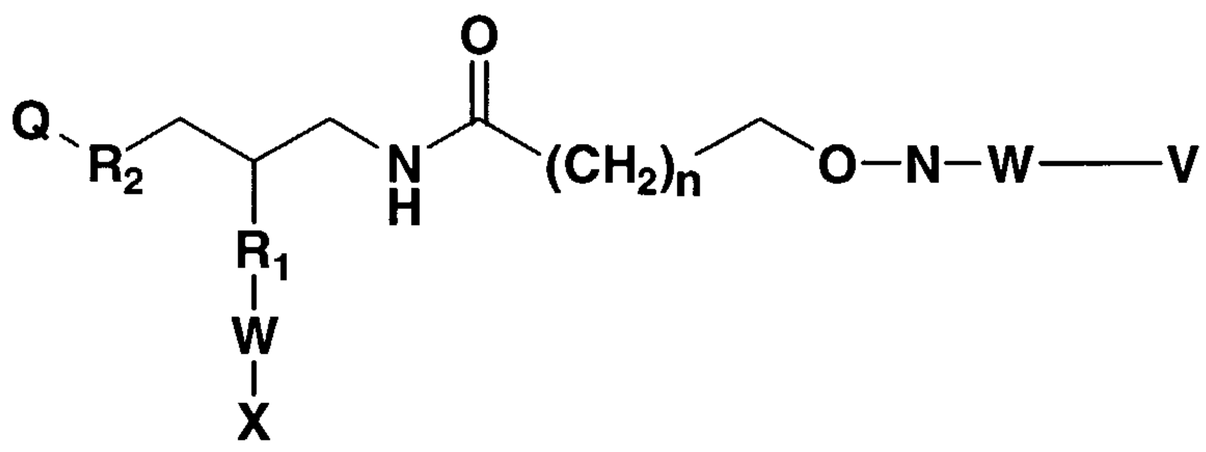
In one embodiment, compounds having Formula 52, 53, 54, 55, 56, and 57 are featured wherein each nitrogen adjacent to a carbonyl can independently be substituted for a carbonyl adjacent to a nitrogen or each carbonyl adjacent to a nitrogen can be substituted for a nitrogen adjacent to a carbonyl.

In another embodiment, the invention features a compound having Formula 58:



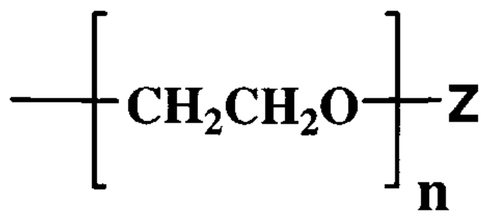
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; Y comprises a linker molecule or amino acid that can be present or absent; V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide; each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100.

In another embodiment, the invention features a compound having Formula 59:



59

wherein each R1 independently comprises O, S, N, substituted N, or a phosphorus containing group; each R2 independently comprises O, S, or N; X comprises H, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, or enzymatic nucleic acid or other biologically active molecule; n is an integer from about 1 to about 50, Q comprises H or a removable protecting group which can be optionally absent, each W independently comprises a linker molecule or chemical linkage that can be present or absent, and V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide, or a compound having Formula 45

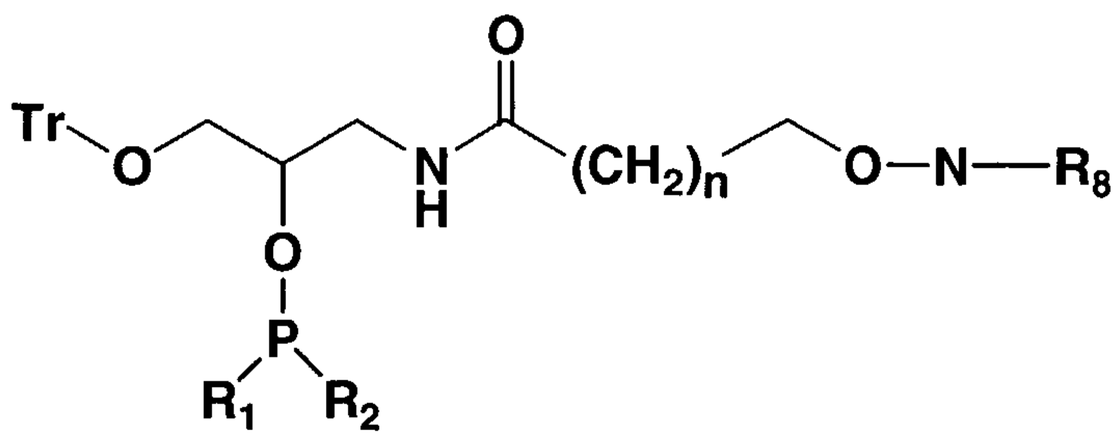


45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid,

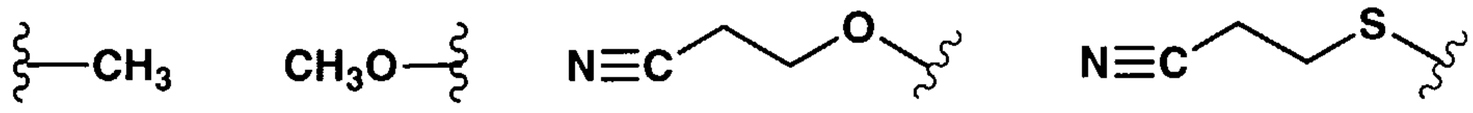
oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100..

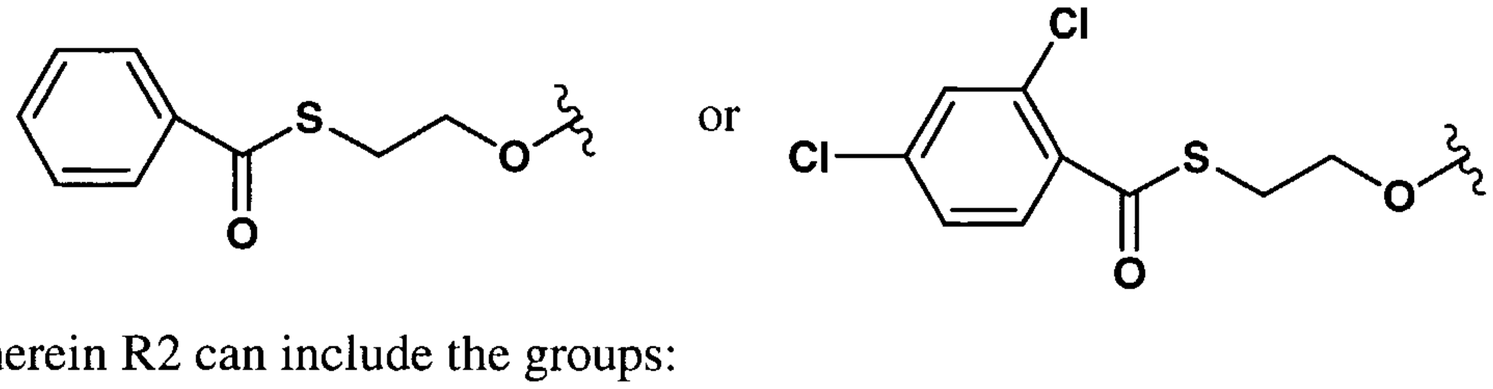
In another embodiment, the invention features a compound having Formula 60:



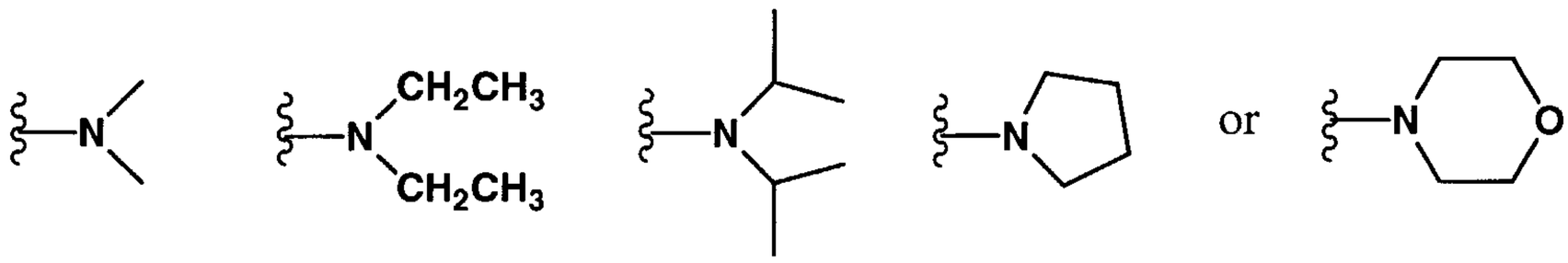
60

wherein R1 can include the groups:



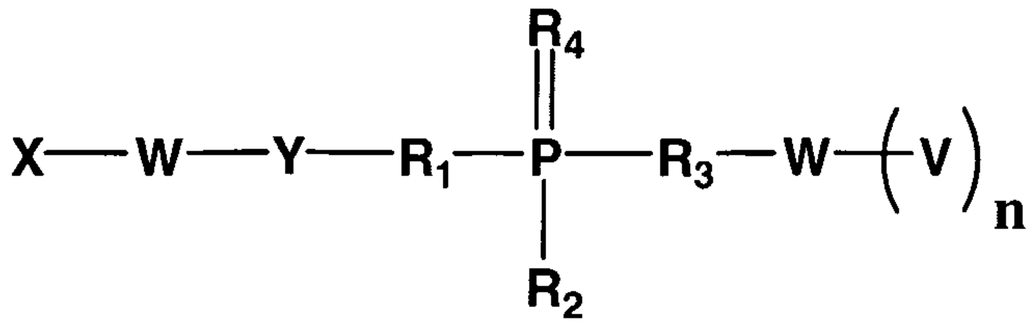


and wherein R2 can include the groups:



and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; n is an integer from about 1 to about 50; and R8 is a nitrogen protecting group, for example a phthaloyl, trifluoroacetyl, FMOC, or monomethoxytrityl group.

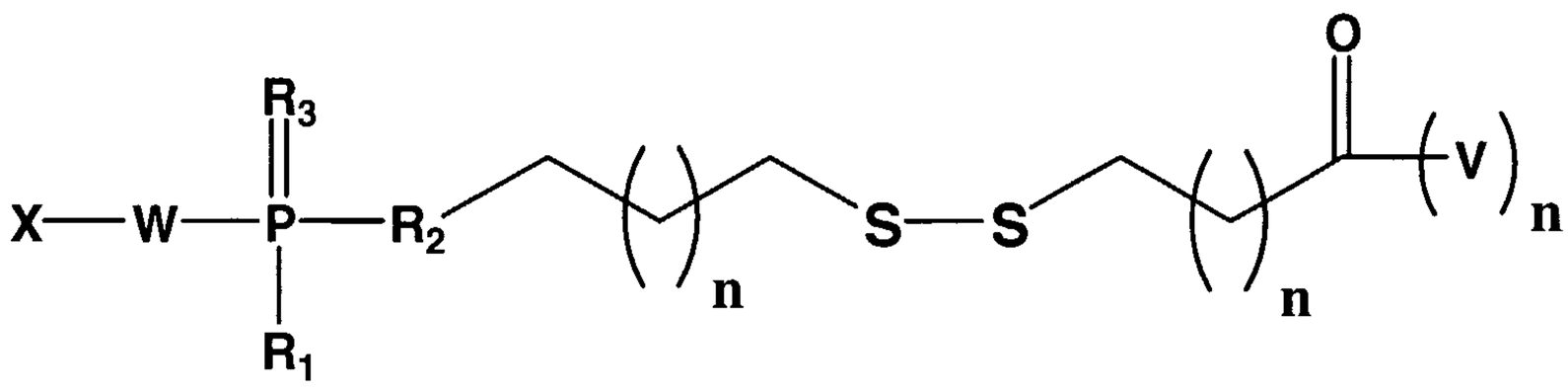
In another embodiment, the invention features a compound having Formula 61:



61

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be the same or different and can be present or absent, Y comprises a linker molecule that can be present or absent; each independently comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide;; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and n is an integer from about 1 to about 10.

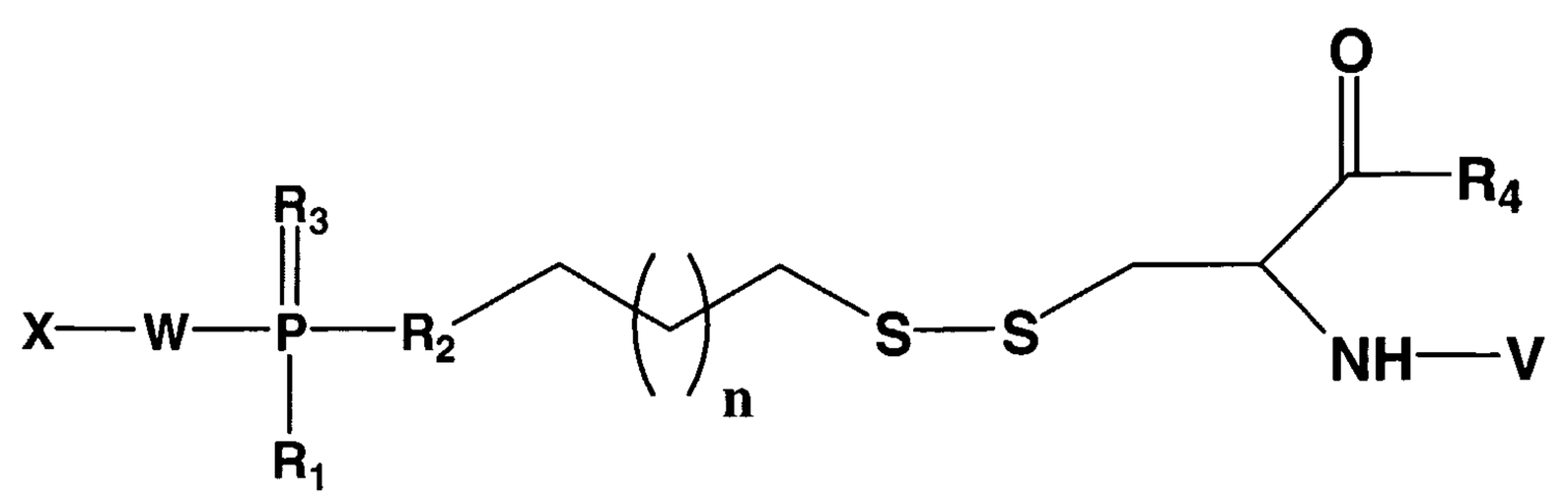
In another embodiment, the invention features a compound having Formula 62:



62

wherein X comprises a biologically active molecule; each independently comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide; W comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, and R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and each n is independently an integer from about 1 to about 10.

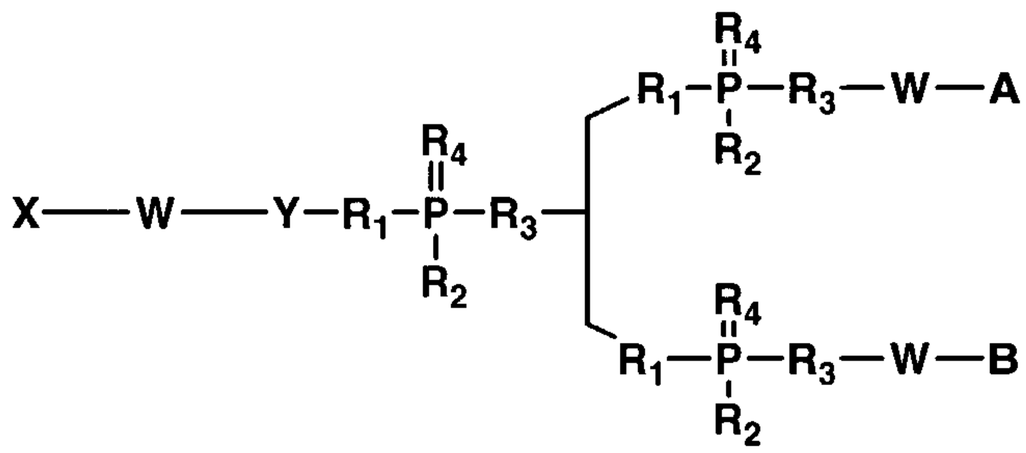
In another embodiment, the invention features a compound having Formula 63:



63

wherein X comprises a biologically active molecule; V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide; W comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, R4 represents an ester, amide, or protecting group, and each n is independently an integer from about 1 to about 10.

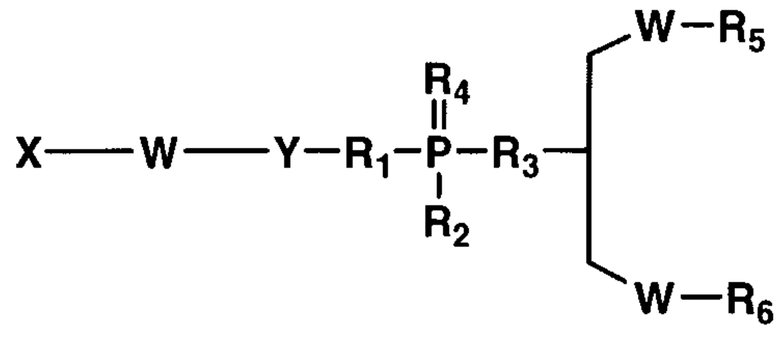
In another embodiment, the invention features a compound having Formula 64:



64

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, A comprises a nitrogen containing group, and B comprises a lipophilic group.

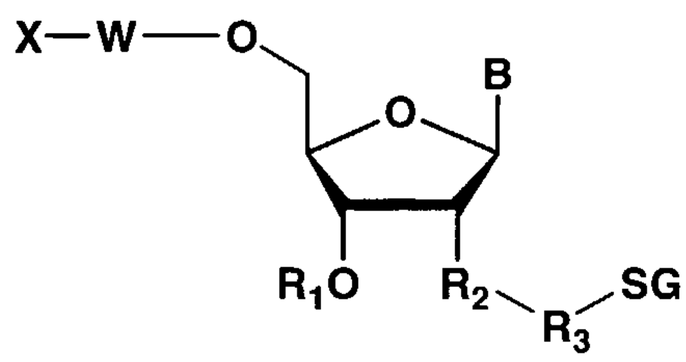
In another embodiment, the invention features a compound having Formula 65:



65

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, RV comprises the lipid or phospholipid component of any of Formulae 47-50, and R6 comprises a nitrogen containing group.

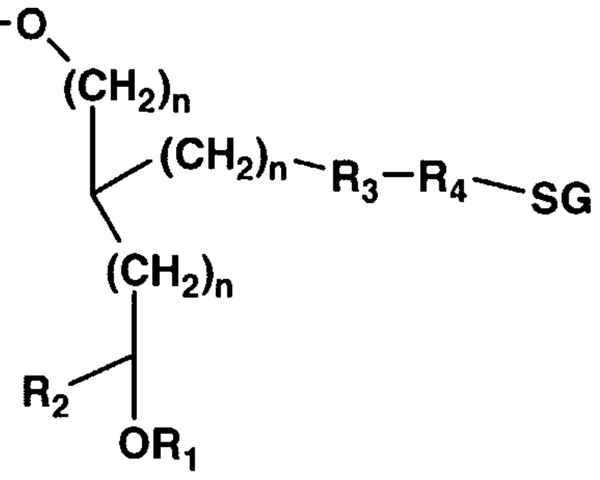
In another embodiment, the invention features a compound having Formula 92:



92

wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; R2 comprises O, NH, S, CO, COO, ON=C, or alkyl; R3 comprises alkyl, akloxy, or an aminoacyl side chain; and SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers.

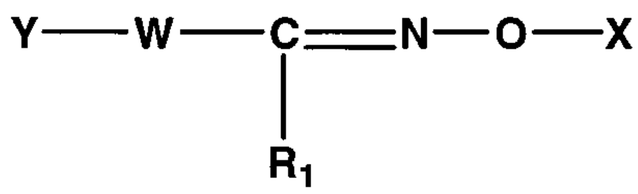
In another embodiment, the invention features a compound having Formula 86:



86

wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; R3 comprises O, NH, S, CO, COO, ON=C, or alkyl; R4 comprises alkyl, akloxy, or an aminoacyl side chain; and SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and each n is independently an integer from about 0 to about 20.

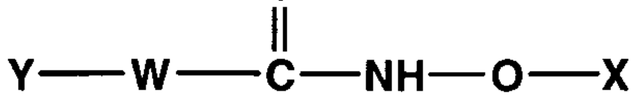
In another embodiment, the invention features a compound having Formula 87:



87

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; W comprises a linker molecule or chemical linkage that can be present or absent; and Y comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody; R1 comprises H, alkyl, or substituted alkyl.

In another embodiment, the invention features a compound having Formula 88:

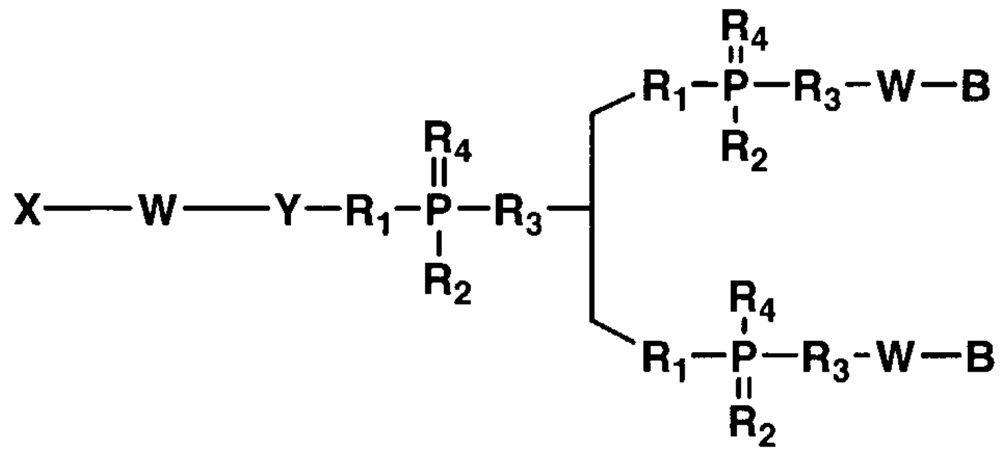


88

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide,

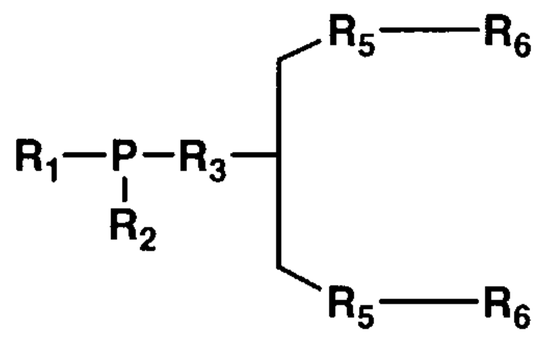
nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody.

In one embodiment, the invention features a method for the synthesis of a compound having Formula 48:



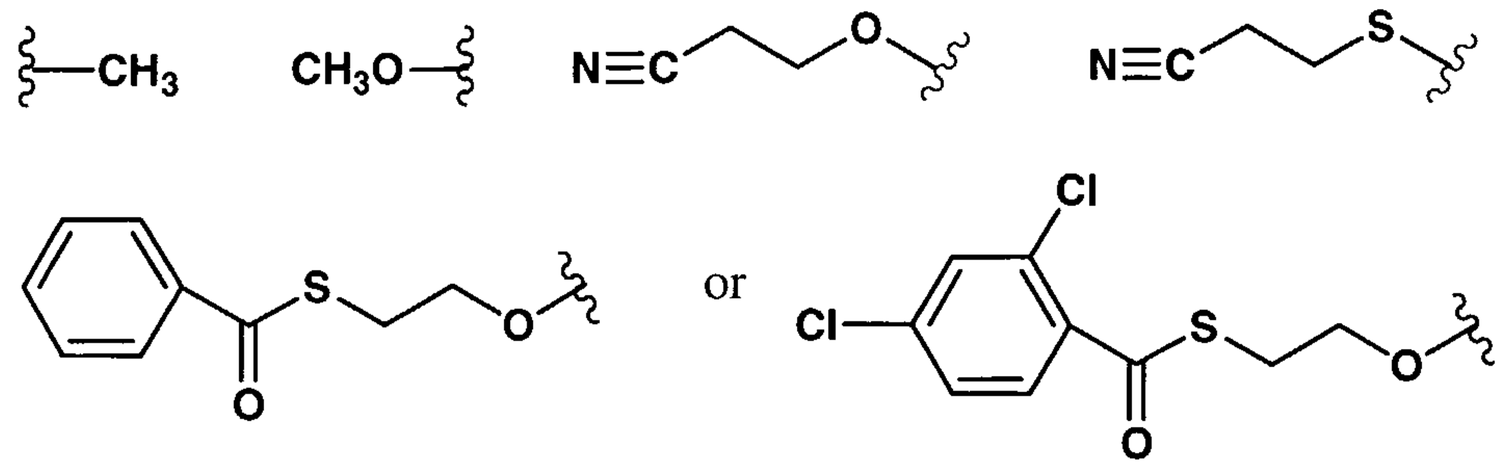
48

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; and each B independently represents a lipophilic group, for example a saturated or unsaturated linear, branched, or cyclic alkyl group, comprising:
(a) introducing a compound having Formula 66:

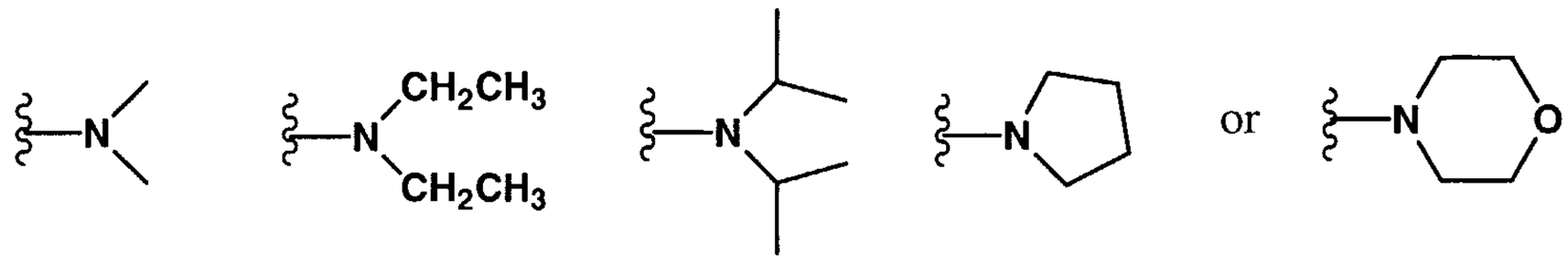


66

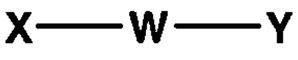
wherein R1 is defined as in Formula 48 and can include the groups:



and wherein R2 is defined as in Formula 48 and can include the groups:

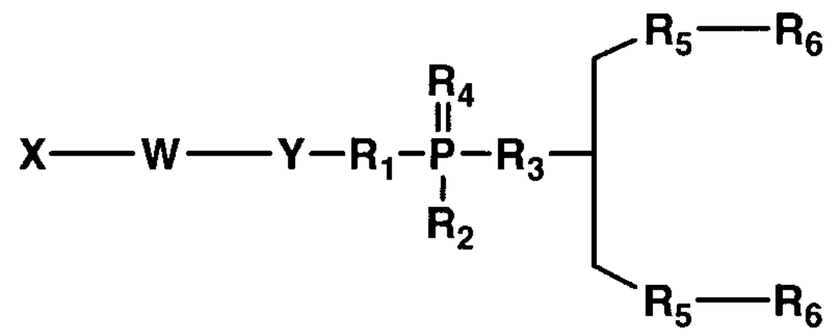


and wherein each R5 independently comprises O, N, or S and each R6 independently comprises a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group, to a compound having Formula 67:



67

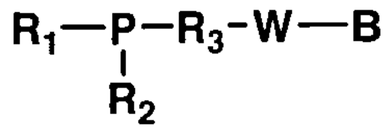
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 68:



68

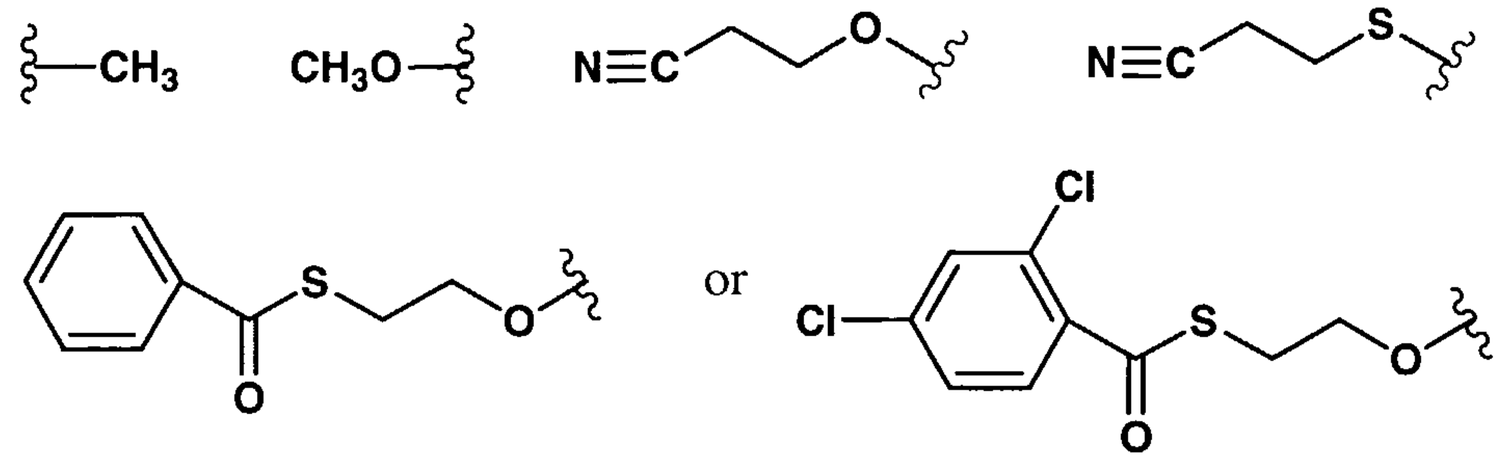
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; and each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N comprising, each R5 independently comprises O, S, or N; and each R6 is independently a removable protecting group, for example a trityl, monomethoxytrityl, or

dimethoxytrityl group; (b) removing R6 from the compound having Formula 26 and (c) introducing a compound having Formula 69:

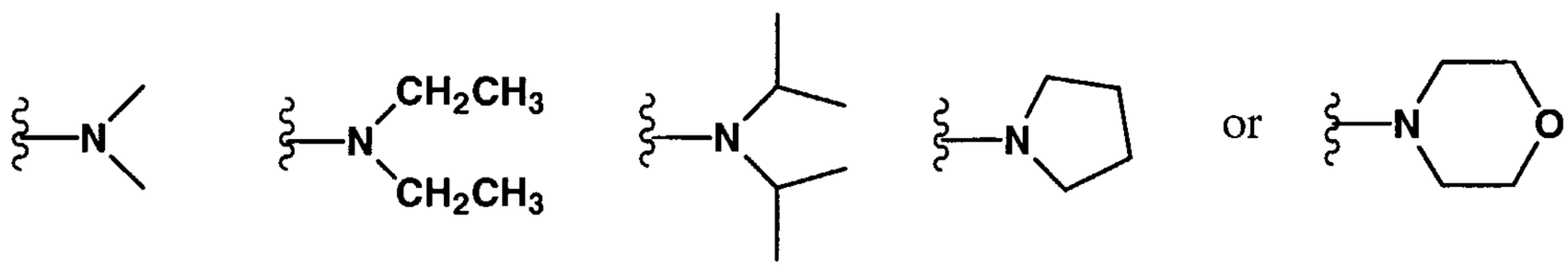


69

wherein R1 is defined as in Formula 48 and can include the groups:

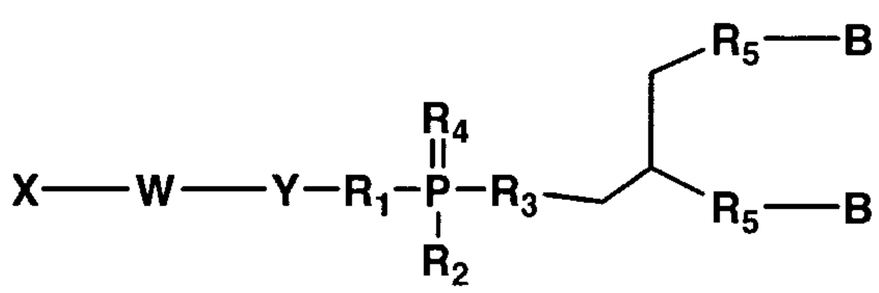


and wherein R2 is defined as in Formula 48 and can include the groups:



and wherein W and B are defined as in Formula 48, to the compound having Formula 68 under conditions suitable for the formation of a compound having Formula 48.

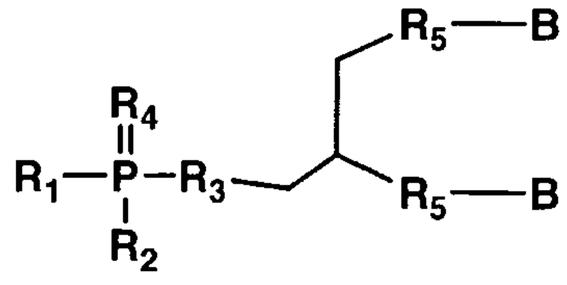
In another embodiment, the invention features a method for the synthesis of a compound having Formula 49:



49

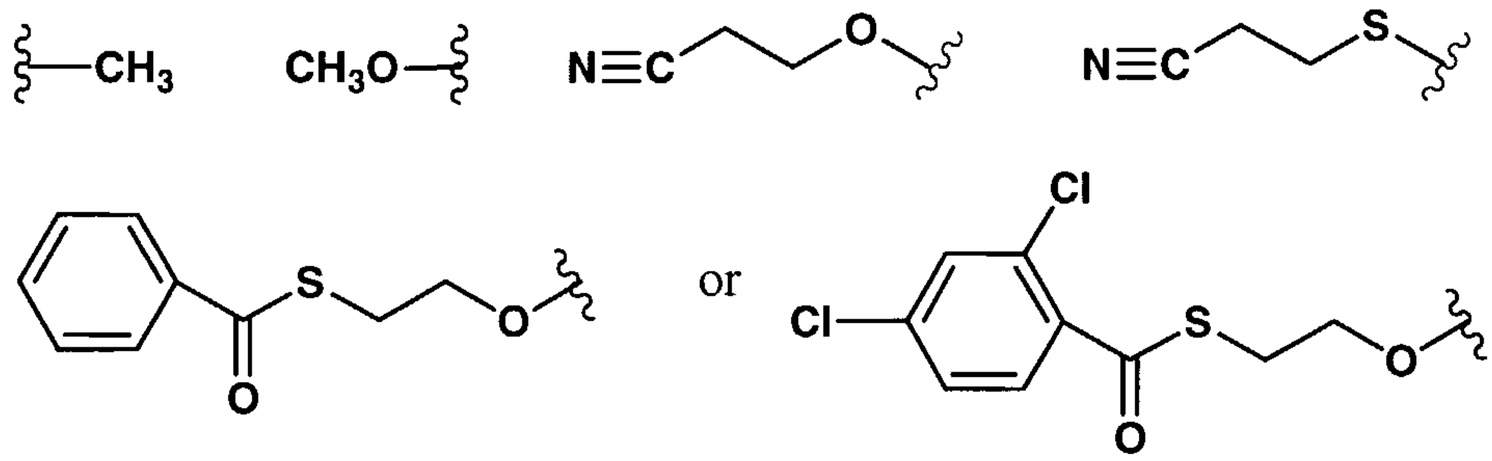
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; each R5 independently comprises O, S, or N; and each B

independently comprises a lipophilic group, for example a saturated or unsaturated linear, branched, or cyclic alkyl group, comprising: (a) coupling a compound having Formula 70:

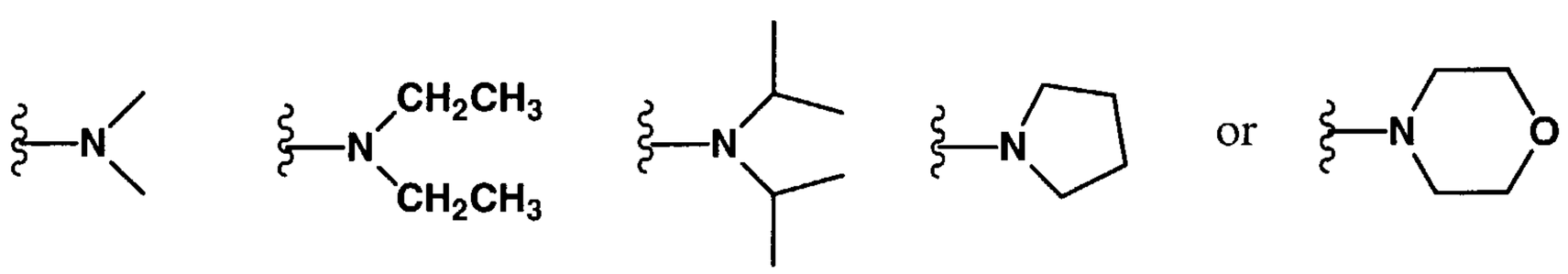


70

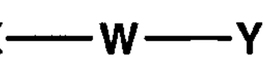
wherein R1 is defined as in Formula 49 and can include the groups:



and wherein R2 is defined as in Formula 49 and can include the groups:



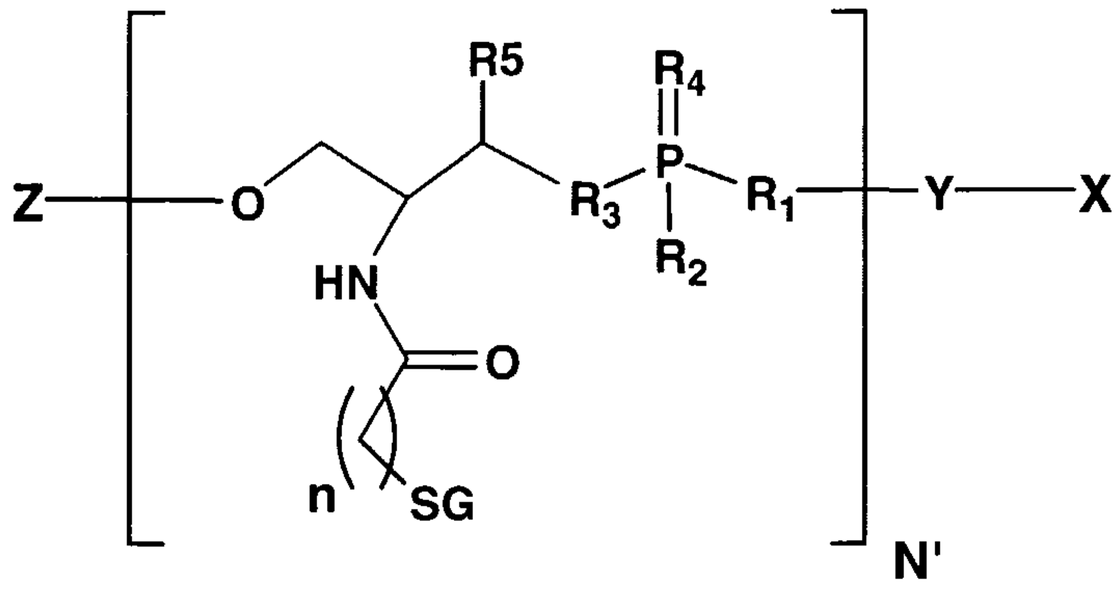
and wherein each R5 independently comprises O, S, or N, and wherein each B independently comprises a lipophilic group, for example a saturated or unsaturated linear, branched, or cyclic alkyl group, with a compound having Formula 67:



67

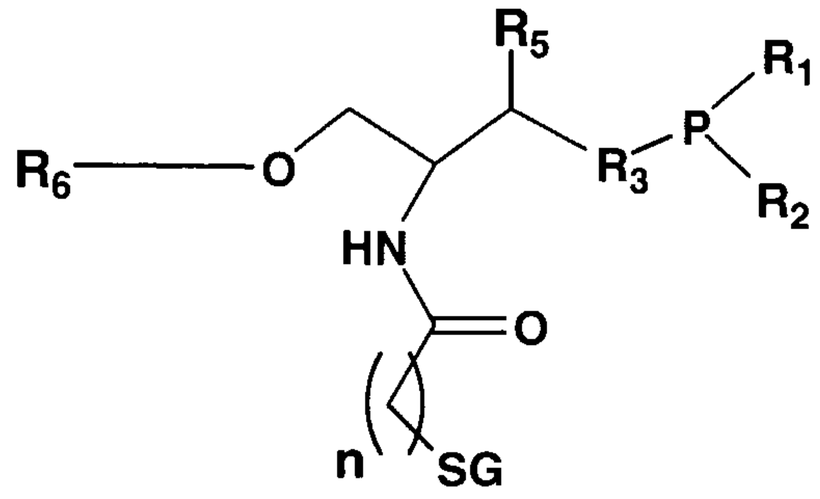
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 49.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 52:



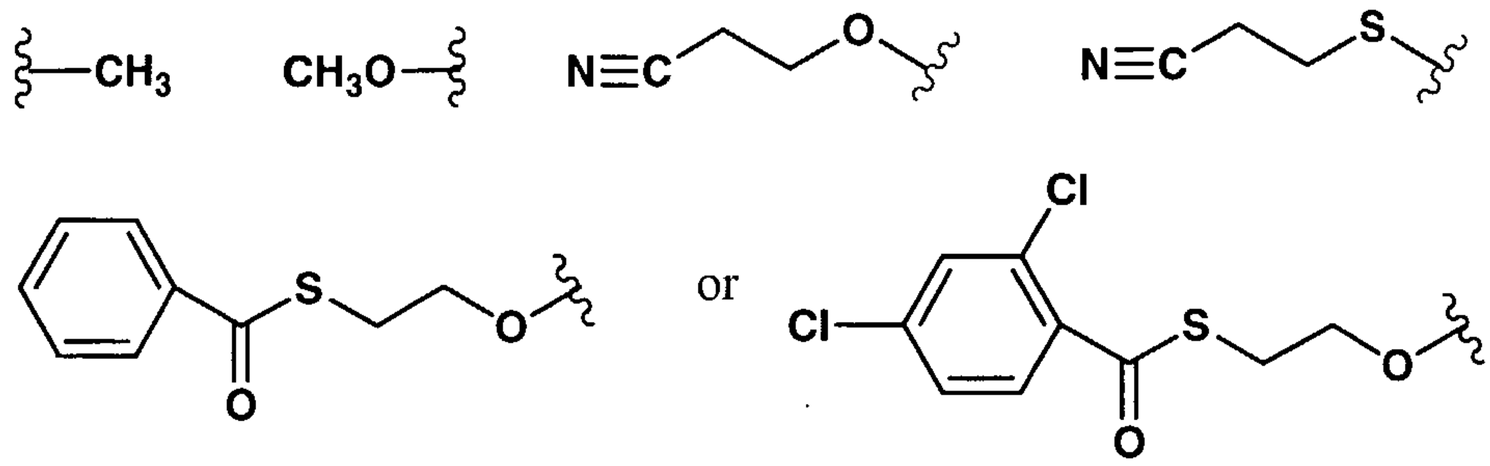
52

wherein X comprises a biologically active molecule; Y comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, n is an integer from about 1 to about 20; and N' is an integer from about 1 to about 20, comprising: (a) coupling a compound having Formula 71:

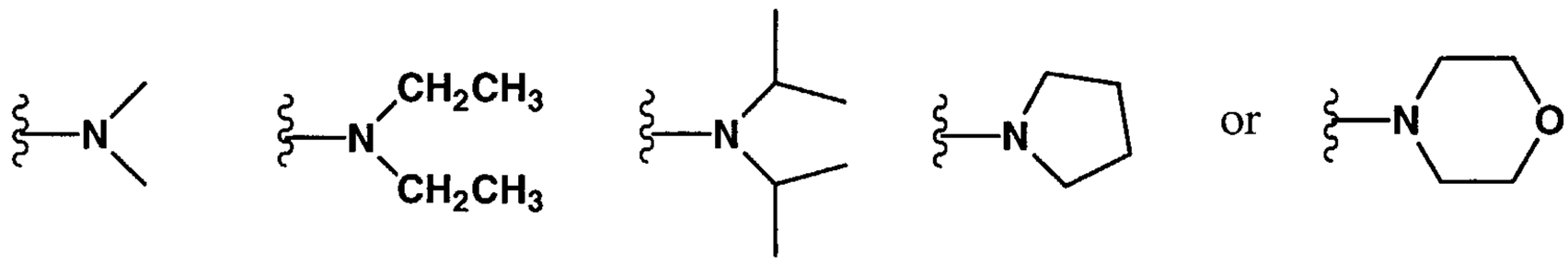


71

wherein R1, R2, R3, R5, SG, and n is as defined in Formula 52, and wherein R1 can include the groups:



and wherein R2 can include the groups:

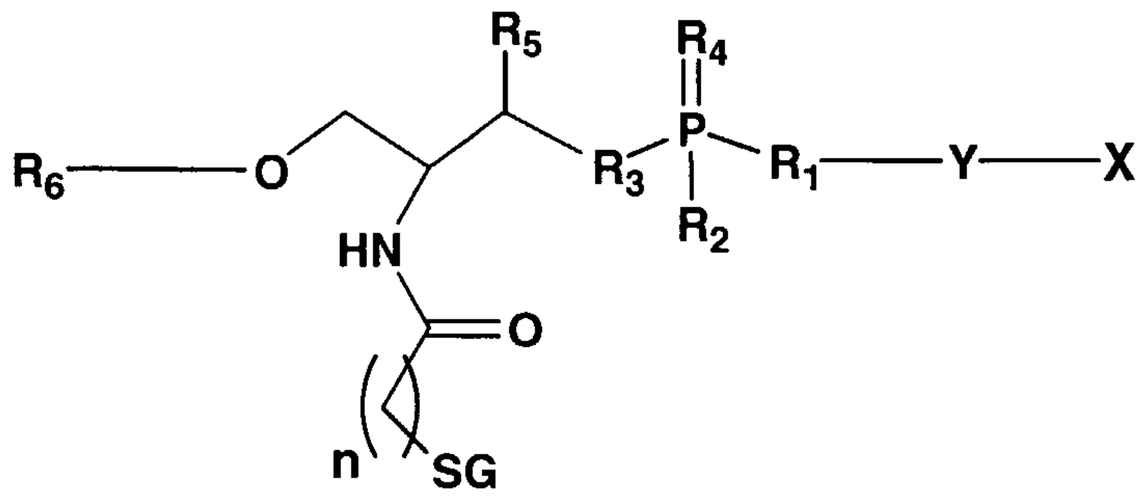


and R6 comprises a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group; with a compound having Formula 72:



72

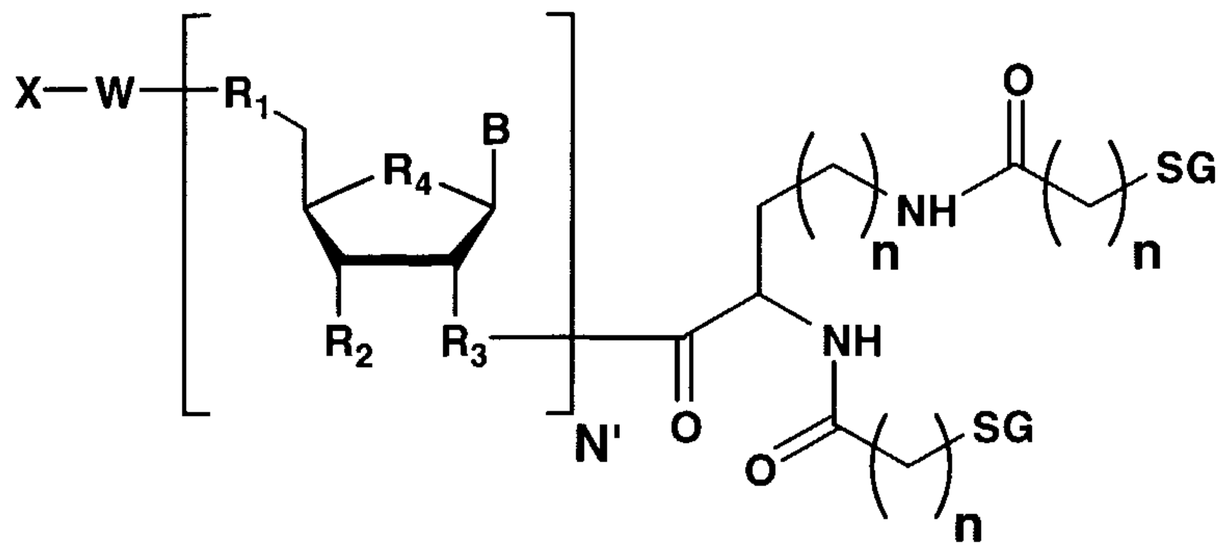
wherein X comprises a biologically active molecule and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 95:



95

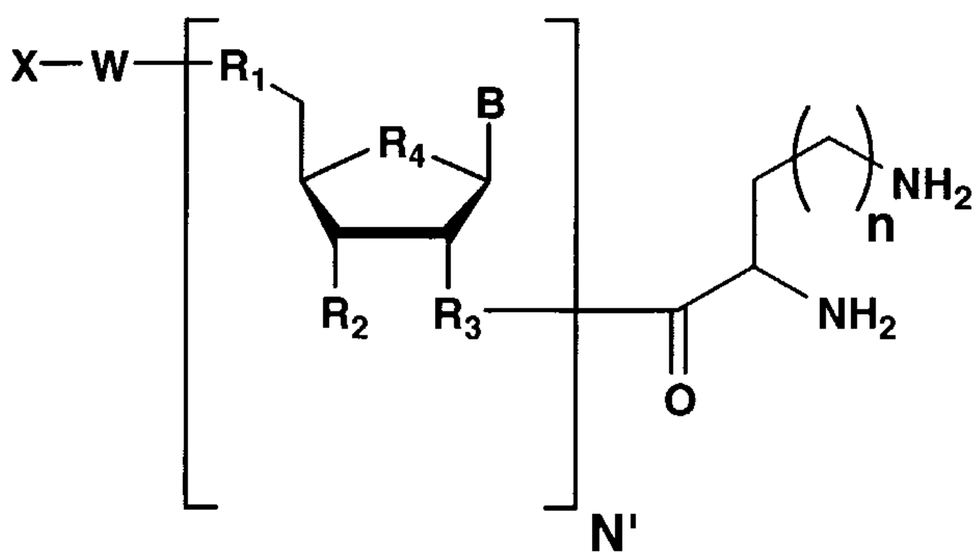
(b) removing R6 from the compound having Formula 95 and (c) optionally coupling a nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label, or optionally; coupling a compound having Formula 71 under and optionally repeating (b) and (c) under conditions suitable for the formation of a compound having Formula 52.

In another embodiment, the invention features a method for synthesizing a compound having Formula 53:



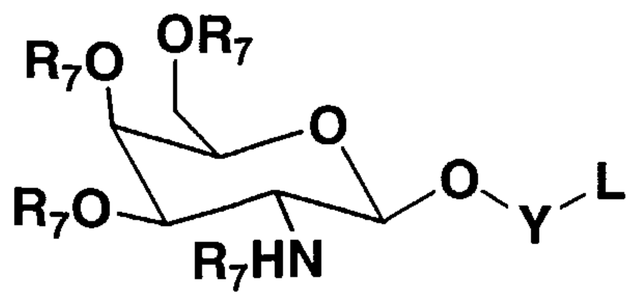
53

wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises N or O-N, each R4 independently comprises O, CH2, S, sulfone, or sulfoxy; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 10, comprising: coupling a compound having Formula 73:



73

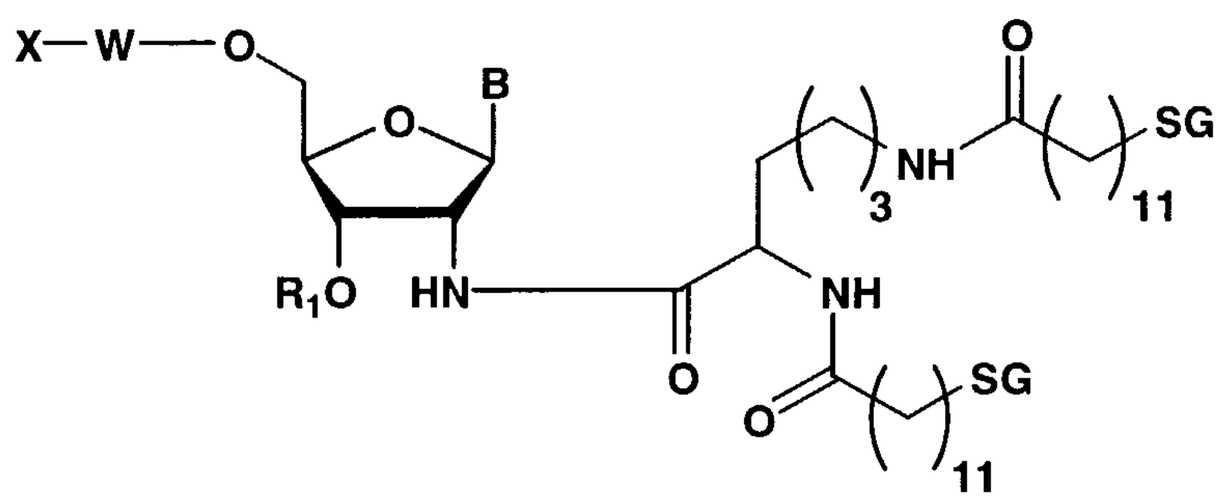
wherein R1, R2, R3, R4, X, W, B, N' and n are as defined in Formula 53, with a sugar, for example a compound having Formula 74:



74

wherein Y comprises a linker molecule or chemical linkage that can be present or absent; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; under conditions suitable for the formation of a compound having Formula 53.

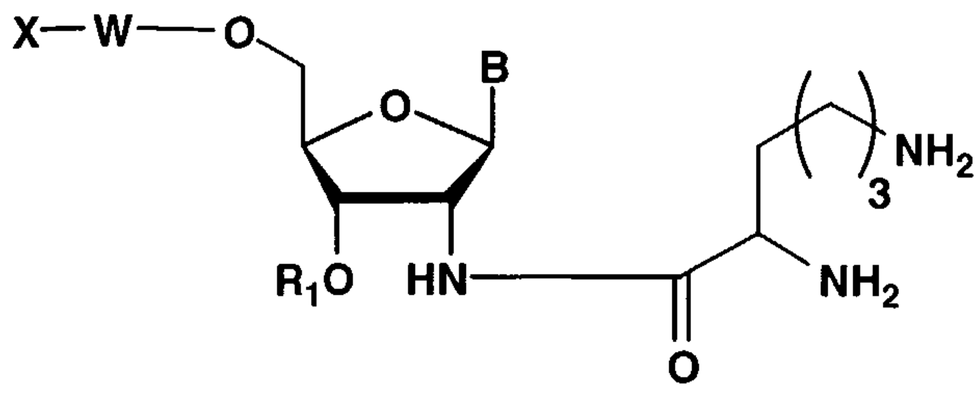
In another embodiment, the invention features a method for the synthesis of a compound having Formula 54:



54

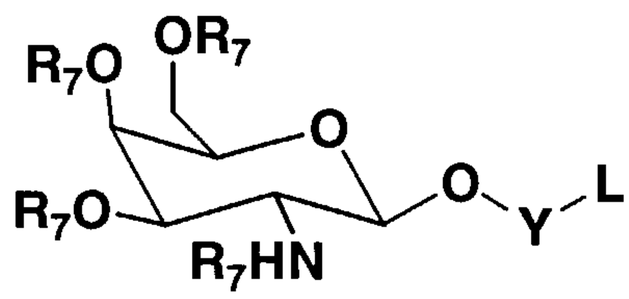
wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, comprising (a) coupling a compound having Formula 75:

45



75

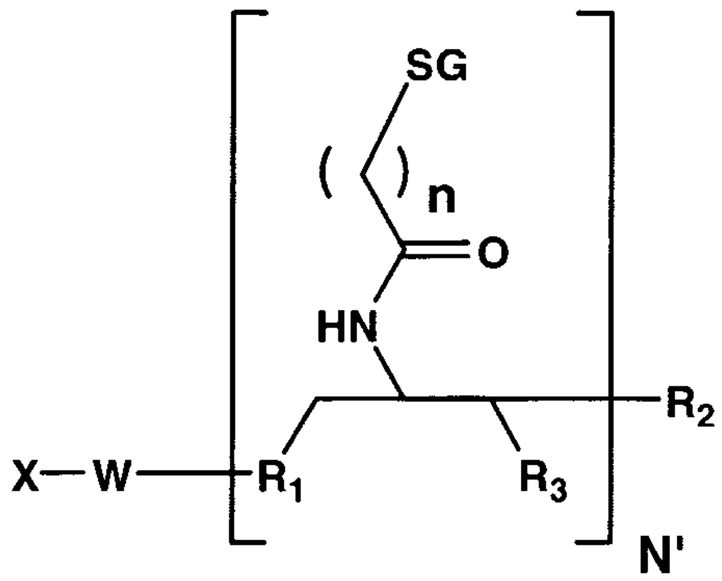
wherein R1, R2, R3, R4, X, W, and B are as defined in Formula 53, with a sugar, for example a compound having Formula 74 .



74

wherein Y comprises a C11 alkyl linker molecule; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; under conditions suitable for the formation of a compound having Formula 54.

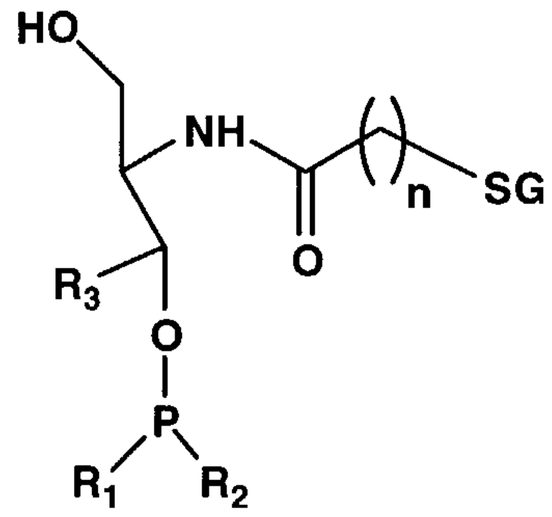
In another embodiment, the invention features a method for the synthesis of a compound having Formula 55:



55

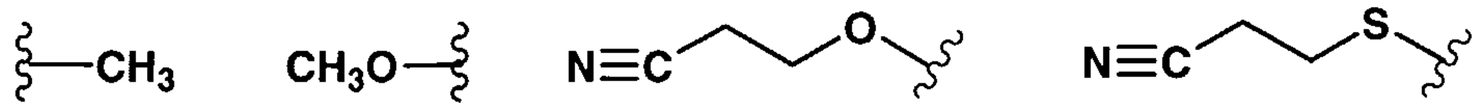
wherein each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; X comprises H, a

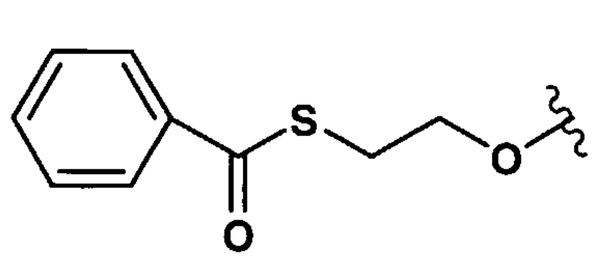
removable protecting group, nucleotide, nucleoside, nucleic acid, oligonucleotide, or enzymatic nucleic acid or biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100, comprising: (a) coupling a compound having Formula 76:



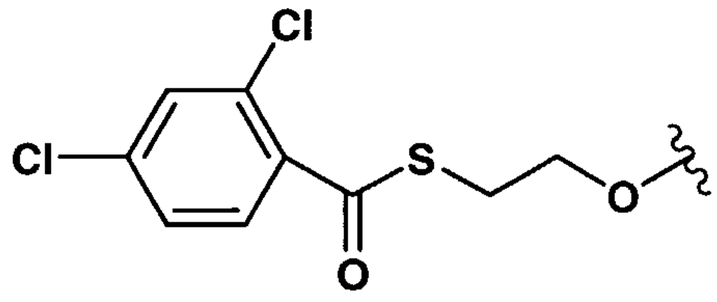
76

wherein R1 can include the groups:

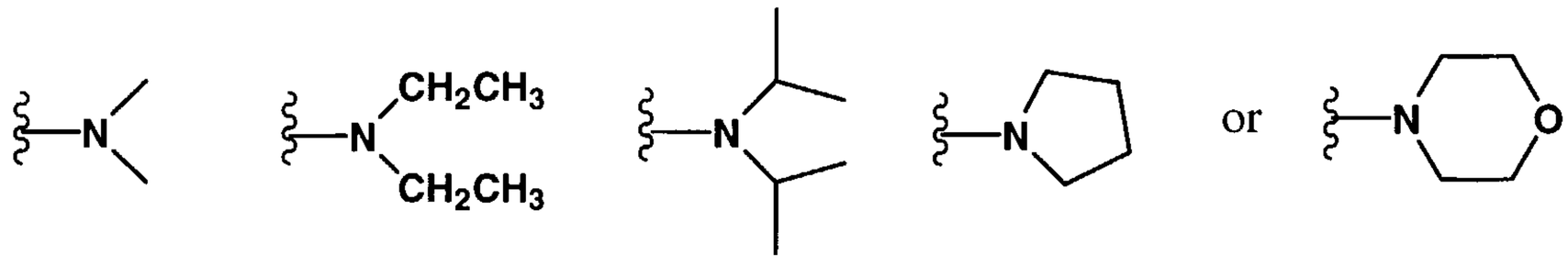




or



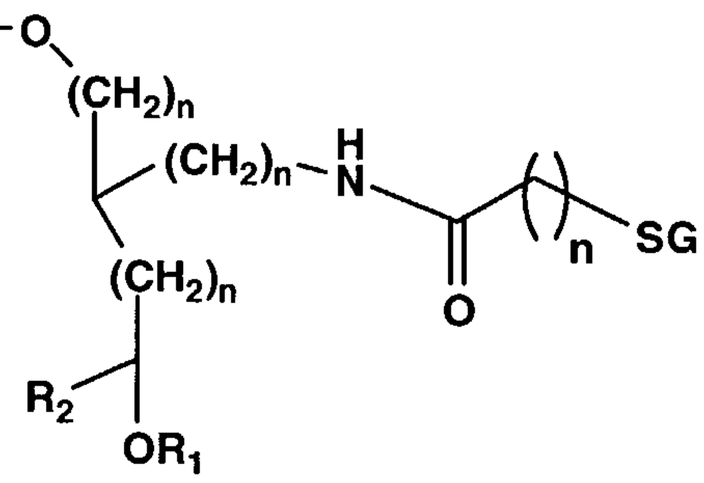
and wherein R2 can include the groups:



and wherein each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and n is an integer from about 1 to about 20, to a compound X-W, wherein X comprises a nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label, and W comprises a linker molecule or chemical linkage that can be present or

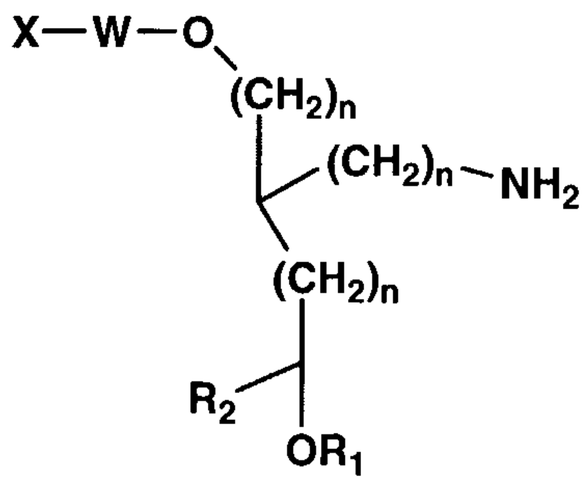
absent; and (b) optionally repeating step (a) under conditions suitable for the formation of a compound having Formula 55.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 56:



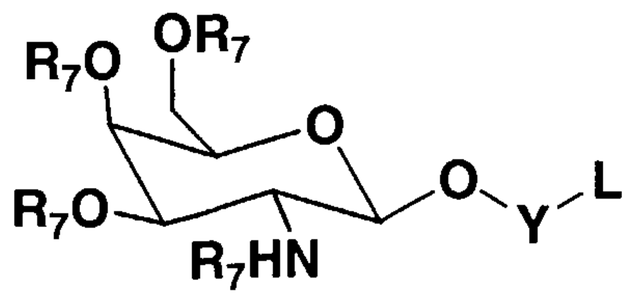
56

wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and each n is independently an integer from about 0 to about 20, comprising: (a) coupling a compound having Formula 77:



77

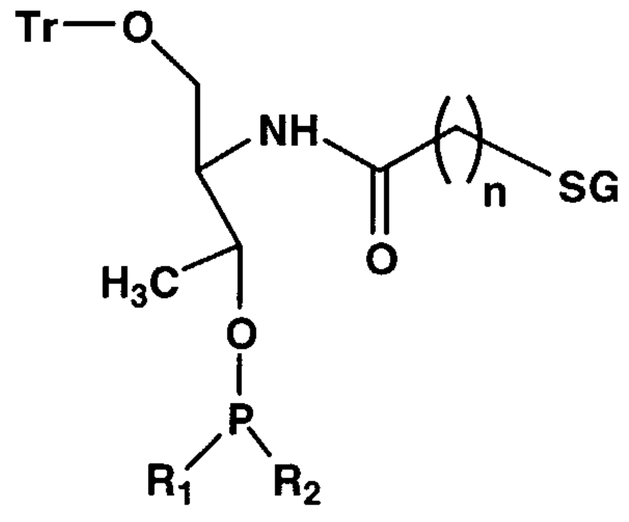
wherein each R1, X, W, and n are as defined in Formula 56, to a sugar, for example a compound having Formula 74:



74

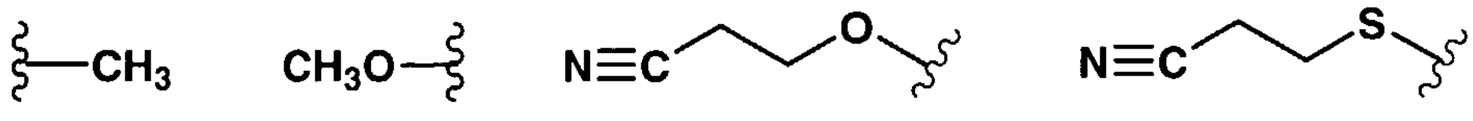
wherein Y comprises an alkyl linker molecule of length n, where n is an integer from about 1 to about 20; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; and (b) optionally coupling X-W, wherein X comprises a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label and W comprises a linker molecule or chemical linkage that can be present or absent, under conditions suitable for the formation of a compound having Formula 56.

In another embodiment, the invention features method for synthesizing a compound having Formula 57:

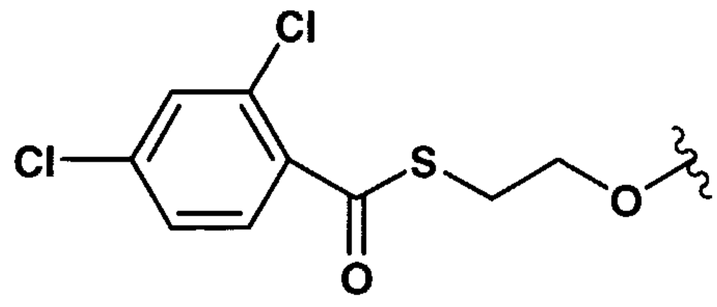


57

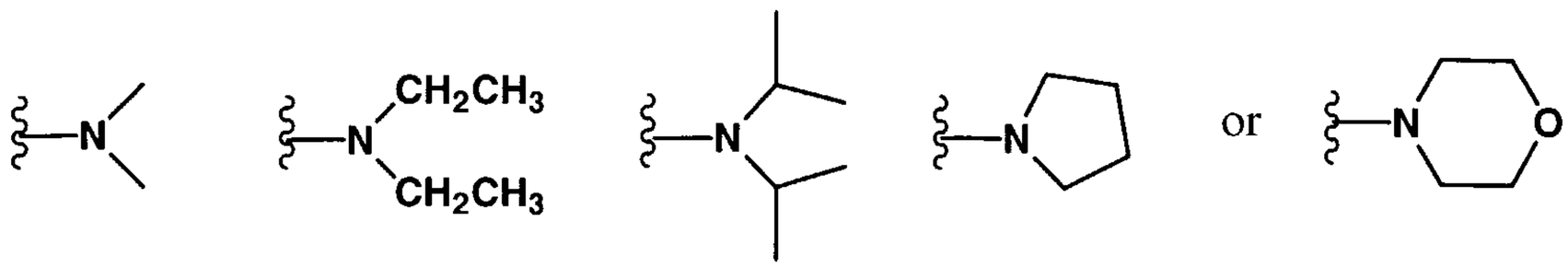
wherein R1 can include the groups:



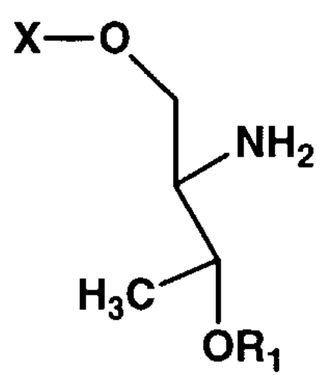
or



and wherein R2 can include the groups:

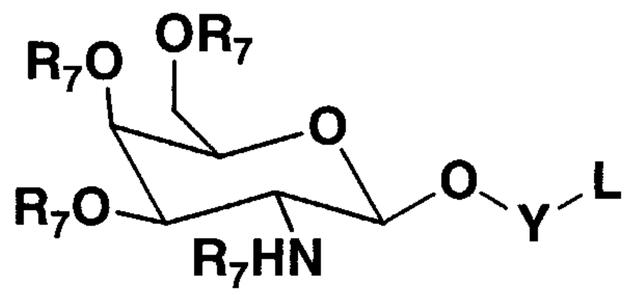


and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and n is an integer from about 1 to about 20, comprising: (a) coupling a compound having Formula 77:



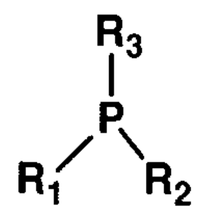
77

wherein R1 and X comprise H, to a sugar, for example a compound having Formula 74:



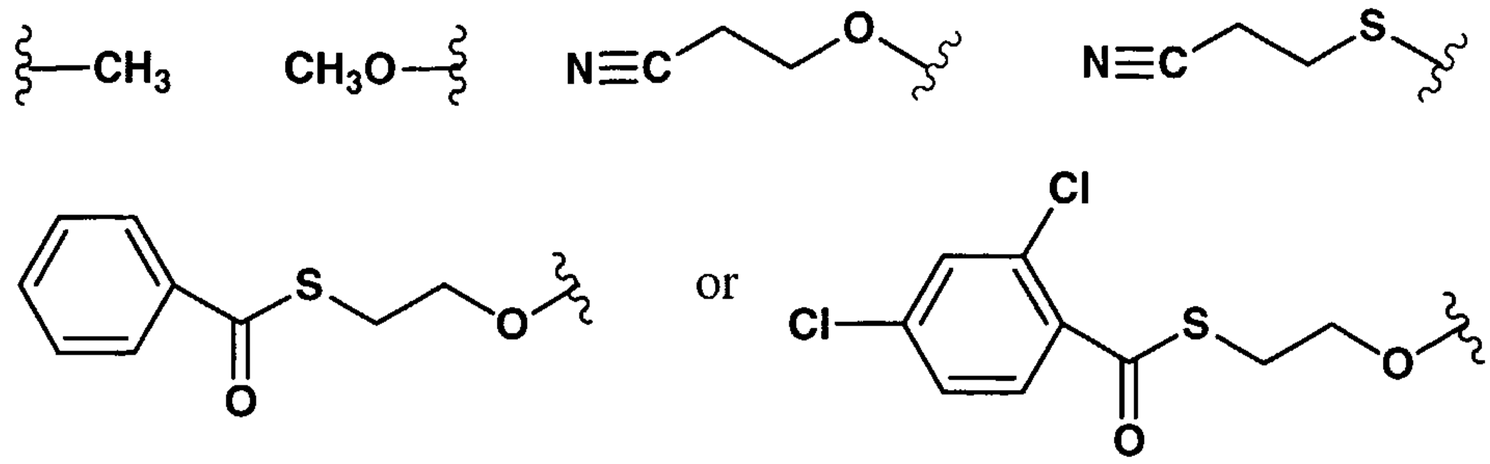
74

wherein Y comprises an alkyl linker molecule of length n, where n is an integer from about 1 to about 20; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; and (b) introducing a trityl group, for example a dimethoxytrityl, monomethoxytrityl, or trityl group to the primary hydroxyl of the product of (a) and (c) introducing a phosphorus containing group having Formula 78:

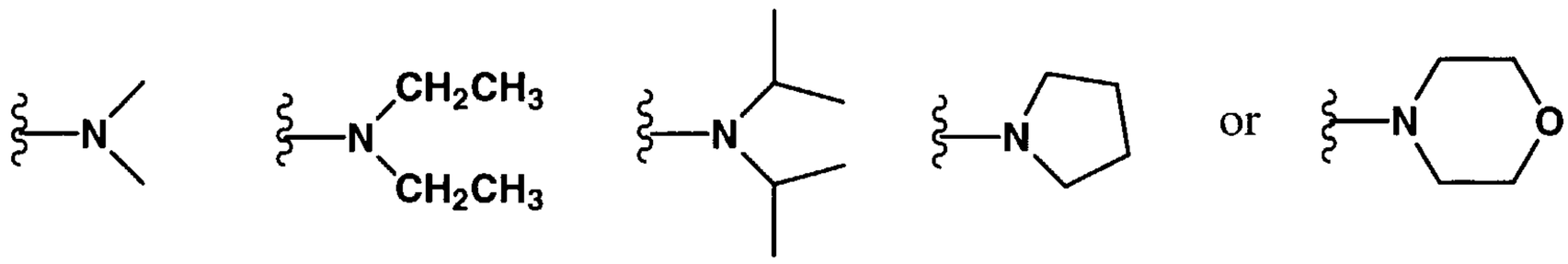


78

wherein R1 can include the groups:

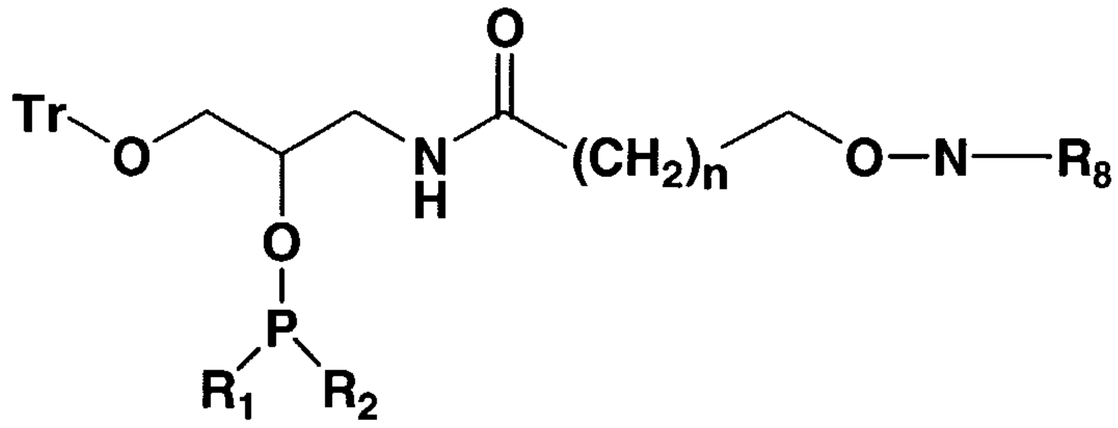


and wherein each R2 and R3 independently can include the groups:

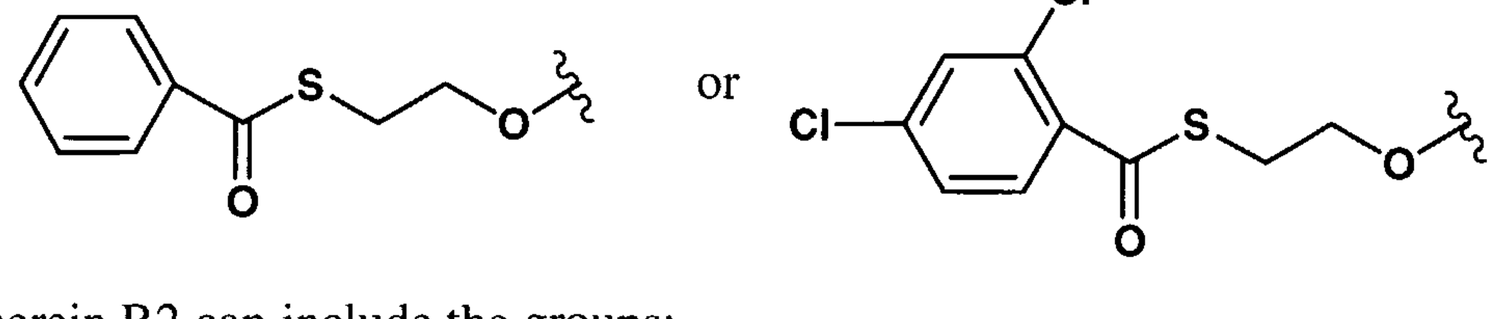


to the secondary hydroxyl of the product of (b) under conditions suitable for the formation of a compound having Formula 57.

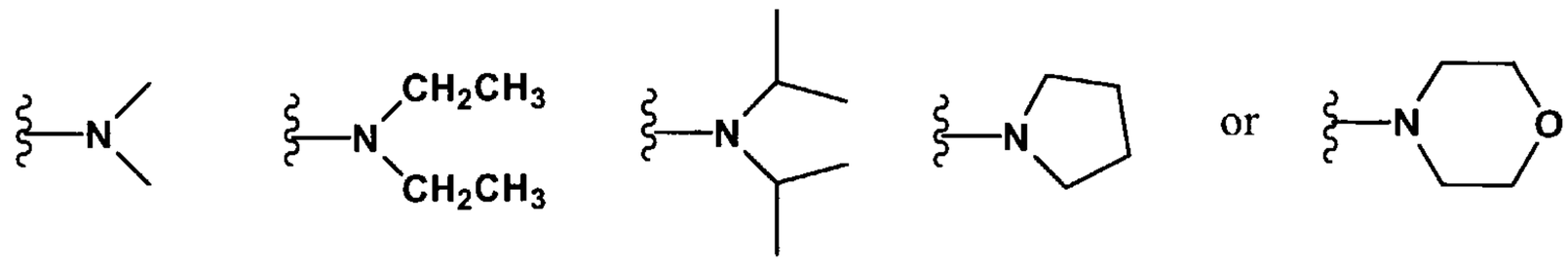
In another embodiment, the invention features a method for synthesizing a compound having Formula 60:



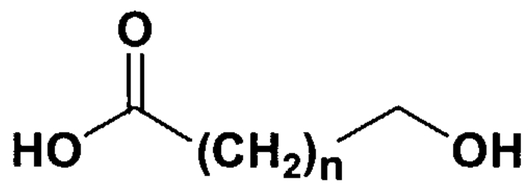
wherein R1 can include the groups:



and wherein R2 can include the groups:

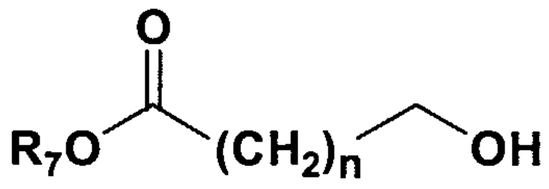


and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; n is an integer from about 1 to about 50; and R8 is a nitrogen protecting group, for example a phthaloyl, trifluoroacetyl, FMOC, or monomethoxytrityl group, comprising: (a) introducing carboxy protection to a compound having Formula 79:



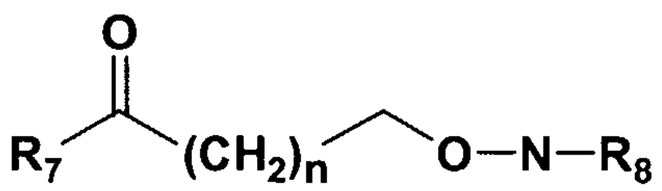
79

wherein n is an integer from about 1 to about 50, under conditions suitable for the formation of a compound having Formula 80:



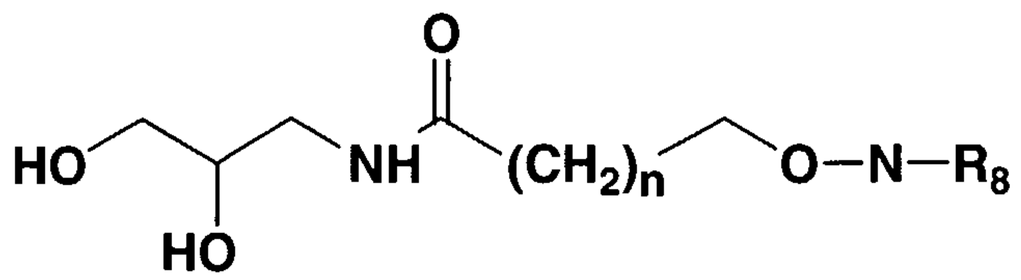
80

wherein n is an integer from about 1 to about 50 and R7 is a carboxylic acid protecting group, for example a benzyl group; (b) introducing a nitrogen containing group to the product of (a) under conditions suitable for the formation of a compound having Formula 81:



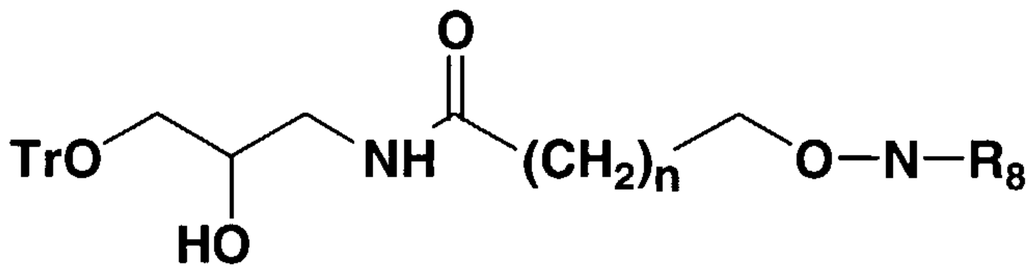
82

wherein n and R7 are as defined in Formula 80 and R8 is a nitrogen protecting group, for example a phthaloyl, trifluoroacetyl, FMOC, or monomethoxytrityl group; (c) removing the carboxylic acid protecting group from the product of (b) and introducing aminopropanediol under conditions suitable for the formation of a compound having Formula 82:



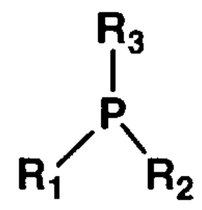
82

wherein n and R8 are as defined in Formula 81; (d) introducing a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl to the product of (c) under conditions suitable for the formation of a compound having Formula 83:



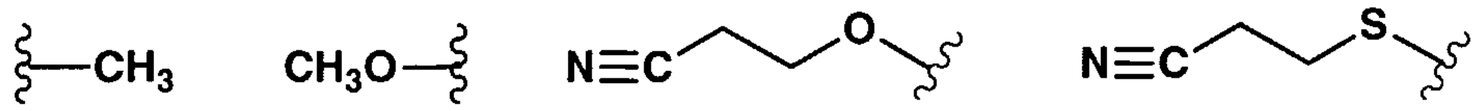
83

wherein Tr, n and R8 are as defined in Formula 60; and (e) introducing a phosphorus containing group having Formula 78:

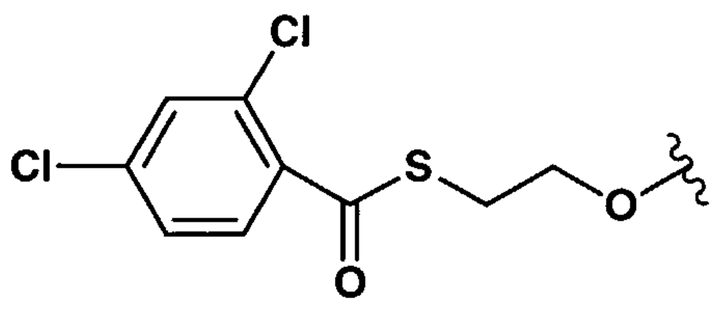


78

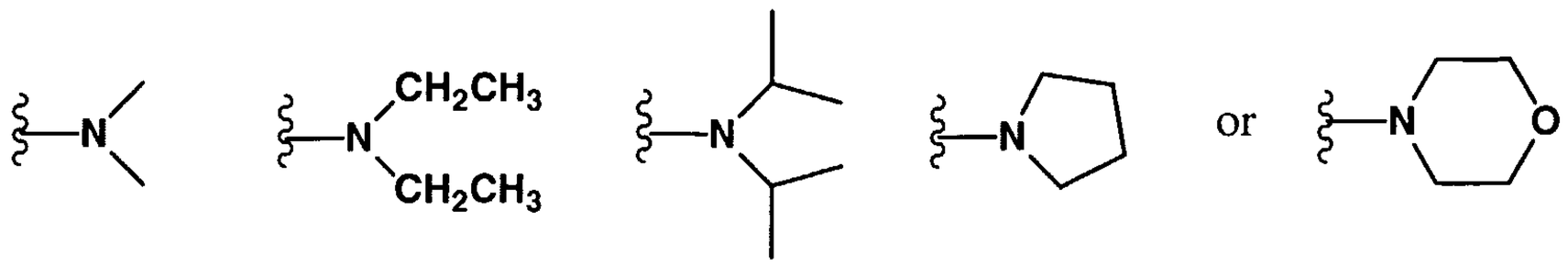
wherein R1 can include the groups:



or

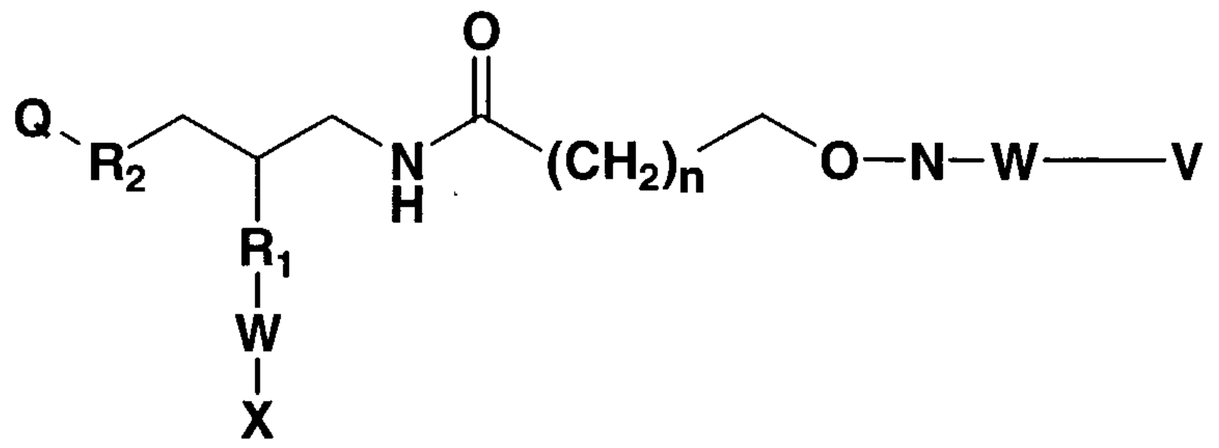


and wherein each R2 and R3 independently can include the groups:



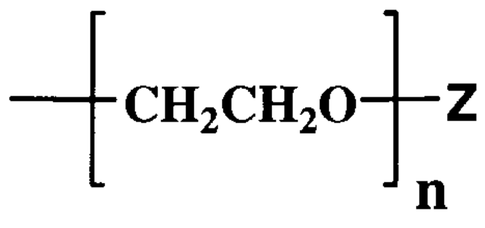
to the product of (d) under conditions suitable for the formation of a compound having Formula 60.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 59:



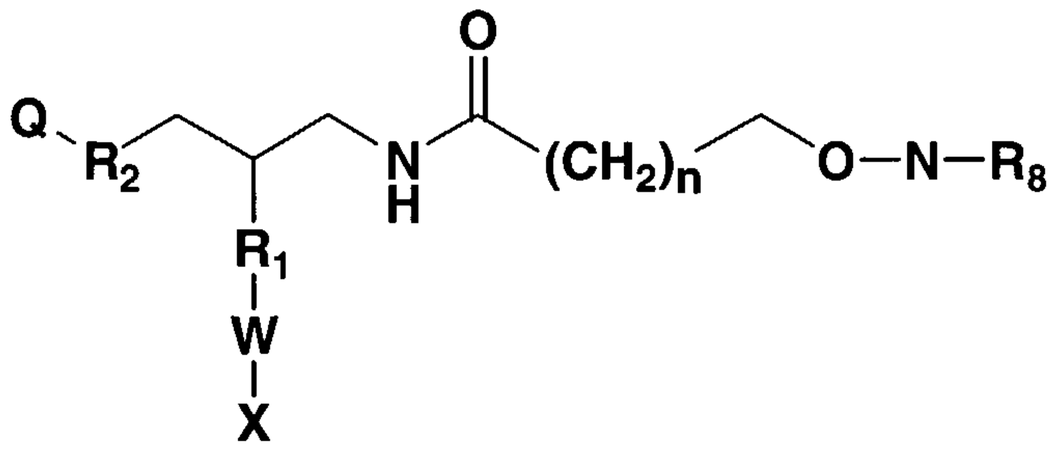
59

wherein each R1 independently comprises O, S, N, substituted N, or a phosphorus containing group; each R2 independently comprises O, S, or N; X comprises H, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid or biologically active molecule; n is an integer from about 1 to about 50, Q comprises H or a removable protecting group which can be optionally absent, each W independently comprises a linker molecule or chemical linkage that can be present or absent, and V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide, or a compound having Formula 45:



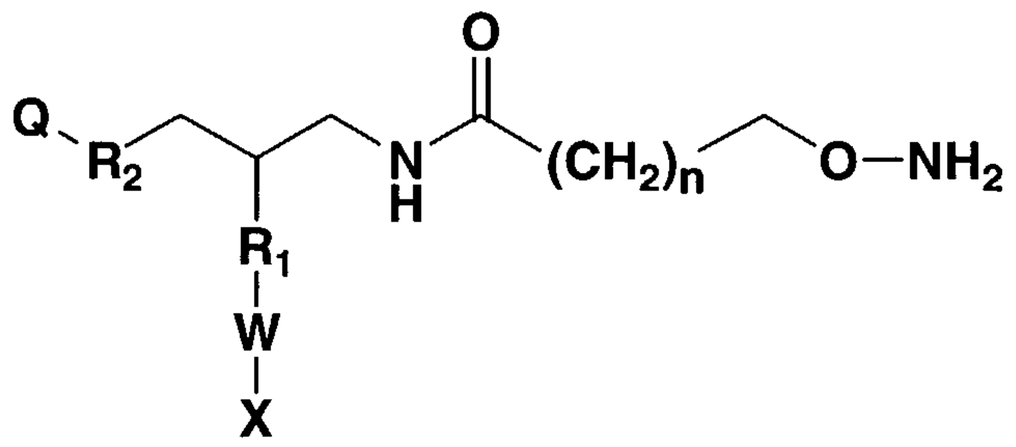
45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100, comprising: (a) removing R8 from a compound having Formula 84:



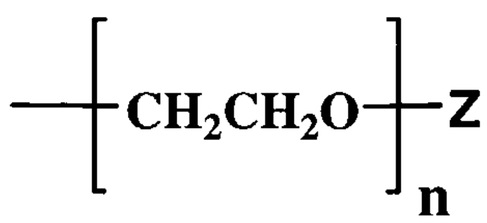
84

wherein Q, X, W, R1, R2, and n are as defined in Formula 59 and R8 is a nitrogen protecting group, for example a phthaloyl, trifluoroacetyl, FMOC, or monomethoxytrityl group, under conditions suitable for the formation of a compound having Formula 85:



85

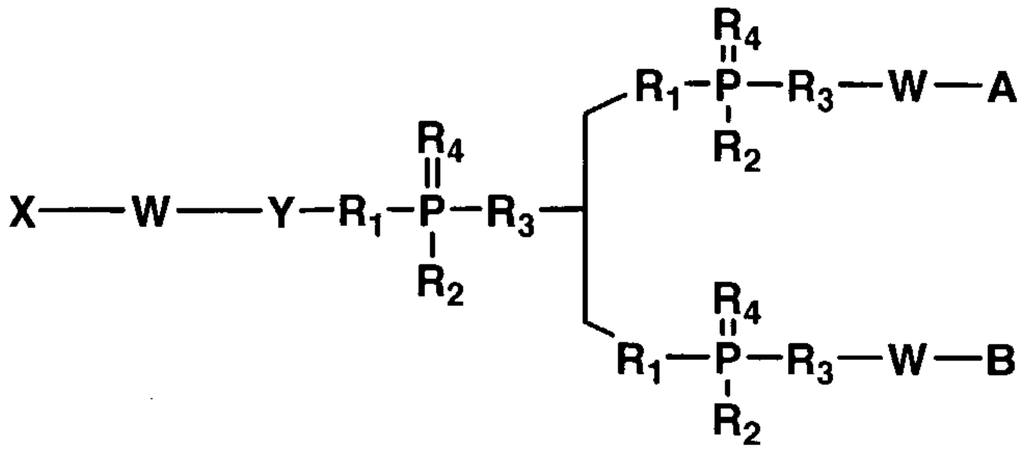
wherein Q, X, W, R1, R2, and n are as defined in Formula 59; (b) introducing a group V to the product of (a) via the formation of an oxime linkage, wherein V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide, or a compound having Formula 45:



45

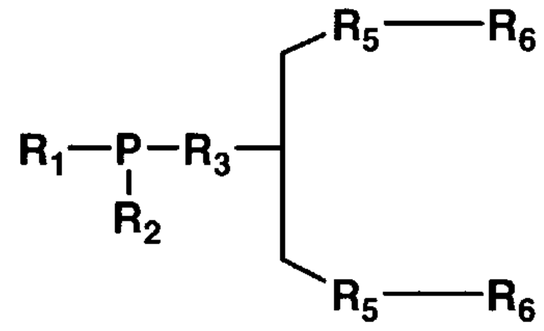
wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100, under conditions suitable for the formation of a compound having Formula 59.

In another embodiment, the invention features a method for synthesizing a compound having Formula 64:



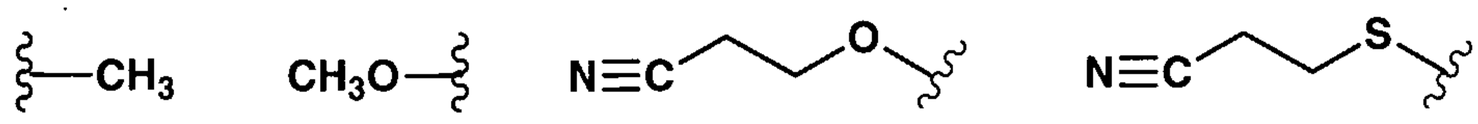
64

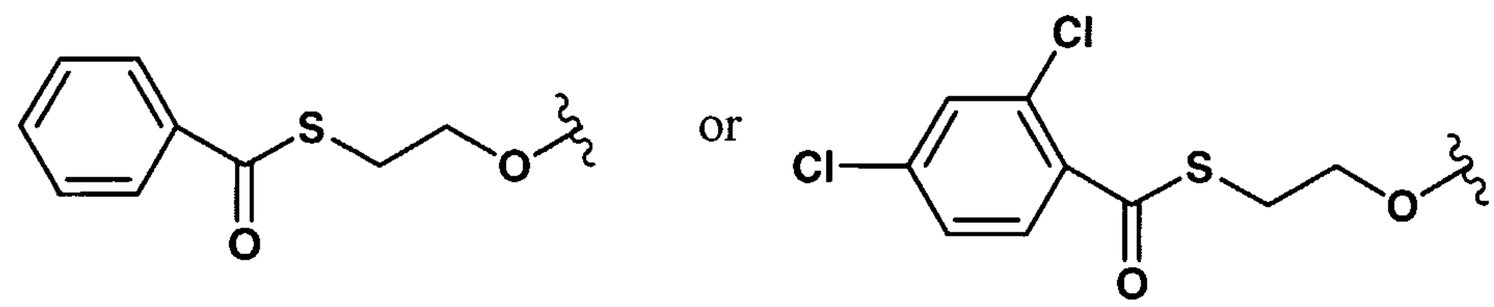
wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, A comprises a nitrogen containing group, and B comprises a lipophilic group, comprising: (a) introducing a compound having Formula 66:



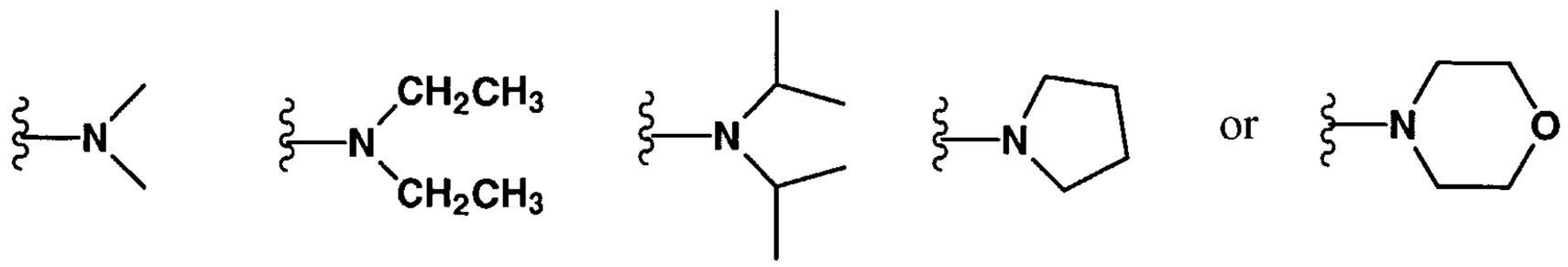
66

wherein R1 is defined as in Formula 64 and can include the groups:

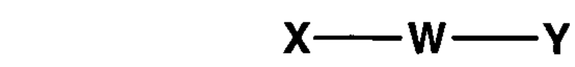




and wherein R2 is defined as in Formula 64 and can include the groups:

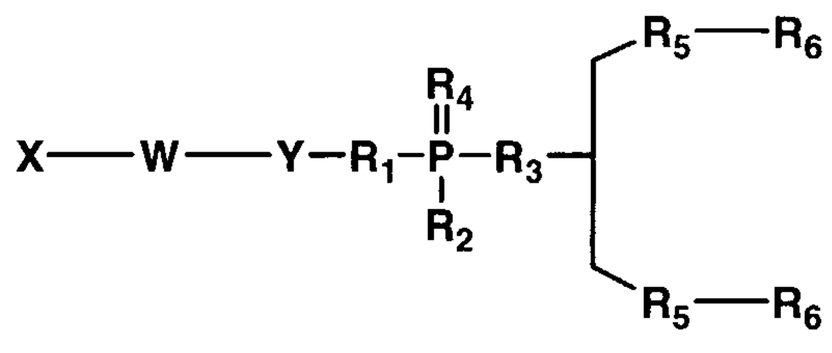


and wherein each R5 independently comprises O, N, or S and each R6 independently comprises a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group, to a compound having Formula 67:



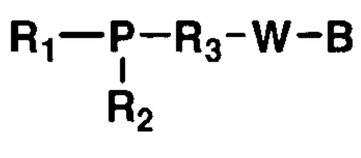
67

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 68:



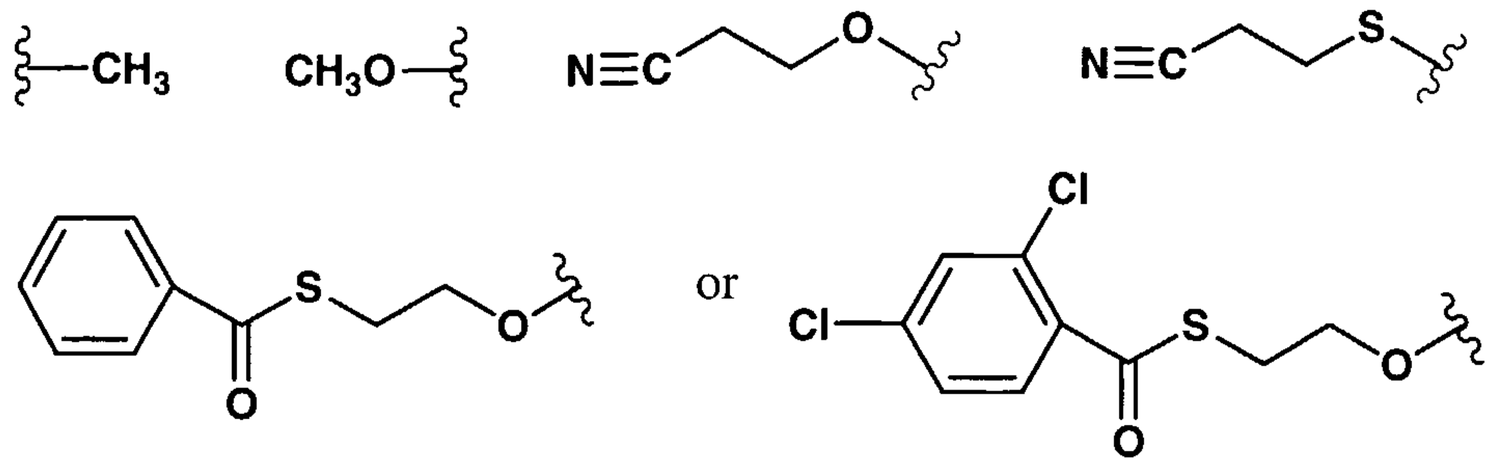
68

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; and each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N comprising, each R5 independently comprises O, S, or N; and each R6 is independently a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group; (b) removing R6 from the compound having Formula 68 and (c) introducing a compound having Formula 69:

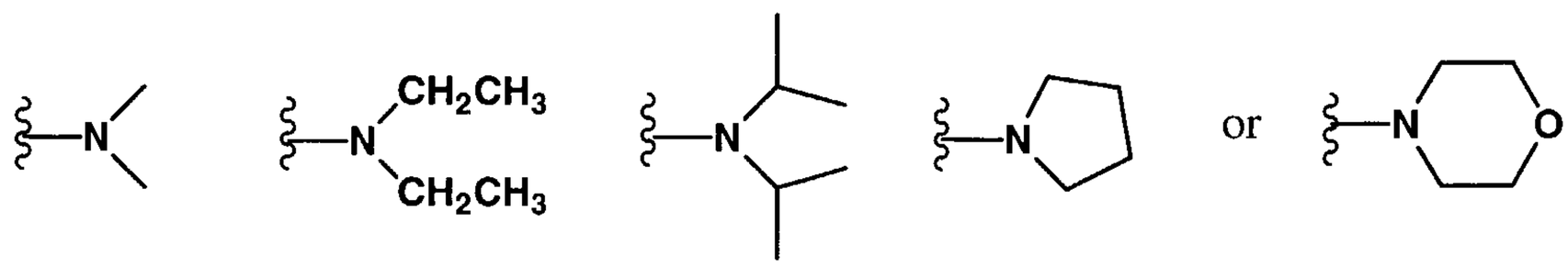


69

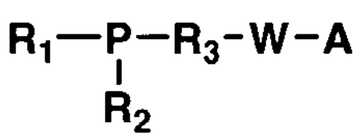
wherein R1 is defined as in Formula 64 and can include the groups:



and wherein R2 is defined as in Formula 64 and can include the groups:

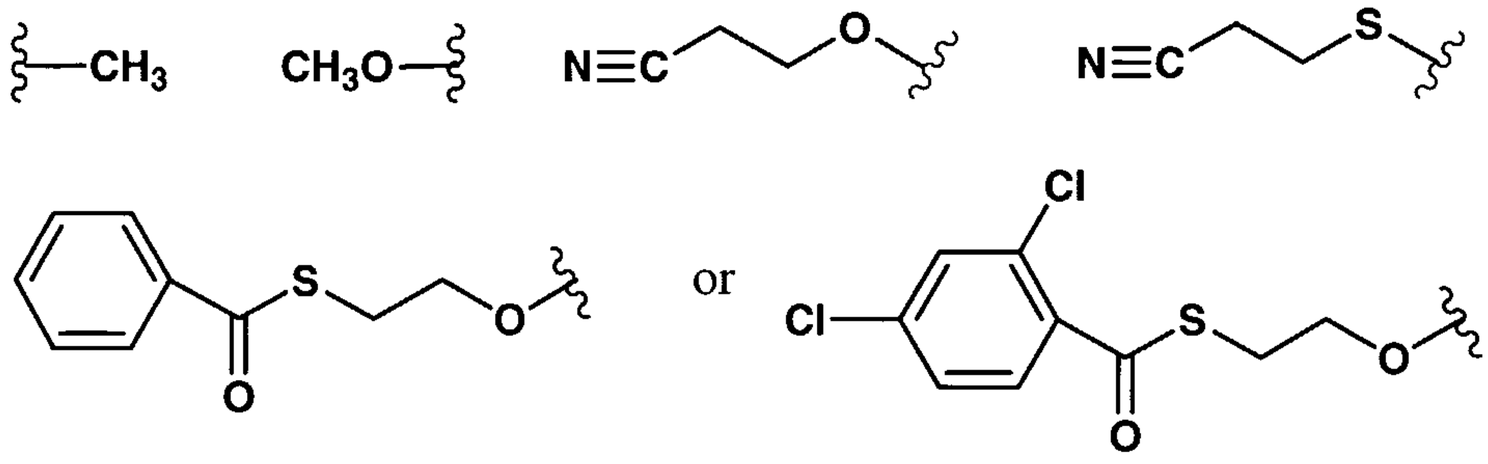


and wherein R3, W and B are defined as in Formula 64; and introducing a compound having Formula 69':

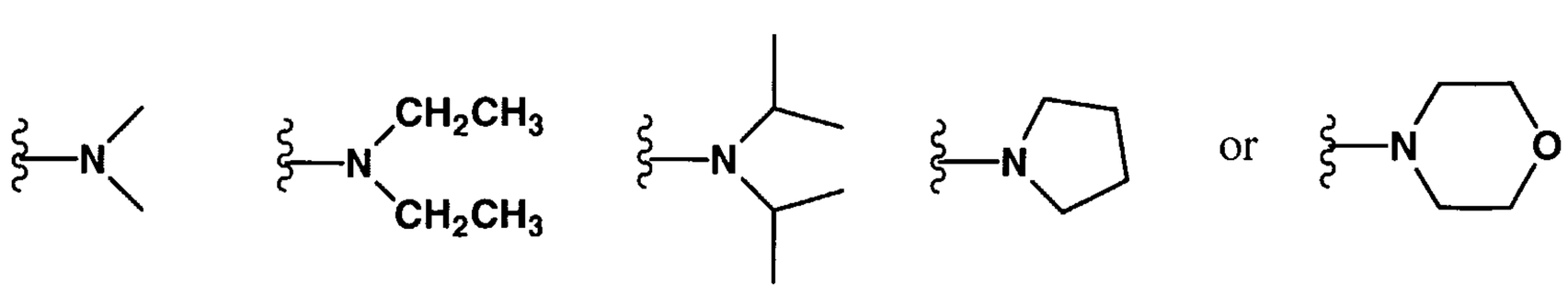


69'

wherein R1 is defined as in Formula 64 and can include the groups:

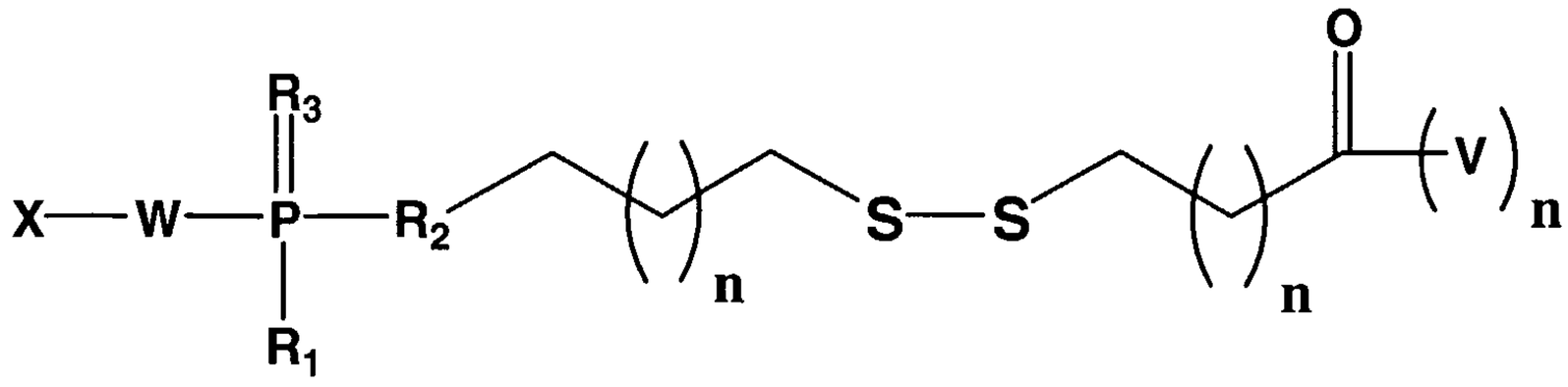


and wherein R2 is defined as in Formula 48 and can include the groups:



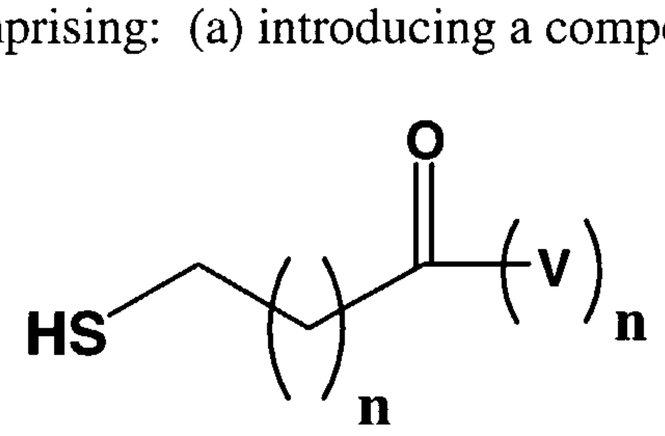
and wherein R3, W and A are defined as in Formula 64; to the compound having Formula 68 under conditions suitable for the formation of a compound having Formula 64.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 62:



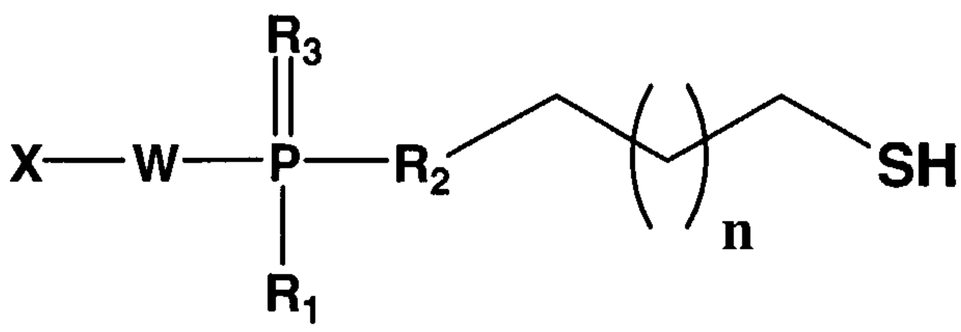
62

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; each 5 independently comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide, Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide;; each R1, R2, and R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and each n is independently an integer from about 1 to about 10, comprising: (a) introducing a compound having Formula 93:



93

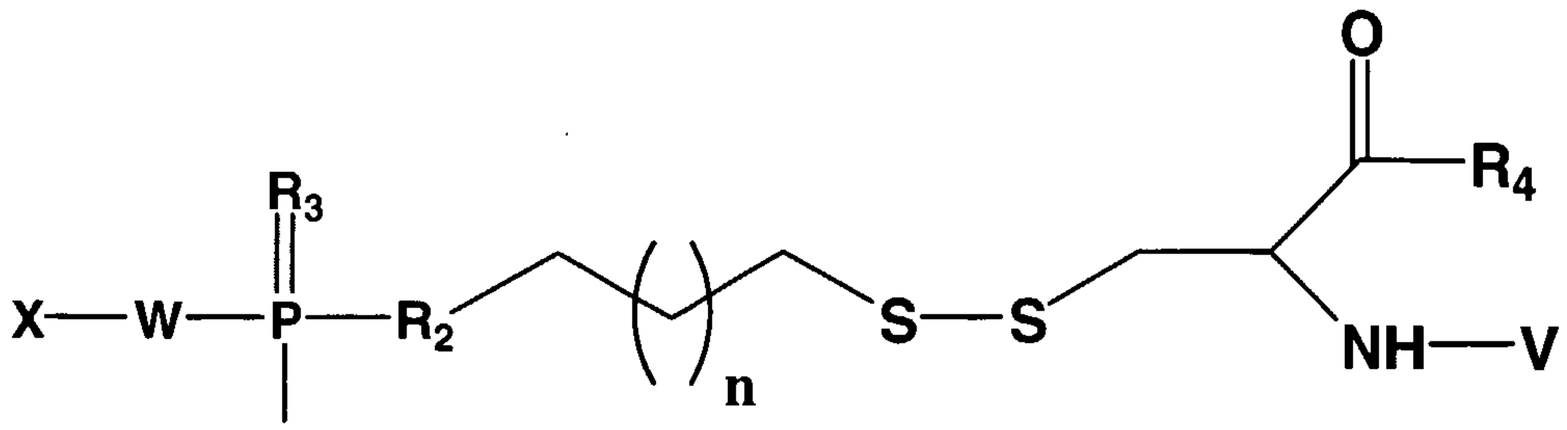
wherein V and n are as defined in Formula 62, to a compound having Formula 86:



86

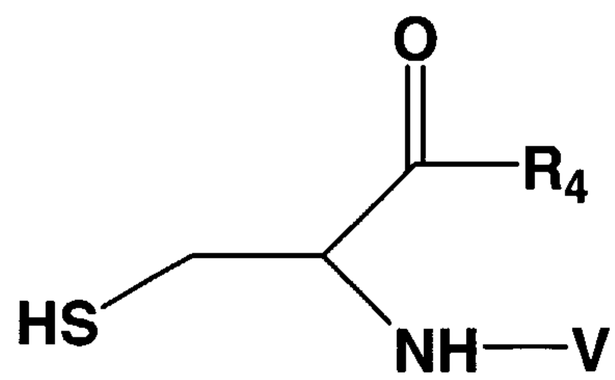
wherein X, W, R1, R2, R3, and n are as defined in Formula 62, under conditions suitable for the formation of a compound having Formula 62.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 63:



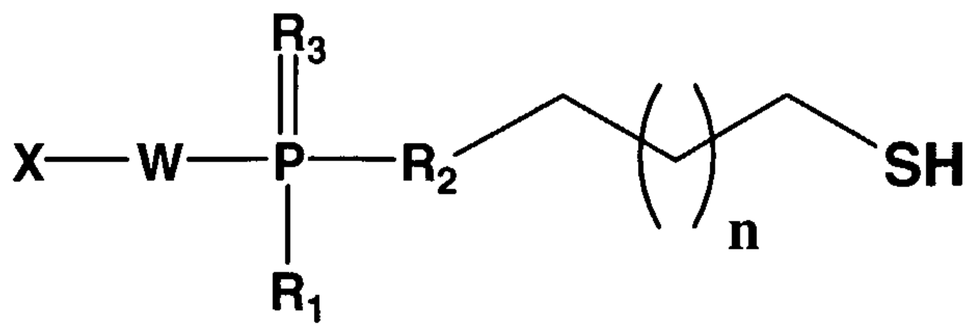
63

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; V comprises a protein or peptide, for example Human serum albumin protein, Antennapedia peptide,. Kaposi fibroblast growth factor peptide, Caiman crocodylus Ig(5) light chain peptide, HIV envelope glycoprotein gp41 peptide, HIV-1 Tat peptide, Influenza hemagglutinin envelope glycoprotein peptide, or transportan A peptide;; each R1, R2, and R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, R4 represents an ester, amide, or protecting group, and each n is independently an integer from about 1 to about 10, comprising: (a) introducing a compound having Formula 96:



96

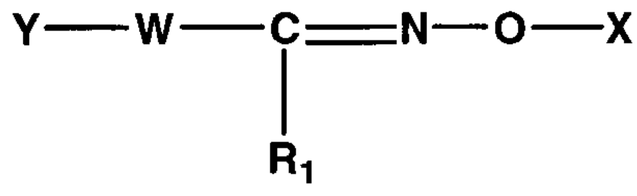
wherein V and R4 are as defined in Formula 63, to a compound having Formula 86:



86

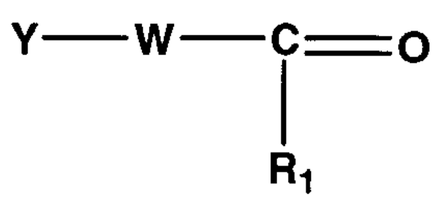
wherein X, W, R1, R2, R3, and n are as defined in Formula 63, under conditions suitable for the formation of a compound having Formula 63.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 87:



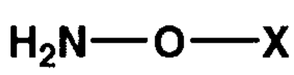
87

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; W comprises a linker molecule or chemical linkage that can be present or absent; and Y comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody; R1 comprises H, alkyl, or substituted alkyl, comprising (a) coupling a compound having Formula 89:



89

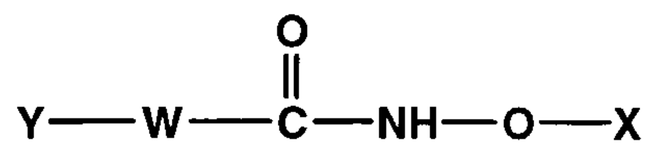
wherein Y, W and R are as defined in Formula 87, with a compound having Formula 90:



90

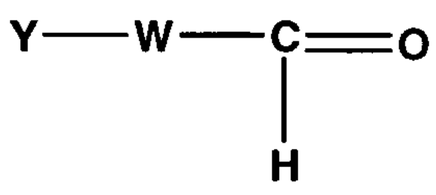
wherein X is as defined in Formula 87, under conditions suitable for the formation of a compound having Formula 87, for example by post-synthetic conjugation of a compound having Formula 89 with a compound having Formula 90, wherein X of compound 90 comprises an enzymatic nucleic acid molecule and Y of Formula 89 comprises a peptide.

In another embodiment, the invention features a method for the synthesis of a compound having Formula 88:



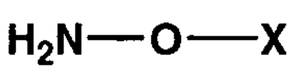
88

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody, comprising (a) coupling a compound having Formula 91:



91

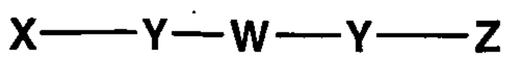
wherein Y and W are as defined in Formula 88, with a compound having Formula 90:



90

wherein X is as defined in Formula 88, under conditions suitable for the formation of a compound having Formula 88, for example by post-synthetic conjugation of a compound having Formula 91 with a compound having Formula 90, wherein X of compound 90 comprises an enzymatic nucleic acid molecule and Y of Formula 91 comprises a peptide.

In one embodiment, the invention features a compound having Formula 94,



94

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; each Y independently comprises a linker or chemical linkage that can be present or absent, W comprises a biodegradable nucleic acid linker molecule, and Z comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody.

In another embodiment, W of a compound having Formula 94 of the invention comprises 5'-cytidine-deoxythymidine-3', 5'-deoxythymidine-cytidine-3', 5'-cytidine-deoxyuridine-3', 5'-deoxyuridine-cytidine-3', 5'-uridine-deoxythymidine-3', or 5'-deoxythymidine-uridine-3'.

In yet another embodiment, W of a compound having Formula 94 of the invention comprises 5'-adenosine-deoxythymidine-3', 5'-deoxythymidine-adenosine-3', 5'-adenosine-deoxyuridine-3', or 5'-deoxyuridine-adenosine-3'.

In another embodiment, Y of a compound having Formula 94 of the invention comprises a phosphorus containing linkage, phoshoramidate linkage, phosphodiester linkage, phosphorothioate linkage, amide linkage, ester linkage, carbamate linkage, disulfide linkage, oxime linkage, or morpholino linkage.

In another embodiment, compounds having Formula 89 and 91 of the invention are synthesized by periodate oxidation of an N-terminal Serine or Threonine residue of a peptide or protein.

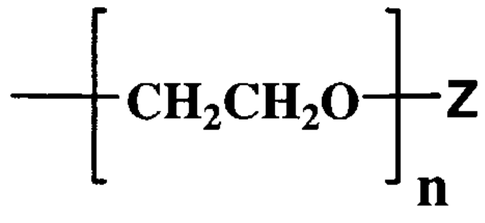
In one embodiment, X of compounds having Formulae 43, 44, 46-52, 58, 61-65, 85-88, 92, 94, and 95 of the invention comprises an enzymatic nucleic acid.

In another embodiment, X of compounds having Formulae 43, 44, 46-52, 58, 61-65, 85-88, 92, 94, and 95 of the invention comprises an antibody. In yet another embodiment, X of compounds having Formulae 43, 44, 46-52, 58, 61-65, 85-88, 92, 94, and 95 of the invention comprises an interferon.

In another embodiment, X of compounds having Formulae 43, 44, 46-52, 58, 61-65, 85-88, 92, 94, and 95 of the invention comprises an antisense nucleic acid, dsRNA, ssRNA, decoy, triplex oligonucleotide, aptamer, or 2,5-A chimera.

In one embodiment, W and/or Y of compounds having Formulae 43, 44, 46-56, 58-59, 61-65, 67, 68, 69, 72, 73, 75, 77, 84-89, 91-92, 94, and 95 of the invention comprises a degradable or cleavable linker, for example a nucleic acid sequence comprising ribonucleotides and/or deoxynucleotides, such as a dimer, trimer, or tetramer. A non limiting example of a nucleic acid cleavable linker is an adenosine-deoxythymidine (A-dT) dimer or a cytidine-deoxythymidine (C-dT) dimer. In yet another embodiment, W and/or V of compounds having Formulae 43, 44, 48-51, 58, 63-65, and 96 of the invention comprises a N-hydroxy succinimide (NHS) ester linkage, oxime linkage, disulfide linkage, phosphoramidate, phosphorothioate, phosphorodithioate, phosphodiester linkage, or NHC(O), $CH_3NC(O)$, CONH, $C(O)NCH_3$, S, SO, SO_2, O, NH, NCH_3 group. In another embodiment, the degradable linker, W and/or Y, of compounds having Formulae 43, 44, 46-56, 58-59, 61-65, 67, 68, 69, 72, 73, 75, 77, 84-89, 91-92, 94, and 95 of the invention comprises a linker that is susceptible to cleavage by carboxypeptidase activity.

In another embodiment, W and/or Y of Formulae 43, 44, 46-56, 58-59, 61-65, 67, 68, 69, 72, 73, 75, 77, 84-89, 91-92, 94, and 95 comprises a polyethylene glycol linker having Formula 45:



45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

In one embodiment, the nucleic acid conjugates of the instant invention are assembled by solid phase synthesis, for example on an automated peptide synthesizer, for example a Miligen 9050 synthesizer and/or an automated oligonucleotide synthesizer such as an ABI 394, 390Z, or Pharmacia OligoProcess, OligoPilot, OligoMax, or AKTA synthesizer. In another embodiment, the nucleic acid conjugates of the invention are

assembled post synthetically, for example, following solid phase oligonucleotide synthesis (see for example **Figure 15**).

In another embodiment, V of compounds having Formula 58-63 and 96 comprise peptides having SEQ ID NOS: 14-23 (**Table 3**).

In one embodiment, the nucleic acid conjugates of the instant invention are assembled post synthetically, for example, following solid phase oligonucleotide synthesis.

The present invention provides compositions and conjugates comprising nucleosidic and non-nucleosidic derivatives. The present invention also provides nucleic acid, polynucleotide and oligonucleotide derivatives including RNA, DNA, and PNA based conjugates. The attachment of compounds of the invention to nucleosides, nucleotides, non-nucleosides, and nucleic acid molecules is provided at any position within the molecule, for example, at internucleotide linkages, nucleosidic sugar hydroxyl groups such as 5', 3', and 2'-hydroxyls, and/or at nucleobase positions such as amino and carbonyl groups.

The exemplary conjugates of the invention are described as compounds of the formulae herein, however, other peptide, protein, phospholipid, and poly-alkyl glycol derivatives are provided by the invention, including various analogs of the compounds of formulae 1-96, including but not limited to different isomers of the compounds described herein.

In one embodiment, the present invention features molecules, compositions and conjugates of molecules, for example, non-nucleosidic small molecules, nucleosides, nucleotides, and nucleic acids, such as enzymatic nucleic acid molecules, antisense nucleic acids, 2-5A antisense chimeras, triplex oligonucleotides, decoys, siRNA, allozymes, aptamers, and antisense nucleic acids containing RNA cleaving chemical groups.

The exemplary folate conjugates of the invention are described as compounds shown by formulae herein, however, other folate and antifolate derivatives are provided by the invention, including various folate analogs of the formulae of the invention, including dihydrofloates, tetrahydrofolates, tetrahydorpterins, folinic acid, pteropolyglutamic acid, 1-deza, 3-deaza, 5-deaza, 8-deaza, 10-deaza, 1,5-deaza, 5,10 dideaza, 8,10-dideaza, and 5,8-dideaza folates, antifolates, and pteroic acids. As used

herein, the term "folate" is meant to refer to folate and folate derivatives, including pteroic acid derivatives and analogs.

The present invention features compositions and conjugates to facilitate delivery of molecules into a biological system such as cells. The conjugates provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes. The present invention encompasses the design and synthesis of novel agents for the delivery of molecules, including but not limited to small molecules, lipids, nucleosides, nucleotides, nucleic acids, negatively charged polymers and other polymers, for example proteins, peptides, carbohydrates, or polyamines. In general, the transporters described are designed to be used either individually or as part of a multi-component system. The compounds of the invention generally shown in Formulae herein are expected to improve delivery of molecules into a number of cell types originating from different tissues, in the presence or absence of serum.

In another embodiment, the present invention features methods to modulate gene expression, for example, genes involved in the progression and/or maintenance of cancer or in a viral infection. For example, in one embodiment, the invention features the use of one or more of the nucleic acid-based molecules and methods independently or in combination to inhibit the expression of the gene(s) encoding proteins associated with cancerous conditions, for example breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancer associated genes.

In another embodiment, the invention features the use of one or more of the nucleic acid-based molecules and methods independently or in combination to inhibit the expression of the gene(s) encoding viral proteins, for example HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, west nile virus, Ebola virus, foot and mouth virus, and papilloma virus associated genes.

In one embodiment, the invention features the use of an enzymatic nucleic acid molecule conjugate comprising compounds of formulae 1-96, preferably in the hammerhead, NCH, G-cleaver, amberzyme, zinzyme and/or DNAzyme motif, to inhibit the expression of cancer and virus associated genes.

In another embodiment, the invention features the use of an enzymatic nucleic acid molecule as a conjugate. These enzymatic nucleic acids can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under

physiological conditions. **Table I** summarizes some of the characteristics of these enzymatic nucleic acids. Without being bound by any particular theory, in general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA destroys its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets. Thus, a single enzymatic nucleic acid molecule is able to cleave many molecules of target RNA. In addition, the enzymatic nucleic acid is a highly specific inhibitor of gene expression, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of an enzymatic nucleic acid.

In one embodiment of the invention described herein, the enzymatic nucleic acid molecule component of the conjugate is formed in a hammerhead or hairpin motif, but can also be formed in the motif of a hepatitis delta virus, group I intron, group II intron or RNase P RNA (in association with an RNA guide sequence), Neurospora VS RNA, DNAzymes, NCH cleaving motifs, or G-cleavers. Examples of such hammerhead motifs are described by Dreyfus, supra, Rossi et al., 1992, AIDS Research and Human Retroviruses 8, 183; of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, Feldstein et al., 1989, Gene 82, 53, Haseloff and Gerlach, 1989, Gene, 82, 43, and Hampel et al., 1990 Nucleic Acids Res. 18, 299; Chowrira & McSwiggen, US. Patent No. 5,631,359; of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNase P motif by Guerrier-Takada et al., 1983 Cell 35, 849; Forster and Altman, 1990, Science 249, 783; Li and Altman, 1996, Nucleic Acids Res. 24, 835; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799; Guo and Collins, 1995, EMBO. J. 14, 363); Group II introns are described by Griffin et al., 1995, Chem. Biol. 2, 761; Michels and Pyle, 1995, Biochemistry 34, 2965; Pyle et al., International PCT Publication No. WO 96/22689; of the Group I intron by Cech et al., U.S. Patent 4,987,071 and of DNAzymes by Usman et al., International PCT Publication No. WO 95/11304; Chartrand et al., 1995, NAR 23, 4092; Breaker et al., 1995, Chem. Bio. 2, 655; Santoro et al., 1997, PNAS 94, 4262, and Beigelman et al., International PCT

publication No. WO 99/55857. NCH cleaving motifs are described in Ludwig & Sproat, International PCT Publication No. WO 98/58058; and G-cleavers are described in Kore et al., 1998, Nucleic Acids Research 26, 4116-4120 and Eckstein et al., International PCT Publication No. WO 99/16871. Additional motifs such as the Aptazyme (Breaker et al., WO 98/43993), Amberzyme (Class I motif; Figure 3; Beigelman et al., U.S. Serial No. 09/301,511) and Zinzyme (Figure 4) (Beigelman et al., U.S. Serial No. 09/301,511), all incorporated by reference herein including drawings, can also be used in the present invention. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule (Cech et al., U.S. Patent No. 4,987,071).

In one embodiment of the present invention, a nucleic acid molecule component of a conjugate of the instant invention can be between 12 and 100 nucleotides in length. For example, enzymatic nucleic acid molecules of the invention are preferably between 15 and 50 nucleotides in length, more preferably between 25 and 40 nucleotides in length, e.g., 34, 36, or 38 nucleotides in length (for example see Jarvis et al., 1996, J. Biol. Chem., 271, 29107-29112). Exemplary DNAzymes of the invention are preferably between 15 and 40 nucleotides in length, more preferably between 25 and 35 nucleotides in length, e.g., 29, 30, 31, or 32 nucleotides in length (see for example Santoro et al., 1998, Biochemistry, 37, 13330-13342; Chartrand et al., 1995, Nucleic Acids Research, 23, 4092-4096). Exemplary antisense molecules of the invention are preferably between 15 and 75 nucleotides in length, more preferably between 20 and 35 nucleotides in length, e.g., 25, 26, 27, or 28 nucleotides in length (see, for example, Woolf et al., 1992, PNAS., 89, 7305-7309; Milner et al., 1997, Nature Biotechnology, 15, 537-541). Exemplary triplex forming oligonucleotide molecules of the invention are preferably between 10 and 40 nucleotides in length, more preferably between 12 and 25 nucleotides in length, e.g., 18, 19, 20, or 21 nucleotides in length (see for example Maher et al., 1990, Biochemistry, 29, 8820-8826; Strobel and Dervan, 1990, Science, 249, 73-75). Those skilled in the art will recognize that all that is required is for the nucleic acid molecule to be of sufficient length and suitable conformation for the nucleic acid molecule to catalyze a reaction contemplated herein. The length of the nucleic acid molecules described and exemplified herein are not limiting within the general size ranges stated.

The conjugates of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or

tissues. The conjugates and/or conjugate complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection or infusion pump, with or without their incorporation in biopolymers. The compositions and conjugates of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with the levels of a pathogenic protein, the patient can be treated, or other appropriate cells can be treated, as is evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the described molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules can be used in combination with one or more known therapeutic agents to treat breast, lung, prostate, colorectal, brain, esophageal, bladder, pancreatic, cervical, head and neck, and ovarian cancer, melanoma, lymphoma, glioma, multidrug resistant cancers, and/or HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, west nile virus, Ebola virus, foot and mouth virus, and papilloma virus infection.

Included in another embodiment are a series of multi-domain cellular transport vehicles (MCTV) including one or more compounds of Formulae 1-96 herein that enhance the cellular uptake and transmembrane permeability of negatively charged molecules in a variety of cell types. The compounds of the invention are used either alone or in combination with other compounds with a neutral or a negative charge including but not limited to neutral lipid and/or targeting components, to improve the effectiveness of the formulation or conjugate in delivering and targeting the predetermined compound or molecule to cells. Another embodiment of the invention encompasses the utility of these compounds for increasing the transport of other impermeable and/or lipophilic compounds into cells. Targeting components include ligands for cell surface receptors including, peptides and proteins, glycolipids, lipids, carbohydrates, and their synthetic variants, for example folate receptors.

In another embodiment, the compounds of the invention are provided as a surface component of a lipid aggregate, such as a liposome encapsulated with the predetermined molecule to be delivered. Liposomes, which can be unilamellar or multilamellar, can introduce encapsulated material into a cell by different mechanisms. For example, the liposome can directly introduce its encapsulated material into the cell cytoplasm by fusing with the cell membrane. Alternatively, the liposome can be compartmentalized into an

acidic vacuole (i.e., an endosome) and its contents released from the liposome and out of the acidic vacuole into the cellular cytoplasm.

In one embodiment the invention features a lipid aggregate formulation of the compounds described herein, including phosphatidylcholine (of varying chain length; e.g., egg yolk phosphatidylcholine), cholesterol, a cationic lipid, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polythyleneglycol-2000 (DSPE-PEG2000). The cationic lipid component of this lipid aggregate can be any cationic lipid known in the art such as dioleoyl 1,2,-diacyl-3-trimethylammonium-propane (DOTAP). In another embodiment this cationic lipid aggregate comprises a covalently bound compound described in any of the Formulae herein.

In another embodiment, polyethylene glycol (PEG) is covalently attached to the compounds of the present invention. The attached PEG can be any molecular weight but is preferably between 2000-50,000 daltons.

The compounds and methods of the present invention are useful for introducing nucleotides, nucleosides, nucleic acid molecules, lipids, peptides, proteins, and/or non-nucleosidic small molecules into a cell. For example, the invention can be used for nucleotide, nucleoside, nucleic acid, lipids, peptides, proteins, and/or non-nucleosidic small molecule delivery where the corresponding target site of action exists intracellularly.

In one embodiment, the compounds of the instant invention provide conjugates of molecules that can interact with cellular receptors, such as high affinity folate receptors and ASGPr receptors, and provide a number of features that allow the efficient delivery and subsequent release of conjugated compounds across biological membranes. The compounds utilize chemical linkages between the receptor ligand and the compound to be delivered of length that can interact preferentially with cellular receptors. Furthermore, the chemical linkages between the ligand and the compound to be delivered can be designed as degradable linkages, for example by utilizing a phosphate linkage that is proximal to a nucleophile, such as a hydroxyl group. Deprotonation of the hydroxyl group or an equivalent group, as a result of pH or interaction with a nuclease, can result in nucleophilic attack of the phosphate resulting in a cyclic phosphate intermediate that can be hydrolyzed. This cleavage mechanism is analogous RNA cleavage in the presence of a base or RNA nuclease. Alternately, other degradable linkages can be selected that respond to various factors such as UV irradiation, cellular nucleases, pH, temperature etc. The use of degradable linkages allows the delivered compound to be released in a

predetermined system, for example in the cytoplasm of a cell, or in a particular cellular organelle.

The present invention also provides ligand derived phosphoramidites that are readily conjugated to compounds and molecules of interest. Phosphoramidite compounds of the invention permit the direct attachment of conjugates to molecules of interest without the need for using nucleic acid phosphoramidite species as scaffolds. As such, the used of phosphoramidite chemistry can be used directly in coupling the compounds of the invention to a compound of interest, without the need for other condensation reactions, such as condensation of the ligand to an amino group on the nucleic acid, for example at the N6 position of adenosine or a 2'-deoxy-2'-amino function. Additionally, compounds of the invention can be used to introduce non-nucleic acid based conjugated linkages into oligonucleotides that can provide more efficient coupling during oligonucleotide synthesis than the use of nucleic acid-based phosphoramidites. This improved coupling can take into account improved steric considerations of abasic or non-nucleosidic scaffolds bearing pendant alkyl linkages.

Compounds of the invention utilizing triphosphate groups can be utilized in the enzymatic incorporation of conjugate molecules into oligonucleotides. Such enzymatic incorporation is useful when conjugates are used in post-synthetic enzymatic conjugation or selection reactions, (see for example Matulic-Adamic *et al.,* 2000, *Bioorg. Med. Chem. Lett.,* 10, 1299-1302; Lee *et al.,* 2001, *NAR.*, 29, 1565-1573; Joyce, 1989, *Gene,* 82, 83-87; Beaudry *et al.,* 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.,* 1994, *TIBTECH* 12, 268; Bartel *et al.,*1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.,* 1995, *FASEB J.,* 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442; Santoro *et al.,* 1997, *Proc. Natl. Acad. Sci.,* 94, 4262; Tang *et al.,* 1997, *RNA* 3, 914; Nakamaye & Eckstein, 1994, *supra*; Long & Uhlenbeck, 1994, supra; Ishizaka et al., 1995, *supra*; Vaish *et al.,* 1997, *Biochemistry* 36, 6495; Kuwabara *et al.,* 2000, *Curr. Opin. Chem. Biol.,* 4, 669).

Compounds of the invention can be used to detect the presence of a target molecule in a biological system, such as tissue, cell or cell lysate. Examples of target molecules include nucleic acids, proteins, peptides, antibodies, polysaccharides, lipids, hormones, sugars, metals, microbial or cellular metabolites, analytes, pharmaceuticals, and other organic and inorganic molecules or other biomolecules in a sample. The compounds of the instant invention can be conjugated to a predetermined compound or molecule that is capable of interacting with the target molecule in the system and providing a detectable signal or response. Various compounds and molecules known in

the art that can be used in these applications include but are not limited to antibodies, labeled antibodies, allozymes, aptamers, labeled nucleic acid probes, molecular beacons, fluorescent molecules, radioisotopes, polysaccharides, and any other compound capable of interacting with the target molecule and generating a detectable signal upon target interaction. For example, such compounds are described in Application entitled "NUCLEIC ACID SENSOR MOLECULES", USSN 09/800,594 filed on March 6, 2001 (Not yet assigned; Attorney Docket No. MBHB00-816-A 700.001) with inventors Nassim Usman and James A. McSwiggen, which is incorporated by reference in its entirety, including the drawings.

The term "biodegradable nucleic acid linker molecule" as used herein, refers to a nucleic acid molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule. The stability of the biodegradable nucleic acid linker molecule can be modulated by using various combinations of ribonucleotides, deoxyribonucleotides, and chemically modified nucleotides, for example 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus based linkage, for example a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siRNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

The term "nitrogen containing group" as used herein refers to any chemical group or moiety comprising a nitrogen or substituted nitrogen. Non-limiting examples of nitrogen containing groups include amines, substituted amines, amides, alkylamines, amino acids such as arginine or lysine, polyamines such as spermine or spermidine, cyclic amines such as pyridines, pyrimidines including uracil, thymine, and cytosine, morpholines, phthalimides, and heterocyclic amines such as purines, including guanine and adenine.

The term "target molecule" as used herein, refers to nucleic acid molecules, proteins, peptides, antibodies, polysaccharides, lipids, sugars, metals, microbial or cellular metabolites, analytes, pharmaceuticals, and other organic and inorganic molecules that are present in a system.

By "inhibit" or "down-regulate" it is meant that the expression of the gene, or level of RNAs or equivalent RNAs encoding one or more protein subunits, or activity of one or more protein subunits, such as pathogenic protein, viral protein or cancer related protein subunit(s), is reduced below that observed in the absence of the compounds or combination of compounds of the invention. In one embodiment, inhibition or down-regulation with an enzymatic nucleic acid molecule preferably is below that level observed in the presence of an enzymatically inactive or attenuated molecule that is able to bind to the same site on the target RNA, but is unable to cleave that RNA. In another embodiment, inhibition or down-regulation with antisense oligonucleotides is preferably below that level observed in the presence of, for example, an oligonucleotide with scrambled sequence or with mismatches. In another embodiment, inhibition or down-regulation of viral or oncogenic RNA, protein, or protein subunits with a compound of the instant invention is greater in the presence of the compound than in its absence.

By "up-regulate" is meant that the expression of the gene, or level of RNAs or equivalent RNAs encoding one or more protein subunits, or activity of one or more protein subunits, such as viral or oncogenic protein subunit(s), is greater than that observed in the absence of the compounds or combination of compounds of the invention. For example, the expression of a gene, such as a viral or cancer related gene, can be increased in order to treat, prevent, ameliorate, or modulate a pathological condition caused or exacerbated by an absence or low level of gene expression.

By "modulate" is meant that the expression of the gene, or level of RNAs or equivalent RNAs encoding one or more protein subunits, or activity of one or more protein subunit(s) of a protein, for example a viral or cancer related protein is up-regulated or down-regulated, such that the expression, level, or activity is greater than or less than that observed in the absence of the compounds or combination of compounds of the invention.

The term "enzymatic nucleic acid molecule" as used herein refers to a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave target RNA. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% can also be useful in this invention (see for example Werner and Uhlenbeck, 1995, Nucleic Acids Research, 23, 2092-2096; Hammann et al., 1999, Antisense and Nucleic Acid Drug Dev., 9, 25-31). The nucleic acids can be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving and/or ligation activity to the molecule (Cech et al., U.S. Patent No. 4,987,071; Cech et al., 1988, 260 JAMA 3030).

The term "nucleic acid molecule" as used herein, refers to a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and can comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

The term "enzymatic portion" or "catalytic domain" as used herein refers to that portion/region of the enzymatic nucleic acid molecule essential for cleavage of a nucleic acid substrate (for example see Figure 1).

The term "substrate binding arm" or "substrate binding domain" as used herein refers to that portion/region of a enzymatic nucleic acid which is able to interact, for example via complementarity (i.e., able to base-pair with), with a portion of its substrate. Preferably, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 can be base-paired (see for example Werner and Uhlenbeck, 1995, Nucleic Acids Research, 23, 2092-2096; Hammann et al., 1999, Antisense and Nucleic Acid Drug Dev., 9, 25-31). Examples of such arms are shown generally in Figures 1-4. That is, these arms contain sequences within a enzymatic nucleic acid which are intended to bring enzymatic nucleic acid and target RNA together through complementary base-pairing interactions. The enzymatic nucleic acid of the invention can have binding arms that are contiguous or non-contiguous and can be of varying lengths. The length of the binding arm(s) are preferably greater than or equal to four nucleotides and of sufficient length to stably interact with the target RNA; preferably 12-100 nucleotides; more preferably 14-24 nucleotides long (see for example Werner and Uhlenbeck, supra; Hamman et al., supra; Hampel et al., EP0360257; Berzal-Herrance et al., 1993, EMBO J., 12, 2567-73). If two binding arms are chosen, the design is such that the length of the binding arms are symmetrical (i.e., each of the binding arms is of the same length; e.g., five and five nucleotides, or six and six nucleotides, or seven and seven nucleotides long) or asymmetrical (i.e., the binding arms are of different length; e.g., six and three nucleotides; three and six nucleotides long; four and five nucleotides long; four and six nucleotides long; four and seven nucleotides long; and the like).

The term "Inozyme" or "NCH" motif as used herein, refers to an enzymatic nucleic acid molecule comprising a motif as is generally described as NCH Rz in **Figure 1**. Inozymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCH/, where N is a nucleotide, C is cytidine and H is adenosine, uridine or cytidine, and / represents the cleavage site. H is used interchangeably with X. Inozymes can also possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCN/, where N is a nucleotide, C is cytidine, and / represents the cleavage site. "I" in Figure 2 represents an Inosine nucleotide, preferably a ribo-Inosine or xylo-Inosine nucleoside.

The term "G-cleaver" motif as used herein, refers to an enzymatic nucleic acid molecule comprising a motif as is generally described as G-cleaver Rz in **Figure 1**. G-

cleavers possess endonuclease activity to cleave RNA substrates having a cleavage triplet NYN/, where N is a nucleotide, Y is uridine or cytidine and / represents the cleavage site. G-cleavers can be chemically modified as is generally shown in Figure 2.

The term "amberzyme" motif as used herein, refers to an enzymatic nucleic acid molecule comprising a motif as is generally described in **Figure 2**. Amberzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NG/N, where N is a nucleotide, G is guanosine, and / represents the cleavage site. Amberzymes can be chemically modified to increase nuclease stability through substitutions as are generally shown in Figure 3. In addition, differing nucleoside and/or non-nucleoside linkers can be used to substitute the 5'-gaaa-3' loops shown in the figure. Amberzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

The term "zinzyme" motif as used herein, refers to an enzymatic nucleic acid molecule comprising a motif as is generally described in **Figure 3**. Zinzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet including but not limited to YG/Y, where Y is uridine or cytidine, and G is guanosine and / represents the cleavage site. Zinzymes can be chemically modified to increase nuclease stability through substitutions as are generally shown in **Figure 3**, including substituting 2'-O-methyl guanosine nucleotides for guanosine nucleotides. In addition, differing nucleotide and/or non-nucleotide linkers can be used to substitute the 5'-gaaa-2' loop shown in the figure. Zinzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

The term 'DNAzyme' as used herein, refers to an enzymatic nucleic acid molecule that does not require the presence of a 2'-OH group for its activity. In particular embodiments the enzymatic nucleic acid molecule can have an attached linker(s) or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. DNAzymes can be synthesized chemically or expressed endogenously in vivo, by means of a single stranded DNA vector or equivalent thereof. An example of a DNAzyme is shown in **Figure 4** and is generally reviewed in Usman et al., International PCT Publication No. WO 95/11304; Chartrand et al., 1995, NAR 23, 4092; Breaker et al., 1995, Chem. Bio. 2, 655; Santoro et al., 1997, PNAS 94, 4262; Breaker, 1999, Nature Biotechnology, 17, 422-423; and Santoro et. al., 2000, J. Am. Chem. Soc., 122, 2433-39. Additional DNAzyme motifs can be selected for using techniques similar to those described in these references, and hence, are within the scope of the present invention.

The term "sufficient length" as used herein, refers to an oligonucleotide of length great enough to provide the intended function under the expected condition, i.e., greater than or equal to 3 nucleotides. For example, for binding arms of enzymatic nucleic acid "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected binding conditions. Preferably, the binding arms are not so long as to prevent useful turnover of the nucleic acid molecule.

The term "stably interact" as used herein, refers to interaction of the oligonucleotides with target nucleic acid (e.g., by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions) that is sufficient to the intended purpose (e.g., cleavage of target RNA by an enzyme).

The term "homology" as used herein, refers to the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

The term "antisense nucleic acid", as used herein, refers to a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 Nature 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 Science 261, 1004 and Woolf et al., US patent No. 5,849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk et al., 1999, J. Biol. Chem., 274, 21783-21789, Delihas et al., 1997, Nature, 15, 751-753, Stein et al., 1997, Antisense N. A. Drug Dev., 7, 151, Crooke, 2000, Methods Enzymol., 313, 3-45; Crooke, 1998, Biotech. Genet. Eng. Rev., 15, 121-157, Crooke, 1997, Ad. Pharmacol., 40, 1-49. In addition, antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target RNA. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof.

The term "RNase H activating region" as used herein, refers to a region (generally greater than or equal to 4-25 nucleotides in length, preferably from 5-11 nucleotides in

length) of a nucleic acid molecule capable of binding to a target RNA to form a non-covalent complex that is recognized by cellular RNase H enzyme (see for example Arrow et al., US 5,849,902; Arrow et al., US 5,989,912). The RNase H enzyme binds to the nucleic acid molecule-target RNA complex and cleaves the target RNA sequence. The RNase H activating region comprises, for example, phosphodiester, phosphorothioate (preferably at least four of the nucleotides are phosphorothiote substitutions; more specifically, 4-11 of the nucleotides are phosphorothiote substitutions); phosphorodithioate, 5'-thiophosphate, or methylphosphonate backbone chemistry or a combination thereof. In addition to one or more backbone chemistries described above, the RNase H activating region can also comprise a variety of sugar chemistries. For example, the RNase H activating region can comprise deoxyribose, arabino, fluoroarabino or a combination thereof, nucleotide sugar chemistry. Those skilled in the art will recognize that the foregoing are non-limiting examples and that any combination of phosphate, sugar and base chemistry of a nucleic acid that supports the activity of RNase H enzyme is within the scope of the definition of the RNase H activating region and the instant invention.

The term "2-5A antisense chimera" as used herein, refers to an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which, in turn, cleaves the target RNA (Torrence et al., 1993 Proc. Natl. Acad. Sci. USA 90, 1300; Silverman et al., 2000, Methods Enzymol., 313, 522-533; Player and Torrence, 1998, Pharmacol. Ther., 78, 55-113).

The term "triplex forming oligonucleotides" as used herein, refers to an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 Proc. Natl. Acad. Sci. USA 89, 504; Fox, 2000, Curr. Med. Chem., 7, 17-37; Praseuth et. al., 2000, Biochim. Biophys. Acta, 1489, 181-206).

The term "gene" it as used herein, refers to a nucleic acid that encodes an RNA, for example, nucleic acid sequences including but not limited to structural genes encoding a polypeptide.

The term "pathogenic protein" as used herein, refers to endogenous or exongenous proteins that are associated with a disease state or condition, for example a particular cancer or viral infection.

The term "complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., enzymatic nucleic acid cleavage, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, CSH Symp. Quant. Biol. LII pp.123-133; Frier et al., 1986, Proc. Nat. Acad. Sci. USA 83:9373-9377; Turner et al., 1987, J. Am. Chem. Soc. 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

The term "RNA" as used herein, refers to a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" or "2'-OH" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribo-furanose moiety.

The term "decoy RNA" as used herein, refers to a RNA molecule or aptamer that is designed to preferentially bind to a predetermined ligand. Such binding can result in the inhibition or activation of a target molecule. The decoy RNA or aptamer can compete with a naturally occurring binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990, Cell, 63, 601-608). This is but a specific example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art, see for example Gold et al., 1995, Annu. Rev. Biochem., 64, 763; Brody and Gold, 2000, J. Biotechnol., 74, 5; Sun, 2000, Curr. Opin. Mol. Ther., 2, 100; Kusser, 2000, J. Biotechnol., 74, 27; Hermann and Patel, 2000, Science, 287, 820; and Jayasena, 1999, Clinical Chemistry, 45, 1628. Similarly, a decoy RNA can be designed to bind to a receptor and block the binding of an effector molecule or a decoy RNA can be designed to bind to receptor of interest and prevent interaction with the receptor.

The term "single stranded RNA" (ssRNA) as used herein refers to a naturally occurring or synthetic ribonucleic acid molecule comprising a linear single strand, for

example a ssRNA can be a messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) etc. of a gene.

The term "single stranded DNA" (ssDNA) as used herein refers to a naturally occurring or synthetic deoxyribonucleic acid molecule comprising a linear single strand, for example, a ssDNA can be a sense or antisense gene sequence or EST (Expressed Sequence Tag).

The term "double stranded RNA" or "dsRNA" as used herein refers to a double stranded RNA molecule capable of RNA interference, including short interfering RNA (siRNA).

The term "short interfering RNA" or "siRNA" as used herein refers to a double stranded nucleic acid molecule capable of RNA interference "RNAi", see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir et al., 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914. As used herein, siRNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides.

The term "allozyme" as used herein refers to an allosteric enzymatic nucleic acid molecule, see for example see for example George et al., US Patent Nos. 5,834,186 and 5,741,679, Shih et al., US Patent No. 5,589,332, Nathan et al., US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker et al., International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger et al., International PCT publication No. WO 99/29842.

The term "cell" as used herein, refers to its usual biological sense, and does not refer to an entire multicellular organism. The cell can, for example, be in vitro, e.g., in cell culture, or present in a multicellular organism, including,, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

The term "highly conserved sequence region" as used herein, refers to a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

The term "non-nucleotide" as used herein, refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine.

The term "nucleotide" as used herein, refers to a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, supra; Eckstein *et al.,* International PCT Publication No. WO 92/07065; Usman *et al.,* International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.,* 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, for example, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin *et al.,* 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

The term "nucleoside" as used herein, refers to a heterocyclic nitrogenous base in N-glycosidic linkage with a sugar. Nucleosides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleoside sugar moiety. Nucleosides generally comprise a base and sugar group. The nucleosides can be unmodified or modified at the sugar, and/or base moiety, (also referred to interchangeably as nucleoside analogs, modified nucleosides, non-natural nucleosides, non-standard nucleosides and other; see for example, Usman and McSwiggen, supra; Eckstein *et al.,* International PCT Publication No. WO 92/07065; Usman *et al.,* International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.,* 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin *et al.,* 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleoside bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

The term "cap structure" as used herein, refers to chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see for example Wincott *et al.,* WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both terminus. In non-limiting examples,

the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott *et al.,* International PCT publication No. WO 97/26270, incorporated by reference herein).

The term "abasic" as used herein, refers to sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative (for more details see Wincott *et al.,* International PCT publication No. WO 97/26270).

The term "unmodified nucleoside" as used herein, refers to one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of β-D-ribo-furanose.

The term "modified nucleoside" as used herein, refers to any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

The term "consists essentially of" as used herein, is meant that the active nucleic acid molecule of the invention, for example, an enzymatic nucleic acid molecule, contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind RNA such that cleavage at the target site occurs. Other sequences can be present which do not interfere with such cleavage. Thus, a core region can, for example, include one or more loop, stem-loop structure, or linker which does not prevent enzymatic activity. For example, a core sequence for a hammerhead enzymatic nucleic acid can comprise a conserved sequence, such as 5'-CUGAUGAG-3' and 5'-CGAA-3' connected by "X", where X is 5'-GCCGUUAGGC-3' (SEQ ID NO 1), or any other Stem II region known in the art, or a nucleotide and/or non-nucleotide linker. Similarly, for other nucleic acid molecules of the instant invention, such as Inozyme, G-cleaver, amberzyme, zinzyme, DNAzyme, antisense, 2-5A antisense, triplex forming nucleic acid, and decoy nucleic acids, other sequences or non-nucleotide linkers can be present that do not interfere with the function of the nucleic acid molecule.

Sequence X can be a linker of ≥ 2 nucleotides in length, preferably 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 26, 30, where the nucleotides can preferably be internally base-paired to form a stem of preferably ≥ 2 base pairs. In yet another embodiment, the nucleotide linker X can be a nucleic acid aptamer, such as an ATP aptamer, HIV Rev aptamer (RRE), HIV Tat aptamer (TAR) and others (for a review see Gold et al., 1995, Annu. Rev. Biochem., 64, 763; and Szostak & Ellington, 1993, in The RNA World, ed. Gesteland and Atkins, pp. 511, CSH Laboratory Press). A "nucleic acid aptamer" as used herein is meant to indicate a nucleic acid sequence capable of interacting with a ligand. The ligand can be any natural or a synthetic molecule, including but not limited to a resin, metabolites, nucleosides, nucleotides, drugs, toxins, transition state analogs, peptides, lipids, proteins, amino acids, nucleic acid molecules, hormones, carbohydrates, receptors, cells, viruses, bacteria and others.

Alternatively or in addition, sequence X can be a non-nucleotide linker. Non-nucleotides can include abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, Nucleic Acids Res. 1990, 18:6353 and Nucleic Acids Res. 1987, 15:3113; Cload and Schepartz, J. Am. Chem. Soc. 1991, 113:6324; Richardson and Schepartz, J. Am. Chem. Soc. 1991, 113:5109; Ma et al., Nucleic Acids Res. 1993, 21:2585 and Biochemistry 1993, 32:1751; Durand et al., Nucleic Acids Res. 1990, 18:6353; McCurdy et al., Nucleosides & Nucleotides 1991, 10:287; Jschke et al., Tetrahedron Lett. 1993, 34:301; Ono et al., Biochemistry 1991, 30:9914; Arnold et al., International Publication No. WO 89/02439; Usman et al., International Publication No. WO 95/06731; Dudycz et al., International Publication No. WO 95/11910 and Ferentz and Verdine, J. Am. Chem. Soc. 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine. Thus, in a preferred embodiment, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

The term "patient" as used herein, refers to an organism, which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. Preferably, a

patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells.

The term "enhanced enzymatic activity" as used herein, includes activity measured in cells and/or in vivo where the activity is a reflection of both the catalytic activity and the stability of the nucleic acid molecules of the invention. In this invention, the product of these properties can be increased in vivo compared to an all RNA enzymatic nucleic acid or all DNA enzyme. In some cases, the activity or stability of the nucleic acid molecule can be decreased (i.e., less than ten-fold), but the overall activity of the nucleic acid molecule is enhanced, in vivo.

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and can or can not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements can be present.

The term “negatively charged molecules” as used herein, refers to molecules such as nucleic acid molecules (e.g., RNA, DNA, oligonucleotides, mixed polymers, peptide nucleic acid, and the like), peptides (e.g., polyaminoacids, polypeptides, proteins and the like), nucleotides, pharmaceutical and biological compositions, that have negatively charged groups that can ion-pair with the positively charged head group of the cationic lipids of the invention.

The term “coupling” as used herein, refers to a reaction, either chemical or enzymatic, in which one atom, moiety, group, compound or molecule is joined to another atom, moiety, group, compound or molecule.

The terms “deprotection” or “deprotecting” as used herein, refers to the removal of a protecting group.

The term "alkyl" as used herein refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain “isoalkyl”, and cyclic alkyl groups. The term “alkyl” also comprises alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, alkenyl, alkynyl, alkoxy, cycloalkenyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, C1-C6 hydrocarbyl, aryl or substituted aryl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from about 1 to about 7 carbons, more preferably about 1 to about 4 carbons. The alkyl group can be substituted

or unsubstituted. When substituted the substituted group(s) preferably comprise hydroxy, oxy, thio, amino, nitro, cyano, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, silyl, alkenyl, alkynyl, alkoxy, cycloalkenyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, C1-C6 hydrocarbyl, aryl or substituted aryl groups. The term "alkyl" also includes alkenyl groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has about 2 to about 12 carbons. More preferably it is a lower alkenyl of from about 2 to about 7 carbons, more preferably about 2 to about 4 carbons. The alkenyl group can be substituted or unsubstituted. When substituted the substituted group(s) preferably comprise hydroxy, oxy, thio, amino, nitro, cyano, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, silyl, alkenyl, alkynyl, alkoxy, cycloalkenyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, C1-C6 hydrocarbyl, aryl or substituted aryl groups. The term "alkyl" also includes alkynyl groups containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has about 2 to about 12 carbons. More preferably it is a lower alkynyl of from about 2 to about 7 carbons, more preferably about 2 to about 4 carbons. The alkynyl group can be substituted or unsubstituted. When substituted the substituted group(s) preferably comprise hydroxy, oxy, thio, amino, nitro, cyano, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, silyl, alkenyl, alkynyl, alkoxy, cycloalkenyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, C1-C6 hydrocarbyl, aryl or substituted aryl groups. Alkyl groups or moieties of the invention can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from about 1 to about 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

The term “alkoxyalkyl” as used herein refers to an alkyl-O-alkyl ether, for example, methoxyethyl or ethoxymethyl.

The term "alkyl-thio-alkyl" as used herein refers to an alkyl-S-alkyl thioether, for example, methylthiomethyl or methylthioethyl.

The term "amino" as used herein refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms "aminoacyl" and "aminoalkyl" refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

The term "amination" as used herein refers to a process in which an amino group or substituted amine is introduced into an organic molecule.

The term "exocyclic amine protecting moiety" as used herein refers to a nucleobase amino protecting group compatible with oligonucleotide synthesis, for example, an acyl or amide group.

The term "alkenyl" as used herein refers to a straight or branched hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon double bond. Examples of "alkenyl" include vinyl, allyl, and 2-methyl-3-heptene.

The term "alkoxy" as used herein refers to an alkyl group of indicated number of carbon atoms attached to the parent molecular moiety through an oxygen bridge. Examples of alkoxy groups include, for example, methoxy, ethoxy, propoxy and isopropoxy.

The term "alkynyl" as used herein refers to a straight or branched hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon triple bond. Examples of "alkynyl" include propargyl, propyne, and 3-hexyne.

The term "aryl" as used herein refers to an aromatic hydrocarbon ring system containing at least one aromatic ring. The aromatic ring can optionally be fused or otherwise attached to other aromatic hydrocarbon rings or non-aromatic hydrocarbon rings. Examples of aryl groups include, for example, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthalene and biphenyl. Preferred examples of aryl groups include phenyl and naphthyl.

The term "cycloalkenyl" as used herein refers to a C3-C8 cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadiene, cyclohexenyl, 1,3-cyclohexadiene, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

The term "cycloalkyl" as used herein refers to a C3-C8 cyclic hydrocarbon. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

The term "cycloalkylalkyl," as used herein, refers to a C3-C7 cycloalkyl group attached to the parent molecular moiety through an alkyl group, as defined above. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

The terms “halogen” or "halo" as used herein refers to indicate fluorine, chlorine, bromine, and iodine.

The term "heterocycloalkyl," as used herein refers to a non-aromatic ring system containing at least one heteroatom selected from nitrogen, oxygen, and sulfur. The heterocycloalkyl ring can be optionally fused to or otherwise attached to other heterocycloalkyl rings and/or non-aromatic hydrocarbon rings. Preferred heterocycloalkyl groups have from 3 to 7 members. Examples of heterocycloalkyl groups include, for example, piperazine, morpholine, piperidine, tetrahydrofuran, pyrrolidine, and pyrazole. Preferred heterocycloalkyl groups include piperidinyl, piperazinyl, morpholinyl, and pyrolidinyl.

The term "heteroaryl" as used herein refers to an aromatic ring system containing at least one heteroatom selected from nitrogen, oxygen, and sulfur. The heteroaryl ring can be fused or otherwise attached to one or more heteroaryl rings, aromatic or non-aromatic hydrocarbon rings or heterocycloalkyl rings. Examples of heteroaryl groups include, for example, pyridine, furan, thiophene, 5,6,7,8-tetrahydroisoquinoline and pyrimidine. Preferred examples of heteroaryl groups include thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, benzimidazolyl, furanyl, benzofuranyl, thiazolyl, benzothiazolyl, isoxazolyl, oxadiazolyl, isothiazolyl, benzisothiazolyl, triazolyl, tetrazolyl, pyrrolyl, indolyl, pyrazolyl, and benzopyrazolyl.

The term “C1-C6 hydrocarbyl” as used herein refers to straight, branched, or cyclic alkyl groups having 1-6 carbon atoms, optionally containing one or more carbon-carbon double or triple bonds. Examples of hydrocarbyl groups include, for example, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, 3-methylpentyl, vinyl, 2-pentene, cyclopropylmethyl, cyclopropyl, cyclohexylmethyl, cyclohexyl and propargyl. When reference is made herein to C1-C6 hydrocarbyl containing one or two double or triple bonds it is understood that at least two carbons are present in the alkyl for one double or triple bond, and at least four carbons for two double or triple bonds.

The term "protecting group" as used herein, refers to groups known in the art that are readily introduced and removed from an atom, for example O, N, P, or S. Protecting groups are used to prevent undesirable reactions from taking place that can compete with the formation of a specific compound or intermediate of interest. See also "Protective Groups in Organic Synthesis", 3rd Ed., 1999, Greene, T. W. and related publications.

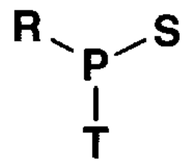
The term "nitrogen protecting group," as used herein, refers to groups known in the art that are readily introduced on to and removed from a nitrogen. Examples of nitrogen protecting groups include Boc, Cbz, benzoyl, and benzyl. See also "Protective Groups in Organic Synthesis", 3rd Ed., 1999, Greene, T. W. and related publications.

The term "hydroxy protecting group," or "hydroxy protection" as used herein, refers to groups known in the art that are readily introduced on to and removed from an oxygen, specifically an –OH group. Examples of hyroxy protecting groups include trityl or substituted trityl goups, such as monomethoxytrityl and dimethoxytrityl, or substituted silyl groups, such as tert-butyldimethyl, trimethylsilyl, or tert-butyldiphenyl silyl groups. See also "Protective Groups in Organic Synthesis", 3rd Ed., 1999, Greene, T. W. and related publications.

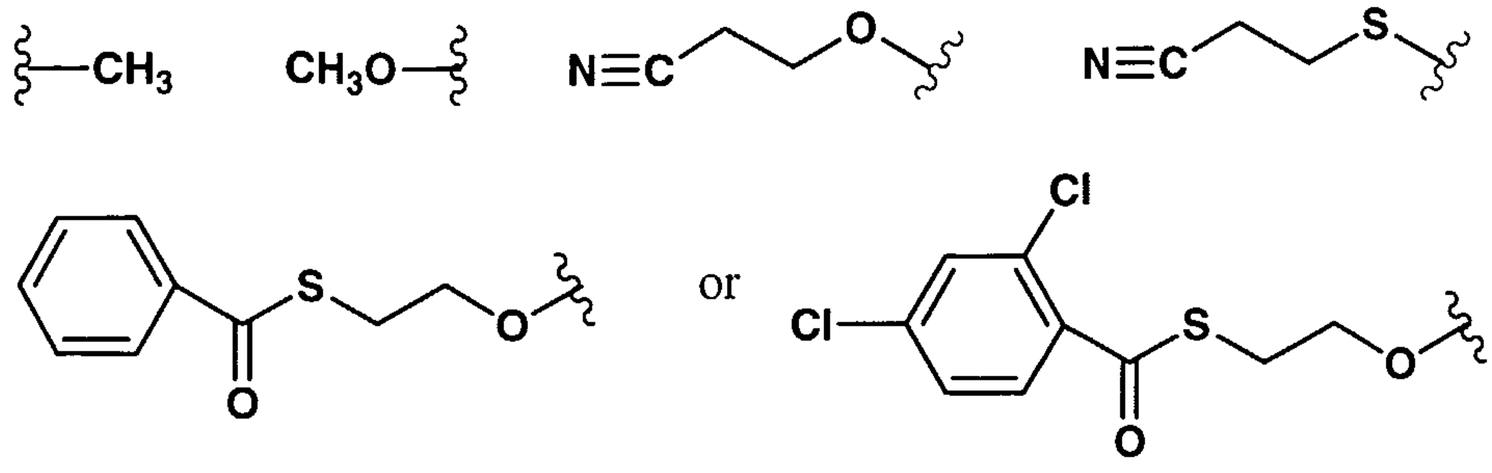
The term "acyl" as used herein refers to -C(O)R groups, wherein R is an alkyl or aryl.

The term "phosphorus containing group" as used herein, refers to a chemical group containing a phosphorus atom. The phosphorus atom can be trivalent or pentavalent, and can be substituted with O, H, N, S, C or halogen atoms. Examples of phosphorus containing groups of the instant invention include but are not limited to phosphorus atoms substituted with O, H, N, S, C or halogen atoms, comprising phosphonate, alkylphosphonate, phosphate, diphosphate, triphosphate, pyrophosphate, phosphorothioate, phosphorodithioate, phosphoramidate, phosphoramidite groups, nucleotides and nucleic acid molecules.

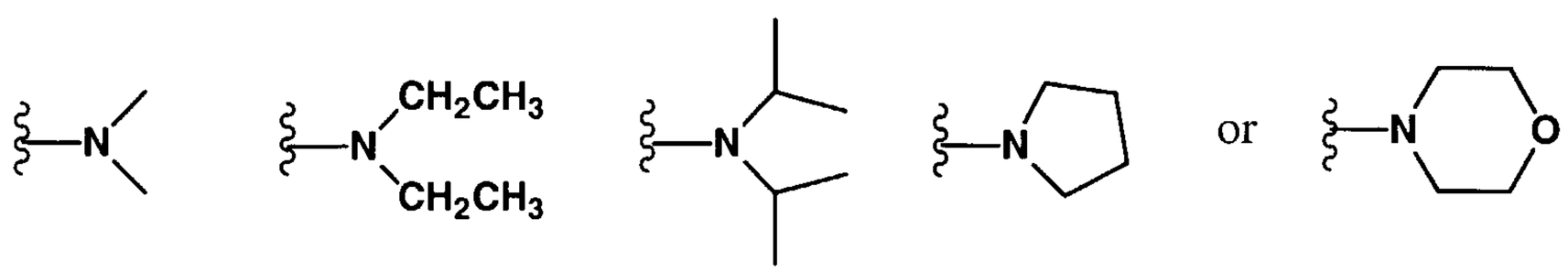
The term "phosphine" or "phosphite" as used herein refers to a trivalent phosphorus species, for example compounds having Formula 97:



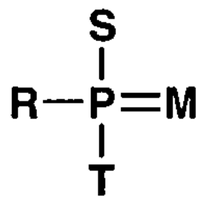
wherein R can include the groups:



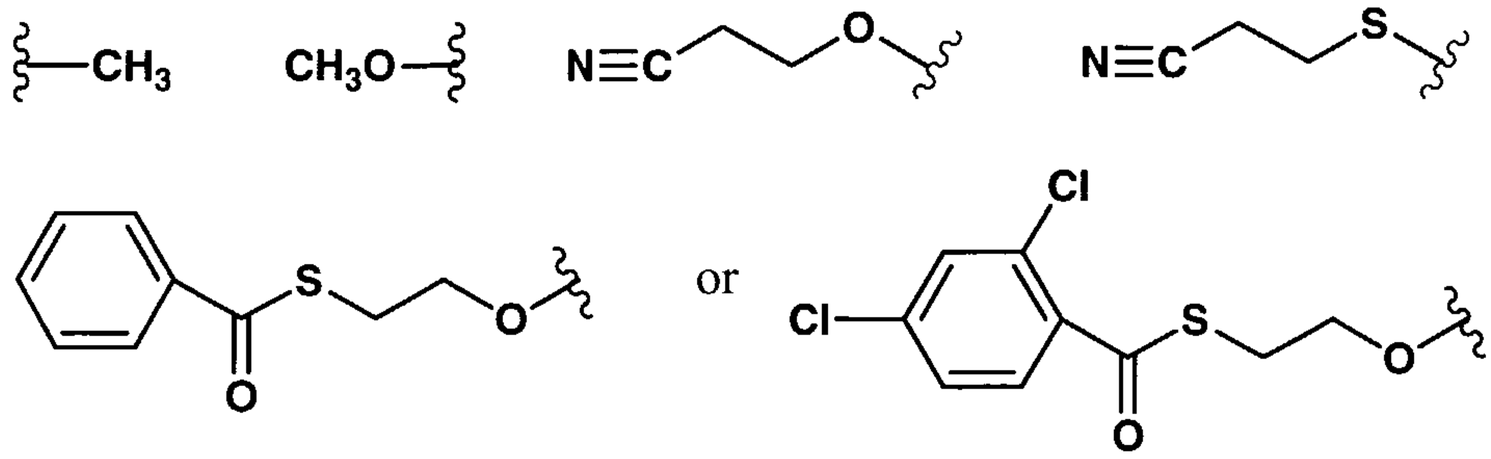
and wherein S and T independently include the groups:



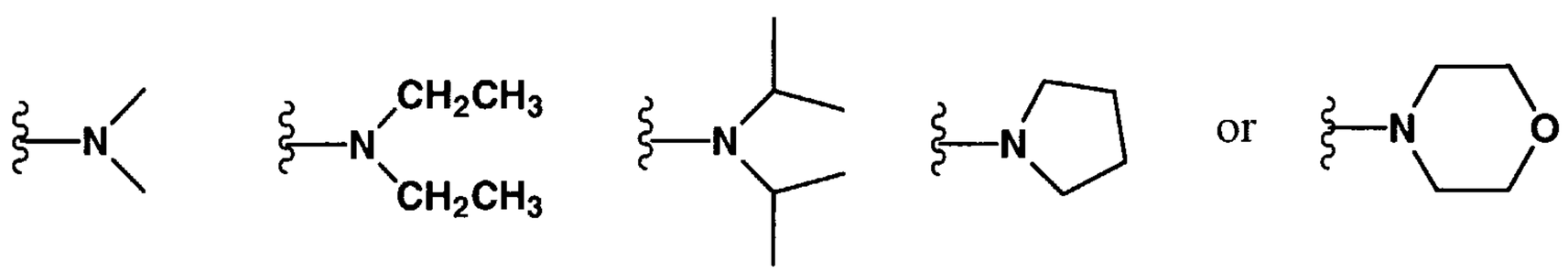
The term "phosphate" as used herein refers to a pentavalent phosphorus species, for example a compound having Formula 98:



wherein R includes the groups:



and wherein S and T each independently can be a sulfur or oxygen atom or a group which can include:



and wherein M comprises a sulfur or oxygen atom. The phosphate of the invention can comprise a nucleotide phosphate, wherein any R, S, or T in Formula 98 comprises a linkage to a nucleic acid or nucleoside.

The term "cationic salt" as used herein refers to any organic or inorganic salt having a net positive charge, for example a triethylammonium (TEA) salt.

The term "degradable linker" as used herein, refers to linker moieties that are capable of cleavage under various conditions. Conditions suitable for cleavage can include but are not limited to pH, UV irradiation, enzymatic activity, temperature, hydrolysis, elimination, and substitution reactions, and thermodynamic properties of the linkage.

The term "photolabile linker" as used herein, refers to linker moieties as are known in the art, that are selectively cleaved under particular UV wavelengths. Compounds of the invention containing photolabile linkers can be used to deliver compounds to a target cell or tissue of interest, and can be subsequently released in the presence of a UV source.

The term "nucleic acid conjugates" as used herein, refers to nucleoside, nucleotide and oligonucleotide conjugates.

The term "folate" as used herein, refers to analogs and derivatives of folic acid, for example antifolates, dihydrofloates, tetrahydrofolates, tetrahydorpterins, folinic acid, pteropolyglutamic acid, 1-deza, 3-deaza, 5-deaza, 8-deaza, 10-deaza, 1,5-deaza, 5,10 dideaza, 8,10-dideaza, and 5,8-dideaza folates, antifolates, and pteroic acid derivatives.

The term "compounds with neutral charge" as used herein, refers to compositions which are neutral or uncharged at neutral or physiological pH. Examples of such compounds are cholesterol and other steroids, cholesteryl hemisuccinate (CHEMS), dioleoyl phosphatidyl choline, distearoylphosphotidyl choline (DSPC), fatty acids such as oleic acid, phosphatidic acid and its derivatives, phosphatidyl serine, polyethylene glycol - conjugated phosphatidylamine, phosphatidylcholine, phosphatidylethanolamine and related variants, prenylated compounds including farnesol, polyprenols, tocopherol, and their modified forms, diacylsuccinyl glycerols, fusogenic or pore forming peptides, dioleoylphosphotidylethanolamine (DOPE), ceramide and the like.

The term "lipid aggregate" as used herein refers to a lipid-containing composition wherein the lipid is in the form of a liposome, micelle (non-lamellar phase) or other aggregates with one or more lipids.

The term "biological system" as used herein, refers to a eukaryotic system or a prokaryotic system, can be a bacterial cell, plant cell or a mammalian cell, or can be of plant origin, mammalian origin, yeast origin, Drosophila origin, or archebacterial origin.

The term "systemic administration" as used herein refers to the in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption

include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged polymers, e.g., nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as the cancer cells.

The term "pharmacological composition" or "pharmaceutical formulation" refers to a composition or formulation in a form suitable for administration, for example, systemic administration, into a cell or patient, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation to reach a target cell (i.e., a cell to which the negatively charged polymer is targeted).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawings will be first described briefly.

Drawings:

Figure 1 shows examples of chemically stabilized ribozyme motifs. HH Rz, represents hammerhead ribozyme motif (Usman et al., 1996, Curr. Op. Struct. Bio., 1, 527); NCH Rz represents the NCH ribozyme motif (Ludwig & Sproat, International PCT Publication No. WO 98/58058); G-Cleaver, represents G-cleaver ribozyme motif (Kore et al., 1998, Nucleic Acids Research 26, 4116-4120, Eckstein et al., International PCT publication No. WO 99/16871). N or n, represent independently a nucleotide which can be same or different and have complementarity to each other; rI, represents ribo-Inosine nucleotide; arrow indicates the site of cleavage within the target. Position 4 of the HH Rz and the NCH Rz is shown as having 2'-C-allyl modification, but those skilled in the art will recognize that this position can be modified with other modifications well known in

the art, so long as such modifications do not significantly inhibit the activity of the ribozyme.

Figure 2 shows an example of the Amberzyme ribozyme motif that is chemically stabilized (see for example Beigelman et al., International PCT publication No. WO 99/55857).

Figure 3 shows an example of the Zinzyme A ribozyme motif that is chemically stabilized (see for example Beigelman et al., Beigelman et al., International PCT publication No. WO 99/55857).

Figure 4 shows an example of a DNAzyme motif described by Santoro et al., 1997, PNAS, 94, 4262.

Figure 5 shows a synthetic scheme for the synthesis of a folate conjugate of the instant invention.

Figure 6 shows representative examples of fludarabine-folate conjugate molecules of the invention.

Figure 7 shows a synthetic scheme for post-synthetic modification of a nucleic acid molecule to produce a folate conjugate.

Figure 8 shows a synthetic scheme for generating a protected pteroic acid synthon of the invention.

Figure 9 shows a synthetic scheme for generating a 2-dithiopyridyl activated folic acid synthon of the invention.

Figure 10 shows a synthetic scheme for generating an oligonucleotide or nucleic acid-folate conjugate.

Figure 11 shows a an alternative synthetic scheme for generating an oligonucleotide or nucleic acid-folate conjugate.

Figure 12 shows an alternative synthetic scheme for post-synthetic modification of a nucleic acid molecule to produce a folate conjugate.

Figure 13 shows a non-limiting example of a synthetic scheme for the synthesis of a N-acetyl-D-galactosamine-2'-aminouridine phosphoramidite conjugate of the invention.

Figure 14 shows a non-limiting example of a synthetic scheme for the synthesis of a N-acetyl-D-galactosamine-D-threoninol phosphoramidite conjugate of the invention.

Figure 15 shows a non-limiting example of an N-acetyl-D-galactosamine enzymatic nucleic acid conjugate of the invention. **W** shown in the example refers to a biodegradable linker, for example a nucleic acid dimer, trimer, or tetramer comprising ribonucleotides and/or deoxyribonucleotides.

Figure 16 shows a non-limiting example of a synthetic scheme for the synthesis of a dodecanoic acid derived conjugate linker of the invention.

Figure 17 shows a non-limiting example of a synthetic scheme for the synthesis of an oxime linked nucleic acid/peptide conjugate of the invention.

Figure 18 shows non-limiting examples of phospholipid derived nucleic acid conjugates of the invention. **W** shown in the examples refers to a biodegradable linker, for example a nucleic acid dimer, trimer, or tetramer comprising ribonucleotides and/or deoxyribonucleotides.

Figure 19 shows a non-limiting example of a synthetic scheme for preparing a phospholipid derived enzymatic nucleic acid conjugates of the invention.

Figure 20 shows a non-limiting example of a synthetic scheme for preparing a polyethylene glycol (PEG) derived enzymatic nucleic acid conjugates of the invention.

Figure 21 shows PK data of a 40K PEG conjugated enzymatic nucleic acid molecule compared to the corresponding non-conjugated enzymatic nucleic acid molecule. The graph is a time course of serum concentration in mice dosed with 30 mg/kg of Angiozyme™ or 40-kDa-PEG-Angiozyme™. The hybridization method was used to quantitate Angiozyme™ levels.

Figure 22 shows PK data of a phospholipid conjugated enzymatic nucleic acid molecule compared to the corresponding non-conjugated enzymatic nucleic acid molecule.

Figure 23 shows a non-limiting example of a synthetic scheme for preparing a poly-N-acetyl-D-galactosamine enzymatic nucleic acid conjugate of the invention.

Figure 24a-b shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker using oxime and morpholino linkages.

Figure 25 shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker using oxime and phosphoramidate linkages.

Figure 26a-b shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker using phosphoramidate linkages.

Figure 27 shows non-limiting examples of phospholipid derived protein/peptide conjugates of the invention. **W** shown in the examples refers to a biodegradable linker, for example a nucleic acid dimer, trimer, or tetramer comprising ribonucleotides and/or deoxyribonucleotides.

Figure 28 shows a non-limiting example of an N-acetyl-D-galactosamine peptide/protein conjugate of the invention, the example shown is with a peptide. **W** shown in the example refers to a biodegradable linker, for example a nucleic acid dimer, trimer, or tetramer comprising ribonucleotides and/or deoxyribonucleotides.

Figure 29 shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker using phosphoramidate linkages via coupling a protein phosphoramidite to a PEG conjugated nucleic acid linker.

Method of Use

The compositions and conjugates of the instant invention can be used to administer pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

Generally, the compounds of the instant invention are introduced by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. For use of a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and the like.

The present invention also includes pharmaceutically acceptable formulations of the compounds described above, preferably in combination with the molecule(s) to be

delivered. These formulations include salts of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

In one embodiment, the invention features the use of the compounds of the invention in a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). In another embodiment, the invention features the use of compounds of the invention covalently attached to polyethylene glycol. These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. Chem. Rev. 1995, 95, 2601-2627; Ishiwataet al., Chem. Pharm. Bull. 1995, 43, 1005-1011). Such compositions have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., Science 1995, 267, 1275-1276; Oku et al.,1995, Biochim. Biophys. Acta, 1238, 86-90). The long-circulating compositions enhance the pharmacokinetics and pharmacodynamics of therapeutic compounds, such as DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et al., J. Biol. Chem. 1995, 42, 24864-24870; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10390; Holland et al., International PCT Publication No. WO 96/10392). Long-circulating compositions are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes a composition(s) prepared for storage or administrationthat includes a pharmaceutically effective amount of the desired compound(s) in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be included in the composition. Examples of such agents include but are not limited to sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be included in the composition.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer. Furthermore, the compounds of the invention and formulations thereof can be administered to a fetus via administration to the mother of a fetus.

The compounds of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained

action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of the invention can also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Compounds of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form will vary depending

upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The compounds of the present invention can also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small refers to nucleic acid motifs less than about 100 nucleotides in length, preferably less than about 80 nucleotides in length, and more preferably less than about 50 nucleotides in length; *e.g.*, antisense oligonucleotides, hammerhead or the NCH ribozymes) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (eg; antisense GeneBlocs) are synthesized using protocols known in the art as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, US patent No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc.

synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. In a non-limiting example, a 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. In a non-limiting example, a 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer includebut are not limited to; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the antisense oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. Standard drying or lyophilization methods known to those skilled in the art can be used.

The method of synthesis used for normal RNA including certain enzymatic nucleic acid molecules follows the procedure as described in Usman *et al.,* 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.,* 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.,* 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.,* 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting

example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The

vial is brought to r.t. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

Inactive hammerhead ribozymes or binding attenuated control ((BAC) oligonucleotides) are synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel, K. J., *et al.,* 1992, *Nucleic Acids Res.*, 20, 3252). Similarly, one or more nucleotide substitutions can be introduced in other enzymatic nucleic acid molecules to inactivate the molecule and such molecules can serve as a negative control.

The average stepwise coupling yields are typically >98% (Wincott *et al.,* 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including, but not limited to, 96 well format, with the ratio of chemicals used in the reaction being adjusted accordingly.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example by ligation (Moore *et al.,* 1992, *Science* 256, 9923; Draper *et al.,* International PCT publication No. WO 93/23569; Shabarova *et al.,* 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.,* 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.,* 1997, *Bioconjugate Chem.* 8, 204).

The nucleic acid molecules of the present invention are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott *et al.,* Supra, the totality of which is hereby incorporated herein by reference) and are re-suspended in water.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases can increase their potency (see *e.g.,* Eckstein *et al.,* International Publication No. WO 92/07065; Perrault *et al.,* 1990 *Nature* 344, 565; Pieken *et al.,* 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.,* International Publication No.

WO 93/15187; and Rossi *et al.,* International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; and Burgin *et al., supra;* all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules herein). Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired. (All these publications are hereby incorporated by reference herein).

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.,* 1996, *Biochemistry* , 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al., International Publication* PCT No. WO 92/07065; Perrault *et al. Nature*, 1990, *344*, 565-568; Pieken *et al. Science,* 1991, *253*, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.* , 1992, *17*, 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, *US Patent* No. 5,334,711 and Beigelman *et al.,* 1995, *J. Biol. Chem.,* 270, 25702; Beigelman *et al.,* International PCT publication No. WO 97/26270; Beigelman *et al.,* US Patent No. 5,716,824; Usman *et al.,* US patent No. 5,627,053; Woolf *et al.,* International PCT Publication No. WO 98/13526; Thompson *et al.,* USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.,* 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic acid Sciences),* 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina *et al.,* 1997, *Bioorg. Med. Chem.,* 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without inhibiting catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, too many of these modifications may cause some toxicity. Therefore, when designing nucleic acid molecules the amount of these internucleotide linkages should be

minimized. Without being bound by any particular theory, the reduction in the concentration of these linkages should lower toxicity resulting in increased efficacy and higher specificity of these molecules.

Nucleic acid molecules having chemical modifications that maintain or enhance activity are provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity can not be significantly lowered. Therapeutic nucleic acid molecules (*e.g.,* enzymatic nucleic acid molecules and antisense nucleic acid molecules) delivered exogenously are optimally stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. The nucleic acid molecules should be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.,* 1995 *Nucleic Acids Res.* 23, 2677; Caruthers *et al.,* 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

Use of the nucleic acid-based molecules of the invention can lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.,* multiple antisense or enzymatic nucleic acid molecules targeted to different genes, nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of molecules (including different motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules can also include combinations of different types of nucleic acid molecules.

In another embodiment, nucleic acid catalysts having chemical modifications that maintain or enhance enzymatic activity are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity of the nucleic acid can not be significantly lowered. As exemplified herein such enzymatic nucleic acids are useful in a cell and/or *in vivo* even if activity over all is reduced 10 fold (Burgin *et al.,* 1996, *Biochemistry*, 35, 14090). Such enzymatic nucleic acids herein are said to "maintain" the enzymatic activity of an all RNA ribozyme or all DNA DNAzyme.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'- cap structure.

In another embodiment the 3'-cap includes, for example 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

In one embodiment, the invention features modified enzymatic nucleic acid molecules with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.,* 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39. These references are hereby incorporated by reference herein.

In connection with 2'-modified nucleotides as described for the invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.,* U.S. Patent 5,672,695 and Matulic-Adamic *et al.,* WO 98/28317, respectively, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid (*e.g.,* antisense and ribozyme) structure can be made to enhance the utility of these molecules. For example, such modifications can enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, including *e.g.,* enhancing penetration of cellular membranes and conferring the ability to recognize and bind to targeted cells.

Use of these molecules can lead to better treatment of disease progression by affording the possibility of combination therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with

known small molecule inhibitors, or intermittent treatment with combinations of enzymatic nucleic acid molecules (including different enzymatic nucleic acid molecule motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules can also include combinations of different types of nucleic acid molecules. Therapies can be devised which include a mixture of enzymatic nucleic acid molecules (including different enzymatic nucleic acid molecule motifs), antisense and/or 2-5A chimera molecules to one or more targets to alleviate symptoms of a disease.

Indications

Particular disease states that can be treated using compounds and compositions of the invention include, but are not limited to, cancers and cancerous conditions such as breast, lung, prostate, colorectal, brain, esophageal, stomach, bladder, pancreatic, cervical, head and neck, and ovarian cancer, melanoma, lymphoma, glioma, multidrug resistant cancers, and/or viral infections including HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, west nile virus, Ebola virus, foot and mouth virus, and papilloma virus infection.

The molecules of the invention can be used in conjunction with other known methods, therapies, or drugs. For example, the use of monoclonal antibodies (eg; mAb IMC C225, mAB ABX-EGF) treatment, tyrosine kinase inhibitors (TKIs), for example OSI-774 and ZD1839, chemotherapy, and/or radiation therapy, are all non-limiting examples of a methods that can be combined with or used in conjunction with the compounds of the instant invention. Common chemotherapies that can be combined with nucleic acid molecules of the instant invention include various combinations of cytotoxic drugs to kill the cancer cells. These drugs include, but are not limited to, paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubin, fluorouracil carboplatin, edatrexate, gemcitabine, vinorelbine etc. Those skilled in the art will recognize that other drug compounds and therapies can be similarly be readily combined with the compounds of the instant invention are hence within the scope of the instant invention.

Diagnostic uses

The compounds of this invention, for example, nucleic acid conjugate molecules, can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of a disease related RNA in a cell. The close relationship between, for example, enzymatic nucleic acid molecule activity and the structure of the

target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple enzymatic nucleic acid molecules conjugates of the invention, one can map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with enzymatic nucleic acid molecules can be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments can lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of enzymatic nucleic acid molecules and/or other chemical or biological molecules). Other in vitro uses of enzymatic nucleic acid molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease-related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with an enzymatic nucleic acid molecule using standard methodology.

In a specific example, enzymatic nucleic acid molecules that are delivered to cells as conjugates and which cleave only wild-type or mutant forms of the target RNA are used for the assay. The first enzymatic nucleic acid molecule is used to identify wild-type RNA present in the sample and the second enzymatic nucleic acid molecule is used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both enzymatic nucleic acid molecules to demonstrate the relative enzymatic nucleic acid molecule efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis requires two enzymatic nucleic acid molecules, two substrates and one unknown sample which is combined into six reactions. The presence of cleavage products is determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher

mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively. The use of enzymatic nucleic acid molecules in diagnostic applications contemplated by the instant invention is more fully described in George et al., US Patent Nos. 5,834,186 and 5,741,679, Shih et al., US Patent No. 5,589,332, Nathan et al., US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker et al., International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger et al., International PCT publication No. WO 99/29842.

Additional Uses

Potential uses of sequence-specific enzymatic nucleic acid molecules of the instant invention that are delivered to cells as conjugates can have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans et al., 1975 Ann. Rev. Biochem. 44:273). For example, the pattern of restriction fragments can be used to establish sequence relationships between two related RNAs, and large RNAs can be specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the enzymatic nucleic acid molecule is ideal for cleavage of RNAs of unknown sequence. Applicant has described the use of nucleic acid molecules to down-regulate gene expression of target genes in bacterial, microbial, fungal, viral, and eukaryotic systems including plant, or mammalian cells.

Example 1: Synthesis of O^1-(4-monomethoxytrityl)-*N*-(6-(*N*-(α-OFm-L-glutamyl)aminocaproyl))-D-threoninol-N^2-*i*Bu-N^{10}-TFA-pteroic acid conjugate 3′-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphor-amidite) (**20**) (**Figure 5**)

General. All reactions were carried out under a positive pressure of argon in anhydrous solvents. Commercially available reagents and anhydrous solvents were used without further purification. ^1H (400.035 MHz) and ^{31}P (161.947 MHz) NMR spectra were recorded in $CDCl_3$, unless stated otherwise, and chemical shifts in ppm refer to TMS and H_3PO_4, respectively. Analytical thin-layer chromatography (TLC) was performed with Merck Art.5554 Kieselgel 60 F_{254} plates and flash column chromatography using Merck 0.040-0.063 mm silica gel 60.

***N*-(*N*-Fmoc-6-aminocaproyl)-D-threoninol (13).** *N*-Fmoc-6-aminocaproic acid (10 g, 28.30 mmol) was dissolved in DMF (50 ml) and *N*-hydroxysuccinimide (3.26 g, 28.30 mmol) and 1,3-dicyclohexylcarbodiimide (5.84 g, 28.3 mmol) were added to the solution. The reaction mixture was stirred at RT (about 23°C) overnight and the

precipitated 1,3-dicyclohexylurea filtered off. To the filtrate D-threoninol (2.98 g, 28.30 mmol) was added and the reaction mixture stirred at RT overnight. The solution was reduced to *ca* half the volume *in vacuo*, the residue diluted with about m ml of ethyl acetate and extracted with about x ml of 5% $NaHCO_3$, followed by washing with brine. The organic layer was dried (Na_2SO_4), evaporated to a syrup and chromatographed by silica gel column chromatography using 1-10% gradient of methanol in ethyl acetate. Fractions containing the product were pooled and evaporated to a white solid (9.94 g, 80%). ^{1}H-NMR (DMSO-d_6-D_2O) δ7.97-7.30 (m, 8H, aromatic), 4.34 (d, J=6.80, 2H, Fm), 4.26 (t, J=6.80, 1H, Fm), 3.9 (m, 1H, H3 Thr), 3.69 (m, 1H, H2 Thr), 3.49 (dd, J=10.6, J=7.0, 1H, H1 Thr), 3.35 (dd, J=10.6, J=6.2, 1H, H1' Thr), 3.01 (m, 2H, *CH_2*CO Acp), 2.17 (m, 2H, *CH_2*NH Acp), 1.54 (m, 2H, CH_2 Acp), 1.45 (m, 2H, CH_2 Acp), 1.27 (m, 2H, CH_2 Acp), 1.04 (d, J=6.4, 3H, CH_3). MS/ESI$^+$ m/z 441.0 $(M+H)^+$.

***O*^{1}-(4-Monomethoxytrityl)-*N*-(*N*-Fmoc-6-aminocaproyl)-D-threoninol (14)**. To the solution of **13** (6 g, 13.62 mmol) in dry pyridine (80 ml) *p*-anisylchlorodiphenyl-methane (6 g, 19.43 mmol) was added and the reaction mixture stirred at RT overnight. Methanol was added (20 ml) and the solution concentrated *in vacuo*. The residual syrup was partitioned between about x ml of dichloromethane and about x ml of 5% $NaHCO_3$, the organic layer was washed with brine, dried (Na_2SO_4) and evaporated to dryness. Flash column chromatography using 1-3% gradient of methanol in dichloromethane afforded **14** as a white foam (6 g, 62%). ^{1}H-NMR (DMSO) δ7.97-6.94 (m, 22H, aromatic), 4.58 (d, 1H, J=5.2, OH), 4.35 (d, J=6.8, 2H, Fm), 4.27 (t, J=6.8, 1H, Fm), 3.97 (m, 2H, H2,H3 Thr), 3.80 (s, 3H, OCH_3), 3.13 (dd, J=8.4, J=5.6, 1H, H1 Thr), 3.01 (m, 2H, *CH_2*CO Acp), 2.92 (m, dd, J=8.4, J=6.4, 1H, H1' Thr), 2.21 (m, 2H, *CH_2*NH Acp), 1.57 (m, 2H, CH_2 Acp), 1.46 (m, 2H, CH_2 Acp), 1.30 (m, 2H, CH_2 Acp), 1.02 (d, J=5.6, 3H, CH_3). MS/ESI$^+$ m/z 735.5 $(M+Na)^+$.

***O*^{1}-(4-Monomethoxytrityl)-*N*-(6-aminocaproyl)-D-threoninol (15)**. **14** (9.1 g, 12.77 mmol) was dissolved in DMF (100 ml) containing piperidine (10 ml) and the reaction mixture was kept at RT for about 1 hour. The solvents were removed *in vacuo* and the residue purified by silica gel column chromatography using 1-10% gradient of methanol in dichloromethane to afford **15** as a syrup (4.46 g, 71%). ^{1}H-NMR δ7.48-6.92 (m, 14H, aromatic), 6.16 (d, J=8.8, 1H, NH), 4.17 (m, 1H, H3 Thr), 4.02 (m, 1H, H2 Thr), 3.86 (s, 3H, OCH_3), 3.50 (dd, J=9.7, J=4.4, 1H, H1 Thr), 3.37 (dd, J=9.7, J=3.4, 1H, H1' Thr), 2.78 (t, J=6.8, 2H, *CH_2*CO Acp), 2.33 (t, J=7.6, 2H, *CH_2*NH Acp), 1.76 (m, 2H, CH_2 Acp), 1.56 (m, 2H, CH_2 Acp), 1.50 (m, 2H, CH_2 Acp), 1.21 (d, J=6.4, 3H, CH_3). MS/ESI$^+$ m/z 491.5 $(M+H)^+$.

O^1-(4-Monomethoxytrityl)-*N*-(6-(*N*-(*N*-Boc-α-OFm-L-glutamyl) aminocaproyl))-D-threoninol (16). To the solution of *N*-Boc-α-OFm-glutamic acid (Bachem) (1.91 g, 4.48 mmol) in DMF (10 ml) *N*-hydroxysuccinimide (518 mg, 4.50 mmol) and 1,3-dicyclohexylcarbodiimide (928 mg, 4.50 mmol) was added and the reaction mixture was stirred at RT overnight. 1,3-Dicyclohexylurea was filtered off and to the filtrate **15** (2 g, 4.08 mmol) and pyridine (2 ml) were added. The reaction mixture was stirred at RT for 3 hours and than concentrated *in vacuo*. The residue was partitioned between ethyl acetate and 5% Na_2HCO_3, the organic layer extracted with brine as previously described, dried (Na_2SO_4) and evaporated to a syrup. Column chromatography using 2-10% gradient of methanol in dichlotomethane afforded **16** as a white foam (3.4 g, 93%). ^{1}H-NMR δ 7.86-6.91 (m, 22H, aromatic), 6.13 (d, J=8.8, 1H, NH), 5.93 (br s, 1H, NH), 5.43 (d, J=8.4, 1H, NH), 4.63 (dd, J=10.6, J=6.4, 1H, Fm), 4.54 (dd, J=10.6, J=6.4, 1H, Fm), 4.38 (m, 1H, Glu), 4.3 (t, J=6.4, 1H, Fm), 4.18 (m, 1H, H3 Thr), 4.01 (m, 1H, H2 Thr), 3.88 (s, 3H, OCH_3), 3.49 (dd, J=9.5, J=4.4, 1H, H1 Thr), 3.37 (dd, J=9.5, J=3.8, 1H, H1' Thr), 3.32 (m, 2H, CH_2CO Acp), 3.09 (br s, 1H, OH), 2.32 (m, 2H, CH_2NH Acp), 2.17 (m, 3H, Glu), 1.97 (m, 1H, Glu), 1.77 (m, 2H, CH_2 Acp), 1.61 (m, 2H, CH_2 Acp), 1.52 (s, 9H, t-Bu), 1.21 (d, J=6.4, 3H, CH_3). MS/ESI$^+$ m/z 920.5 $(M+Na)^+$.

***N*-(6-(*N*-α-OFm-L-glutamyl)aminocaproyl))-D-threoninol hydrochloride (17).** **16** (2 g, 2.23 mmol) was dissolved in methanol (30 ml) containing anisole (10 ml) and to this solution x ml of 4M HCl in dioxane was added. The reaction mixture was stirred for 3 hours at RT and then concentrated *in vacuo*. The residue was dissolved in ethanol and the product precipitated by addition of x ml of ether. The precipitate was washed with ether and dried to give **17** as a colorless foam (1 g, 80%). ^{1}H-NMR (DMSO-d_6-D_2O) δ7.97-7.40 (m, 8H, aromatic), 4.70 (m, 1H, Fm), 4.55 (m, 1H, Fm), 4.40 (t, J=6.4, 1H, Fm), 4.14 (t, J=6.6, 1H, Glu), 3.90 (dd, J=2.8, J=6.4, 1H, H3 Thr), 3.68 (m, 1H, H2 Thr), 3.49 (dd, J=10.6, J=7.0, 1H, H1 Thr), 3.36 (dd, J=10.6, J=6.2, 1H, H1' Thr), 3.07 (m, 2H, CH_2CO Acp), 2.17 m, 3H), 1.93 (m, 2H), 1.45 (m, 2H), 1.27 (m, 2H), 1.04 (d, J=6.4, 3H Thr). MS/ESI$^+$ m/z 526.5 $(M+H)^+$.

***N*-(6-(*N*-α-OFm-L-glutamyl)aminocaproyl))-D-threoninol-N^2-*i*Bu-N^{10}-TFA-pteroic acid conjugate (18).** To the solution of N^2-*i*Bu-N^{10}-TFA-pteroic acid[1] (480 mg, 1 mmol) in DMF (5 ml) 1-hydroxybenzotriazole (203 mg, 1.50 mmol), EDCI (288 mg, 1.50 mmol) and **17** (free base, 631 mg, 1.2 mmol) are added. The reaction mixture is stirred at RT for 2 hours, then concentrated to *ca* 3 ml and loaded on the column of silica gel. Elution with dichloromethane, followed by 1-20% gradient of methanol in dichloromethane afforded **18** (0.5 g, 51%). ^{1}H-NMR (DMSO-d_6-D_2O) δ 9.09 (d, J=6.8, 1H, NH) 8.96 (s, 1H, H7 pteroic acid), 8.02-7.19 (m, 13H, aromatic, NH), 5.30 (s, 2H,

pteroic acid), 4.50 (m, 1H, Glu), 4.41 (d, J=6.8, 2H, Fm), 4.29 (t, J=6.8, 1H, Fm), 3.89 (dd, J=6.2, J=2.8, 1H, H3 Thr), 3.68 (m, 1H, H2 Thr), 3.48 (dd, J=10.4, J=7.0, 1H, H1 Thr), 3.36 (dd, J=10.4, J=6.2, 1H H1' Thr), 3.06 (m, 2H, CH_2CO Acp), 2.84 (m, 1H, *i*Bu), 2.25 (m, 2H, CH_2NH Acp), 2.16 (m, 3H, Glu), 1.99 (m, 1H, Glu), 1.52 (m, 2H Acp), 1.42 (m, 2H Acp), 1.27 (m, 2H Acp), 1.20 (s, 3H *i*Bu), 1.19 (s, 3H, *i*Bu), 1.03 (d, J=6.2, 3H Thr). MS/ESI$^-$ m/z 984.5 $(M-H)^-$.

O^1-(4-monomethoxytrityl)-*N*-(6-(*N*-α-OFm-L-glutamyl)aminocaproyl))-D-threoninol-N^2-*i*Bu-N^{10}-TFA-pteroic acid conjugate (19). To the solution of conjugate **18** (1 g, 1.01 mmol) in dry pyridine (15 ml) *p*-anisylchlorodiphenylmethane (405 mg) was added and the reaction mixture was stirred, protected from moisture, at RT overnight. Methanol (3 ml) was added and the reaction mixture concentrated to a syrup *in vacuo*. The residue was partitioned between dichloromethane and 5% $NaHCO_3$, the organic layer washed with brine, dried (Na_2SO_4) and evaporated to dryness. Column chromatography using 0.5-10% gradient of methanol in dichloromethane afforded **19** as a colorless foam (0.5 g, 39%. ^{1}H-NMR (DMSO-d_6-D_2O δ9.09 (d, J=6.8, 1H, NH) 8.94 (s, 1H, H7 pteroic acid), 8.00-6.93 (m, 27H, aromatic, NH), 5.30 (s, 2H, pteroic acid), 4.50 (m, 1H, Glu), 4.40 (d, J=6.8, 2H, Fm), 4.29 (t, J=6.8, 1H, Fm), 3.94 (m, 2H, H3,H2 Thr), 3.79 (s, 3H, OCH_3) 3.11 (dd, J=8.6, J=5.8, 1H, H1 Thr), 3.04 (m, 2H, CH_2CO Acp), 2.91 (dd, J=8.6, J=6.4, 1H, H1' Thr), 2.85 (m, 1H, *i*Bu), 2.25 (m, 2H, CH_2NH Acp), 2.19 (m, 2H, Glu), 2.13 (m, 1H, Glu), 1.98 (m, 1H, Glu), 1.55 (m, 2H Acp), 1.42 (m, 2H Acp), 1.29(m, 2H Acp), 1.20 (s, 3H *i*Bu), 1.18 (s, 3H, *i*Bu), 1.00 (d, J=6.4, 3H Thr). MS/ESI$^-$ m/z 1257.0 $(M-H)^-$.

O^1-(4-monomethoxytrityl)-*N*-(6-(*N*-α-OFm-L-glutamyl)aminocaproyl))-D-threoninol-N^2-*i*Bu-N^{10}-TFA-pteroic acid conjugate 3'-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphor-amidite) (20). To the solution of **19** (500 mg, 0.40 mmol) in dichloromethane (2 ml) 2-cyanoethyl tetraisopropylphosphordiamidite (152 μL, 0.48 mmol) was added followed by pyridinium trifluoroacetate (93 mg, 0.48 mmol). The reaction mixture was stirred at RT for 1 hour and than loaded on the column of silica gel in hexanes. Elution using ethyl acetate-hexanes 1:1, followed by ethyl acetate and ethyl acetate-acetone 1:1 in the presence of 1% pyridine afforded **20** as a colorless foam (480 mg, 83%). ^{31}P NMR δ 149.4 (s), 149.0 (s).

Example 2: Synthesis of 2-dithiopyridyl activated folic acid (30) (**Figure 9**)

Synthesis of the cysteamine modified folate **30** is presented in Fig. 9. Monomethoxytrityl cysteamine **21** was prepared by selective tritylation of the thiol group of cysteamine with 4-methoxytrityl alcohol in trifluoroacetic acid. Peptide coupling of **21**

with Fmoc-Glu-O*t*Bu (Bachem Bioscience Inc., King of Prussia, PA) in the presence of PyBOP yielded **22** in a high yield. *N*-Fmoc group was removed smoothly with piperidine to give **23**. Condensation of **23** with *p*-(4-methoxytrityl)aminobenzoic acid, prepared by reaction of *p*-aminobenzoic acid with 4-methoxytrityl chloride in pyridine, afforded the fully protected conjugate **24**. Selective cleavage of *N*-MMTr group with acetic acid afforded **25** in quantitative yield. Shiff base formation between **25** and N^2-*i*Bu-6-formylpterin **26**,[9] followed by reduction with borane-pyridine complex proceeded with a good yield to give fully protected cysteamine-folate adduct **27**.[12] The consecutive cleavage of protecting groups of **27** with base and acid yielded thiol derivative **29**. The thiol exchange reaction of **29** with 2,2-dipyridyl disulfide afforded the desired *S*-pyridyl activated synthon **30** as a yellow powder; Isolated as a TEA^+ salt: ^1H NMR spectrum for **10** in D_2O: δ 8.68 (s, 1 H, H-7), 8.10 (d, J = 3.6, 1 H, pyr), 7.61 (d, J = 8.8, 2 H, PABA), 7.43 (m, 1 H, pyr), 7.04 (d, J = 7.6, 1 H, pyr), 6.93 (m, 1 H, pyr), 6.82 (d, J = 8.8, 1 H, PABA), 4.60 (s, 2 H, 6-CH_2), 4.28 (m, 1 H, Glu), 3.30-3.08 (m, 2 H, cysteamine), 3.05 (m, 6 H, TEA), 2.37 (m, 2 H, cysteamine), 2.10 (m, 4 H, Glu), 1.20 (m, 9 H, TEA). MS/ESI$^-$ m/z 608.02 $[M-H]^-$. It is worth noting that the isolation of **30** as its TEA^+ or Na^+ salt made it soluble in DMSO and/or water, which is an important requirement for its use in conjugation reactions.

Example 3: Post synthetic conjugation of enzymatic nucleic acid to form nucleic acid-folate conjugate (33) (**Figure 10**)

Oligonucleotide synthesis, deprotection and purification was performed as described herein. 5'-Thiol-Modifier C6 (Glen Research, Sterling, Virginia) was coupled as the last phosphoramidite to the 5'-end of a growing oligonucleotide chain. After cleavage from the solid support and base deprotection, the disulfide modified enzymatic nucleic acid molecule **31** (Fig. 10) was purified using ion exchange chromatography. The thiol group was unmasked by reduction with dithiothreitol (DTT) to afford **32** which was purified by gel filtration and immediately conjugated with **30**. The resulting conjugate **33** was separated from the excess folate by gel filtration and then purified by RP HPLC using gradient of acetonitrile in 50 mM triethylammonium acetate (TEAA). Desalting was performed by RP HPLC. Reactions were conducted on 400 mg of disulfide modified enzymatic nucleic acid molecule **31** to afford 200-250 mg (50-60% yield) of conjugate **33**. MALDI TOF MS confirmed the structure: **13** $[M-H]^-$ 12084.74 (calc.12083.82). An alternative approach to this synthesis is shown in **Figure 11**.

As shown in Examples 2 and 3, a folate-cysteamine adduct can be prepared by a scaleable solution phase synthesis in a good overall yield. Disulfide conjugation of this

novel targeting ligand to the thiol-modified oligonucleotide is suitable for the multi-gram scale synthesis. The 9-atom spacer provides a useful spatial separation between folate and attached oligonucleotide cargo. Importantly, conjugation of folate to the oligonucleotide through a disulfide bond should permit intermolecular separation which was suggested to be required for the functional cytosolic entry of a protein drug.

Example 4: Synthesis of Galactose and N-acetyl-Galactosamine conjugates (**Figures 13, 14, and 15**)

Applicant has designed both nucleoside and non-nucleoside-*N*-acetyl-D-galactosamine conjugates suitable for incorporation at any desired position of an oligonucleotide. Multiple incorporations of these monomers could result in a "glycoside cluster effect".

All reactions were carried out under a positive pressure of argon in anhydrous solvents. Commercially available reagents and anhydrous solvents were used without further purification. *N*-acetyl-D-galactosamine was purchased from Pfanstiel (Waukegan, IL), folic acid from Sigma (St. Louis, MO), D-threoninol from Aldrich (Milwaukee, WI) and *N*-Boc-α-OFm glutamic acid from Bachem. ^{1}H (400.035 MHz) and ^{31}P (161.947 MHz) NMR spectra were recorded in $CDCl_3$, unless stated otherwise, and chemical shifts in ppm refer to TMS and H_3PO_4, respectively. Analytical thin-layer chromatography (TLC) was performed with Merck Art.5554 Kieselgel 60 F_{254} plates and flash column chromatography using Merck 0.040-0.063 mm silica gel 60. The general procedures for RNA synthesis, deprotection and purification are described herein. MALDI-TOF mass spectra were determined on PerSeptive Biosystems Voyager spectrometer. Electrospray mass spectrometry was run on the PE/Sciex API365 instrument.

2'-(*N*-L-lysyl)amino-5'-*O*-4,4'-dimethoxytrityl-2'-deoxyuridine (2). 2'-(*N*-α,ε-bis-Fmoc-L-lysyl)amino-5'-*O*-4,4'-dimethoxytrityl-2'-deoxyuridine (**1**) (4 g, 3.58 mmol) was dissolved in anhydrous DMF (30 ml) and diethylamine (4 ml) was added. The reaction mixture was stirred at rt for 5 hours and than concentrated (oil pump) to a syrup. The residue was dissolved in ethanol and ether was added to precipitate the product (1.8 g, 75%). ^{1}H-NMR (DMSO-d_6-D_2O) δ 7.70 (d, $J_{6,5}$=8.4, 1H, H6), 7.48-6.95 (m, 13H, aromatic), 5.93 (d, $J_{1',2'}$=8.4, 1H, H1'), 5.41 (d, $J_{5,6}$=8.4, 1H, H5), 4,62 (m, 1H, H2'), 4.19 (d, 1H, $J_{3',2'}$=6.0, H3'), 3.81 (s, 6H, 2×OMe), 3.30 (m, 4H, 2H5', CH_2), 1.60-1.20 (m, 6H, 3×CH_2). MS/ESI$^+$ m/z 674.0 $(M+H)^+$.

***N*-Acetyl-1,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-galactospyranose (3).** *N*-Acetyl-D-galac-tosamine (6.77 g, 30.60 mmol) was suspended in acetonitrile (200 ml) and triethylamine (50 ml, 359 mmol) was added. The mixture was cooled in an ice-bath and

acetic anhydride (50 ml, 530 mmol)) was added dropwise under cooling. The suspension slowly cleared and was then stirred at rt for 2 hours. It was than cooled in an ice-bath and methanol (60 ml) was added and the stirring continued for 15 min. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane and 1 N HCl. Organic layer was washed twice with 5% $NaHCO_3$, followed by brine, dried (Na_2SO_4) and evaporated to dryness to afford 10 g (84%) of **3** as a colorless foam. ^{1}H NMR was in agreement with published data (Findeis, 1994, *Int. J. Peptide Protein Res.*, 43, 477-485.

2-Acetamido-3,4,6-tetra-*O*-acetyl-1-chloro-D-galactospyranose (4). This compound was prepared from **3** as described by Findeis *supra*.

Benzyl 12-Hydroxydodecanoate (5). To a cooled (0 °C) and stirred solution of 12-hydroxydodecanoic acid (10.65 g, 49.2 mmol) in DMF (70 ml) DBU (8.2 ml, 54.1 mmol) was added, followed by benzyl bromide (6.44 ml, 54.1 mmol). The mixture was left overnight at rt, than concentrated under reduced pressure and partitioned between 1 N HCl and ether. Organic phase was washed with saturated $NaHCO_3$, dried over Na_2SO_4 and evaporated . Flash chromatography using 20-30% gradient of ethyl acetate in hexanes afforded benzyl ester as a white powder (14.1 g, 93.4%). ^{1}H-NMR spectral data were in accordance with the published values.[33]

12'-Benzyl hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyrano-se (6). 1-Chloro sugar **4** (4.26 g, 11.67 mmol)and benzyl 12-hydroxydodecanoate (**5**) (4.3 g, 13.03 mmol) were dissolved in nitromethane-toluene 1:1 (122 ml) under argon and $Hg(CN)_2$ (3.51 g, 13.89 mmol) and powdered molecular sieves 4A (1.26 g) were added. The mixture was stirred at rt for 24 h, filtered and the filtrate concentrated under reduced pressure. The residue was partitioned between dichloromethane and brine, organic layer was washed with brine, followed by 0.5 M KBr, dried (Na_2SO_4) and evaporated to a syrup. Flash silica gel column chromatography using 15-30% gradient of acetone in hexanes yielded product **6** as a colorless foam (6 g, 81%). ^{1}H-NMR δ 7.43 (m, ^{5}H, phenyl), 5.60 (d, 1H, $J_{NH,2}$=8.8, NH), 5.44 (d, $J_{4,3}$=3.2, 1H, H4), 5.40 (dd, $J_{3,4}$=3.2, $J_{3,2}$=10.8, 1H, H3), 5.19 (s, 2H, CH_2Ph), 4.80 (d, $J_{1,2}$=8.0, 1H, H1), 4.23 (m, 2H, CH_2), 3.99 (m, 3H, H2, H6), 3.56 (m, 1H, H5), 2.43 (t, J=7.2, 2H,CH_2), 2.22 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.64 (m, 4H, 2×CH_2), 1.33 (br m, 14H, 7×CH_2). MS/ESI$^-$ m/z 634.5 $(M-H)^-$.

12'-Hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranose (7).

Conjugate **6** (2 g, 3.14 mmol)) was dissolved in ethanol (50 ml) and 5% Pd-C (0.3 g) was added. The reaction mixture was hydrogenated overnight at 45 psi H_2, the catalyst was

filtered off and the filtrate evaporated to dryness to afford pure **7** (1.7 g, quantitative) as a white foam. ^{1}H-NMR δ• 5.73 (d, 1H, $J_{NH,2}$=8.4, NH), 5.44 (d, $J_{4,3}$=3.0, 1H, H4), 5.40 (dd, $J_{3,4}$=3.0, $J_{3,2}$=11.2,1H, H3), 4.78 (d, $J_{1,2}$=8.8, 1H, H1), 4.21(m, 2H, CH_2), 4.02 (m, 3H, H2, H6), 3.55 (m, 1H, H5), 2.42 (m, 2H, CH_2), 2.23(s, 3H, Ac), 2.13 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.69 (m, 4H, 2×CH_2), 1.36 (br m, 14H, 7×CH_2). MS/ESI$^-$ m/z 544.0 $(M-H)^-$.

2'-(*N*-α,ε-bis-(12'-Hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galac-topyranose)-L-lysyl)amino-2'-deoxy-5'-*O*-4,4'-dimethoxytrityl uridine (9). **7** (1.05 g, 1.92 mmol) was dissolved in anhydrous THF and *N*-hydroxysuccinimide (0.27 g, 2.35 mmol) and 1,3-dicyclohexylcarbodiimide (0.55 g, 2.67 mmol) were added. The reaction mixture was stirred at rt overnight, then filtered through Celite pad and the filtrate concentrated under reduced pressure. The crude NHSu ester **8** was dissolved in dry DMF (13 ml) containing diisopropylethylamine (0.67 ml, 3.85 mmol) and to this solution nucleoside **2** (0.64 g, 0.95 mmol was added). The reaction mixture was stirred at rt overnight and than concentrated under reduced pressure. The residue was partitioned between water and dichloromethane, the aqueous layer extracted with dichloromethane, the organic layers combined, dried (Na_2SO_4) and evaporated to a syrup. Flash silica gel column chromatography using 2-3% gradient of methanol in ethyl acetate yielded **9** as a colorless foam (1.04 g, 63%). ^{1}H-NMR δ 7.42 (d, $J_{6,5}$=8.4, 1H, H6 Urd), 7.53-6.97 (m, 13H, aromatic), 6.12 (d, $J_{1',2'}$=8.0, 1H, H-1'), 5.41 (m, 3H, H5 Urd, H4 NAcGal), 5.15 (dd, $J_{3,4}$=3.6, $J_{3,2}$=11.2, 2H, H3 NAcGal), 4.87 (dd, $J_{2',3'}$=5.6, $J_{2',1'}$=8.0, 1H, H2'), 4.63 (d, $J_{1,2}$=8.0, 2H, H1 NAcGal), 4.42 (d, $J_{3',2'}$=5.6, 1H, H3'), 4.29-4.04 (m, 9H, H4', H2 NAcGal, H5 NacGal, CH_2), 3.95-3.82 (m, 8H, H6 NAcGal, 2×OMe), 3.62-3.42 (m, 4H, H5', H6 NAcGal), 3.26 (m, 2H, CH_2), 2.40-1.97 (m, 28H, CH_2, Ac), 1.95-1.30 (m, 50H, CH_2). MS/ESI$^-$ m/z 1727.0 $(M-H)^-$.

2'-(*N*-α,ε-bis-(12'-Hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galac-topyranose)-L-lysyl)amino-2'-deoxy-5'-*O*-4,4'-dimethoxytrityl uridine 3'-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (10). Conjugate **9** (0.87 g, 0.50 mmol) was dissolved in dry dichloromethane (10 ml) under argon and diisopropylethylamine (0.36 ml, 2.07 mmol) and 1-methylimidazole (21 μL, 0.26 mmol) were added. The solution was cooled to 0 ^{o}C and 2-cyanoethyl diisopropylchlorophosphoramidite (0.19 ml, 0.85 mmol) was added. The reaction mixture was stirred at rt for 1 hour, than cooled to 0 ^{o}C and quenched with anhydrous ethanol (0.5 ml). After stirring for 10 min the solution was concentrated under reduced pressure (40 ^{o}C) and the residue dissolved in dichloromethane and chromatographed on the column of silica gel using hexanes-ethyl acetate 1:1, followed by ethyl acetate and finally ethyl acetate-acetone 1:1 (1%

triethylamine was added to solvents) to afford the phosphoramidite **10** (680 mg, 69%). ^{31}P-NMR δ 152.0 (s), 149.3 (s). MS/ESI$^-$ m/z 1928.0 (M-H)$^-$.

***N*-(12'-Hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranose)-D-threoninol (11)**. 12'-Hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galac-topyranose **7** (850 mg, 1.56 mmol) was dissolved in DMF (5 ml) and to the solution *N*-hydroxysuccinimide (215 mg, 1.87 mmol) and 1,3-dicyclohexylcarbodimide (386 mg, 1.87 mmol) were added. The reaction mixture was stirred at rt overnight, the precipitate was filtered off and to the filtrate D-threoninol (197 mg, 1.87 mmol) was added. The mixture was stirred at rt overnight and concentrated in vacuo. The residue was partitioned between dichloromethane and 5% $NaHCO_3$, the organic layer was washed with brine, dried (Na_2SO_4) and evaporated to a syrup. Silica gel column chromatography using 1-10% gradient of methanol in dichloromethane afforded **11** as a colorless oil (0.7 g, 71%). ^{1}H-NMR δ 6.35 (d, J=7.6, 1H, NH), 5.77 (d, J=8.0, 1H, NH), 5.44 (d, $^J_{4,3}$=3.6, 1H, H4), 5.37 (dd, $^J_{3,4}$=3.6, $^J_{3,2}$=11.2, 1H, H3), 4.77 (d, $^J_{1,2}$=8.0, 1H, H1), 4.28-4.18 (m, 3H, CH_2, CH), 4.07-3.87 (m, 6H), 3.55 (m, 1H, H5), 3.09 (d, J=3.2, 1H, OH), 3.02 (t, J=4.6, 1H, OH), 2.34 (t, J=7.4 2H, CH_2), 2.23 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.76-1.61 (m, 2×CH_2), 1.35 (m, 14H, 7×CH_2), 1.29 (d, J=6.4, 3H, CH_3). MS/ESI$^-$ m/z (M-H)$^-$.

1-*O*-(4-Monomethoxytrityl)-*N*-(12'-hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranose)-D-threoninol (12). To the solution of **11** (680 mg, 1.1 mmol) in dry pyridine (10 ml) *p*-anisylchlorotriphenylmethane (430 mg, 1.39 mmol) was added and the rection mixture was stirred, protected from moisture, overnight. Methanol (3 ml) was added and the solution stirred for 15 min and evaporated *in vacuo*. The residue was partitioned between dichloromethane and 5% $NaHCO_3$, the organic layer was washed with brine, dried (Na_2SO_4) and evaporated to a syrup. Silica gel column chromatography using 1-3% gradient of methanol in dichloromethane afforded **12** as a white foam (0.75 g, 77%). ^{1}H-NMR δ 7.48-6.92 (m, 14 H, aromatic), 6.15 (d, J=8.8, 1H, NH), 5.56 (d, J=8.0, 1H, NH), 5.45 (d, $^J_{4,3}$=3.2, 1H, H4), 5.40 (dd, $^J_{3,4}$=3.2, $^J_{3,2}$=11.2, 1H, H3), 4.80 (d, $^J_{1,2}$=8.0, 1H, H1), 4.3-4.13 (m, 3H, CH_2, CH), 4.25-3.92 (m, 4H, H6, H2, CH), 3.89 (s, 3H, OMe), 3.54 (m, 2H, H5, CH), 3.36 (dd, J=3.4, J=9.8, 1H, CH), 3.12 (d, J=2.8, 1H, OH), 2.31 (t, J=7.6, 2H, CH_2), 2.22 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.80-1.55 (m, 2×CH_2), 1.37 (m, 14H, 7×CH_2), 1.21 (d, J=6.4, 3H, CH_3). MS/ESI$^-$ m/z 903.5 (M-H)$^-$.

1-*O*-(4-Monomethoxytrityl)-*N*-(12'-hydroxydodecanoyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranose)-D-threoninol 3-*O*-(2-cyanoethyl *N,N*-diisopropylphosphorami-dite) (13). Conjugate **12** (1.2 g, 1.33 mmol) was dissolved in

dry dichloromethane (15 ml) under argon and diisopropylethylamine (0.94 ml, 5.40 mmol) and 1-methylimidazole (55 μL, 0.69 mmol) were added. The solution was cooled to 0 °C and 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.51 ml, 2.29 mmol) was added. The reaction mixture was stirred at rt for 2 hours, than cooled to 0 °C and quenched with anhydrous ethanol (0.5 ml). After stirring for 10 min. the solution was concentrated under reduced pressure (40 °C) and the residue dissolved in dichloromethane and chromatographed on the column of silica gel using 50-80% gradient of ethyl acetate in hexanes (1% triethylamine) to afford the phosphoramidite **13** (1.2 g, 82%). ^{31}P-NMR δ •149.41 (s), 149.23 (s).

Oligonucleotide synthesis

Phosphoramidites **10**, and **13**, were used along with standard 2'-*O*-TBDMS and 2'-*O*-methyl nucleoside phosphoramidites. Synthesis were conducted on a 394 (ABI) synthesizer using modified 2.5 μmol scale protocol with a 5 min coupling step for 2'-*O*-TBDMS protected nucleotides and 2.5 min coupling step for 2'-*O*-methyl nucleosides. Coupling efficiency for the phosphoramidite **10** was lower than 50% while coupling efficiencies for phosphoramidite **13** was typically greater than 95% based on the measurement of released trityl cations. Once the synthesis was completed, the oligonucleotides were deprotected. The 5'-trityl groups were left attached to the oligomers to assist purification. Cleavage from the solid support and the removal of the protecting groups was performed as described herein with the exception of using 20% piperidine in DMF for 15 min for the removal of Fm protection prior methylamine treatment. The 5'-tritylated oligomers were separated from shorter (trityl-off) failure sequences using a short column of SEP-PAK C-18 adsorbent. The bound, tritylated oligomers were detritylated on the column by treatment with 1% trifluoroacetic acid, neutralized with triethylammonium acetate buffer, and than eluted. Further purification was achieved by reverse-phase HPLC. An example of a N-acetyl-D-galactosamine conjugate that can be synthesized using phosphoramidite **13** is shown in **Figure 15**.

Structures of the ribozyme conjugates were confirmed by MALDI-TOF MS.

Monomer synthesis

2'-Amino-2'-deoxyuridine-*N*-acetyl-D-galactosamine conjugate. The bis-Fmoc protected lysine linker was attached to the 2'-amino group of 2'-amino-2'-deoxyuridine using the EEDQ catalyzed peptide coupling. The 5'-OH was protected with 4,4'-dimethoxytrityl group to give **1**, followed by the cleavage of *N*-Fmoc groups with diethylamine to afford synthon **2** in the high overall yield.

2-acetamido-3,4,6-tetra-O-acetyl-1-chloro-D-galactopyranose **4** was synthesized with minor modifications according to the reported procedure (Findeis *supra*). Mercury salt catalyzed glycosylation of **4** with the benzyl ester of 12-hydroxydodecanoic acid **5** afforded glycoside **6** in 81 % yield. Hydrogenolysis of benzyl protecting group yielded **7** in a quantitative yield. The coupling of the sugar derivative with the nucleoside synthon was achieved through preactivation of the carboxylic function of **7** as *N*-hydroxysuccinimide ester **8**, followed by coupling to lysyl-2'-aminouridine conjugate **2**. The final conjugate **9** was than phosphitylated under standard conditions to afford the phosphoramidite **10** in 69% yield.

D-Threoninol-*N*-acetyl-D-galactosamine conjugate Using the similar strategy as described above, D-threoninol was coupled to **7** to afford conjugate **11** in a good yield. Monomethoxytritylation, followed by phosphitylation yielded the desired phosphoramidite **13**.

Example 2: Synthesis of Oxime linked nucleic acid/peptide conjugates (**Figures 16 and 17**)

12-Hydroxydodecanoic acid benzyl ester Benzyl bromide (10.28 ml, 86.45 mmol) was added dropwise to a solution of 12-hydroxydodecanoic acid (17 g, 78.59 mmol) and DBU (12.93 ml, 86.45 mmol) in absolute DMF (120 ml) under vigorous stirring at 0•C. After completeion of the addition reaction mixture was warmed to a room temperature and left overnight under stirring. TLC (hexane-ethylacetate 3:1) indicated complete transformation of the starting material. DMF was removed under reduced pressure and the residue was partitioned between ethyl ether and 1N HCl. Organic phase was separated, washed with saturated aq sodium bicarbonate and dried over sodium sulfate. Sodium sulfate was filtered off, filtrate was evaporated to dryness. The residue was crystallized from hexane to give 21.15 g (92%) of the title compound as a white powder.

12-O-N-Phthaloyl-dodecanoic acid benzyl ester (15). Diethylazodicarboxylate (DEAD, 16.96 ml, 107.7 mmol) was added dropwise to the mixture of 12-Hydroxydodecanoic acid benzyl ester (21g, 71.8 mmol), triphenylphosphine (28.29 g, 107.7 mmol) and N-hydroxyphthalimide (12.88 g, 78.98 mmol) in absolute THF (250 ml) at –20• – -30•C under stirring. The reaction mixture was stirred at this temperature for additional 2-3h, after which time TLC (hexane-ethylacetate 3:1) indicated reaction completion. The solvent was removed in *vacuo* and the residue was treated ether (250 ml). Formed precipitate of triphenylphosphine oxide was filtered off, mother liquor was evaporated to dryness and the residue was dissolved in methylene chloride and purified by flash chromatography on silica gel in hexane-ethyl acetate (7:3). Appropriate fractions were pooled and evaporated to dryness to afford 26.5 g(84.4%) of compound **15**.

12-O-N-Phthaloyl-dodecanoic acid (16). Compound **15** (26.2 g, 59.9 mmol) was dissolved in 225 ml of ethanol-ethylacetate (3.5:1) mixture and 10% Pd/C (2.6 g) was added. The reaction mixture was hydrogenated in Parr apparatus for 3 hours. Reaction mixture was filtered through celite and evaporated to dryness. The residue was crystallized from methanol to provide 15.64 g (75%) of compound **16**.

12-O-N-Phthaloyl-dodecanoic acid 2,3-di-hydroxy-propylamide (18) The mixture of compound **16** (15.03 g, 44.04 mmol), dicyclohexylcarbodiimide (10.9 g, 52.85 mmol) and N-hydroxysuccinimide (6.08 g, 52.85 mmol) in absolute DMF (150 ml) was stirred at room temperature overnight. TLC (methylene chloride –methanol 9:1) indicated complete conversion of the starting material and formation of NHS ester **17**. Then aminopropanediol (4.01 g, 44 mmol) was added and the reaction mixture was stirred at room temperature for another 2 h. The formed precipitate of dicyclohexylurea was removed by filtration, filtrate was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and saturated aq sodium bicarbonate. The whole mixture was filtered to remove any insoluble material and clear layers were separated. Organic phase was concentrated in vacuo until formation of crystalline material. The precipitate was filtered off and washed with cold ethylacetate to produce 10.86 g of compound **17**. Combined mother liquor and washings were evaporated to dryness and crystallized from ethylacetate to afford 3.21 g of compound **18**. Combined yield – 14.07 g (73.5%).

12-O-N-Phthaloyl-dodecanoic acid 2-hydroxy,3-dimethoxytrityloxy-propylamide (19)_Dimethoxytrityl chloride (12.07 g, 35.62 mmol) was added to a stirred solution of compound **18** (14.07 g, 32.38 mmol) in absolute pyridine (130 ml) at 0•C. The reaction solution was kept at 0•C overnight. Then it was quenched with MeOH (10 ml) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate. Organic phase was separated, dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using step gradient of acetone in hexanes (3:7 to 1:1) as an eluent. Appropriate fractions were pooled and evaporated to provide 14.73g (62%) of compound **19**, as a colorless oil.

12-O-N-Phthaloyl-dodecanoic acid 2-O-(cyanoethyl-N.N-diisopropylamino-phosphoramidite),3-dimethoxytrityloxy-propylamide (20). Phosphitylated according to Sanghvi, *et al.,* 2000, *Organic Process Research and Development*, 4, 175-81.

Purified by flash chromatography on silica gel using step gradient of acetone in hexanes (1:4 to 3:7) containing 0.5% of triethylamine. Yield – 82%, colourless oil.

Oxidation of peptides

Peptide (3.3 mg, 3.3 μmol) was dissolved in 10mM AcONa and 2 eq of sodium periodate (100mM soln in water) was added. Final reaction volume – 0.5 ml. After 10 minutes reaction mixture was purified using analytical HPLC on Phenomenex Jupiter 5u C18 300A (150x4.6 mm) column; solvent A: 50mM KH_2PO_4 (pH 3); solvent B: 30% of solvent A in MeCN; gradient B over 30 min. Appropriate fractions were pooled and concentrated on a SpeedVac to dryness. Yield: quantitative.

Conjugation reaction of Herzyme-ONH2-linker with N-glyoxyl peptide (**Figure 17**)

Herzyme (SEQ ID NO: 13) with a 5'-terminal linker (100 OD) was mixed with oxidized peptide (3-5 eq) in 50 mM KH2PO4 (pH3, reaction volume 1 ml) and kept at room temperature for 24-48h. The reaction mixture was purified using analytical HPLC on a Phenomenex Jupiter 5u C18 300A (150x4.6 mm) column; solvent A: 10mM TEAA; solvent B: 10mM TEAA/MeCN. Appropriate fractions were pooled and concentrated on a SpeedVac to dryness to provide desired conjugate. ESMS: calculated: 12699, determined: 12698.

Example 5: Synthesis of Phospholipid enzymatic nucleic acid conjugates (**Figure 19**)

A phospholipid enzymatic nucleic acid conjugate (see **Figure 19**) was prepared by coupling a C18H37 phosphoramidite to the 5'-end of an enzymatic nucleic acid molecule (Angiozyme™, SEQ ID NO: 24) during solid phase oligonucleotide synthesis on an ABI 394 synthesizer using standard synthesis chemistry. A 5'-terminal linker comprising 3'-AdT-di-Glycerol-5', where A is Adenosine, dT is 2'-deoxy Thymidine, and di-Glycerol is a di-DMT-Glycerol linker (Chemgenes CAT number CLP-5215), is used to attach two C18H37 phosphoramidites to the enzymatic nucleic acid molecule using standard synthesis chemistry. Additional equivalents of the C18H37 phosphoramidite were used for the bis-coupling. Similarly, other nucleic acid conjugates as shown in **Figure 18** can be prepared according to similar methodology.

Example 6: Synthesis of PEG enzymatic nucleic acid conjugates (**Figure 20**)

A 40K-PEG enzymatic nucleic acid conjugate (see **Figure 20**) was prepared by post synthetic N-hydroxysuccinimide ester coupling of a PEG derivative (Shearwater Polymers Inc, CAT number PEG2-NHS) to the 5'-end of an enzymatic nucleic acid molecule (Angiozyme™, SEQ ID NO: 24). A 5'-terminal linker comprising 3'-AdT-C6-amine-5', where A is Adenosine, dT-C6-amine is 2'-deoxy Thymidine with a C5 linked six carbon amine linker (Glen Research CAT number 10-1039-05), is used to attach the PEG derivative to the enzymatic nucleic acid molecule using NHS coupling chemistry.

Angiozyme™ with the C6dT-NH2 at the 5' end was synthesized and deprotected using standard oligonucleotide synthesis procedures as described herein. The crude

sample was subsequently loaded onto a reverse phase column and rinsed with sodium chloride solution (0.5 M). The sample was then desalted with water on the column until the concentration of sodium chloride was close to zero. Acetonitrile was used to elute the sample from the column. The crude product was then concentrated and lyophilized to dryness.

The crude material (Angiozyme™) with 5'-amino linker (50 mg) was dissolved in sodium borate buffer (1.0 mL, pH 9.0). The PEG NHS ester (200 mg) was dissolved in anhydrous DMF (1.0 mL). The Angiozyme™ buffer solution was then added to the PEG NHS ester solution. The mixture was immediately vortexed for 5 minutes. Sodium acetate buffer solution (5 mL, pH 5.2) was used to quench the reaction. Conjugated material was then purified by ion-exchange and reverse phase chromatography.

Example 7: Phamacokinetics of PEG ribozyme acid conjugate (**Figure 21**)

Forty-eight female C57Bl/6 mice were given a single subcutaneous (SC) bolus of 30 mg/kg Angiozyme™ and 30 mg/kg Angiozyme™/40K PEG conjugate. Plasma was collected out to 24 hours post ribozyme injection. Plasma samples were analyzed for full length ribozyme by a hybridization assay.

Oligonucleotides complimentary to the 5' and 3' ends of Angiozyme™ were synthesized with biotin at one oligo, and FITC on the other oligo. A biotin oligo and FITC labeled oligo pair are incubated at 1 ug/ml with known concentrations of Angiozyme™ at 75degrees C for 5 min. After 10 minutes at RT, the mixture is allowed to bind to streptavidin coated wells of a 96-wll plate for two hours. The plate is washed with Tris-saline and detergent, and peroxidase labeled anti-FITC antibody is added. After one hour, the wells are washed, and the enzymatic reaction is developed, then read on an ELISA plate reader. Results are shown in **Figure 21**.

Example 8: Phamacokinetics of Phospholipid ribozyme conjugate (**Figure 22**)

Seventy-two female C57Bl/6 mice were given a single intravenous (4) bolus of 30 mg/kg Angiozyme™ and 30 mg/kg Angiozyme™ conjugated with phospholipid (**Figure 19**). Plasma was collected out to 3 hours post ribozyme injection. Plasma samples were analyzed for full length ribozyme by a hybridization assay.

Oligonucleotides complimentary to the 5' and 3' ends of Angiozyme™ were synthesized with biotin at one oligo, and FITC on the other oligo. A biotin oligo and FITC labeled oligo pair are incubated at 1 ug/ml with known concentrations of Angiozyme™ at 75degrees C for 5 min. After 10 minutes at RT, the mixture is allowed to bind to streptavidin coated wells of a 96-wll plate for two hours. The plate is washed with Tris-saline and detergent, and peroxidase labeled anti-FITC antibody is added. After

one hr, the wells are washed, and the enzymatic reaction is developed, then read on an ELISA plate reader. Results are shown in **Figure 22**.

Example 9: Synthesis of Protein or Peptide conjugates with biodegradable linkers **(Figures 24-26, and 29)**

Proteins and peptides can be conjugated with various molecules, including PEG, via biodegradable nucleic acid linker molecules of the invention, using oxime and morpholino linkages. For example, a therapeutic antibody can be conjugated with PEG to improve the **Figure 24** shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker, the example shown is for a protein conjugate. Other conjugates can be synthesized in a similar manner where the protein or peptide is conjugated to molecules other than PEG, such as small molecules, toxins, radioisotopes, peptides or other proteins. (a) The protein of interest, such as an antibody or interferon, is synthesized with a terminal Serine or Threonine moiety that is oxidized, for example with sodium periodate. The oxidized protein is then coupled to a nucleic acid linker molecule that is designed to be biodegradable, for example a cytidine-deoxythymidine, cytidine-deoxyuridine, adenosine-deoxythymidine, or adenosine-deoxyuridine dimer that contains an oxyamino ($O\text{-}NH_2$) function. Other biodegradable nucleic acid linkers can be similarly used, for example other dimers, trimers, tetramers etc. that are designed to be biodegradable. The example shown makes use of a 5'-oxyamino moiety, however, other examples can utilize an oxyamino at other positions within the nucleic acid molecule, for example at the 2'-position, 3'-position, or at a nucleic acid base position. (b) The protein/nucleic acid conjugate is then oxidized to generate a dialdehyde function that is coupled to PEG molecule comprising an amino group (H_2N-PEG), for example a PEG molecule with an amino linker. Other amino containing molecules can be conjugated as shown in the figure, for example small molecules, toxins, or radioisotope labeled molecules.

Proteins and peptides can be conjugated with various molecules, including PEG, via biodegradable nucleic acid linker molecules of the invention, using oxime and phosphoramidate linkages. **Figure 25** shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker, the example shown is for a protein conjugate. Other conjugates can be synthesized in a similar manner where the protein or peptide is conjugated to molecules other than PEG, such as small molecules, toxins, radioisotopes, peptides or other proteins. The protein of interest, such as an antibody or interferon, is synthesized with a terminal Serine or Threonine moiety that is oxidized, for example with sodium periodate. The oxidized

protein is then coupled to a nucleic acid linker molecule that is designed to be biodegradable, for example a cytidine-deoxythymidine, cytidine-deoxyuridine, adenosine-deoxythymidine, or adenosine-deoxyuridine dimer that contains an oxyamino ($O\text{-}NH_2$) function and a terminal phosphate group. Terminal phosphate groups can be introduced during synthesis of the nucleic acid molecule using chemical phosphorylation reagents, such as Glen Research Cat Nos. 10-1909-02, 10-1913-02, 10-1914-02, and 10-1918-02. Other biodegradable nucleic acid linkers can be similarly used, for example other dimers, trimers, tetramers etc. that are designed to be biodegradable. The example shown makes use of a 5'-oxyamino moiety, however, other examples can utilize an oxyamino at other positions within the nucleic acid molecule, for example at the 2'-position, 3'-position, or at a nucleic acid base position. The protein/nucleic acid conjugate terminal phosphate group is then activated with an activator reagent, such as NMI and/or tetrazole, and coupled a PEG molecule comprising an amino group ($H_2N\text{-}PEG$), for example a PEG molecule with an amino linker. Other amino containing molecules can be conjugated as shown in the figure, for example small molecules, toxins, or radioisotope labeled molecules.

Proteins and peptides can be conjugated with various molecules, including PEG, via biodegradable nucleic acid linker molecules of the invention, using phosphoramidate linkages. **Figure 26** shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker, the example shown is for a protein conjugate. Other conjugates can be synthesized in a similar manner where the protein or peptide is conjugated to molecules other than PEG, such as small molecules, toxins, radioisotopes, peptides or other proteins. (a) A nucleic acid linker molecule that is designed to be biodegradable, for example a cytidine-deoxythymidine, cytidine-deoxyuridine, adenosine-deoxythymidine, or adenosine-deoxyuridine dimer, is synthesized with a terminal phosphate group. Other biodegradable nucleic acid linkers can be similarly used, for example other dimers, trimers, tetramers etc. that are designed to be biodegradable. The protein/nucleic acid conjugate terminal phosphate group is then activated with an activator reagent, such as NMI and/or tetrazole, and coupled a PEG molecule comprising an amino group ($H_2N\text{-}PEG$), for example a PEG molecule with an amino linker. Other amino containing molecules can be conjugated as shown in the figure, for example small molecules, toxins, or radioisotope labeled molecules. The terminal protecting group, for example a dimethoxytrityl group, is removed from the conjugate and a terminal phosphite group is introduced with a phosphitylating reagent, such as N,N-diisopropyl-2-cyanoethyl chlorophosphoramidite. (b) The PEG/nucleic acid conjugate is then coupled to a peptide or protein comprising an amino group, such as the amino terminus or amino side chain of a suitably protected

peptide or protein or via an amino linker. The conjugate is then oxidized and any protecting groups are removed to yield the protein/PEG conjugate comprising a biodegradable linker.

Proteins and peptides can be conjugated with various molecules, including PEG, via biodegradable nucleic acid linker molecules of the invention, using phosphoramidate linkages from coupling protein-based phosphoramidites. **Figure 29** shows a non-limiting example of a synthetic approach for synthesizing peptide or protein conjugates to PEG utilizing a biodegradable linker, the example shown is for a protein conjugate. Other conjugates can be synthesized in a similar manner where the protein or peptide is conjugated to molecules other than PEG, such as small molecules, toxins, radioisotopes, peptides or other proteins. The protein of interest, such as an antibody or interferon, is synthesized with a terminal Serine, Threonin, or Tyrosine moiety that is phosphitylated, for example with N,N-diisopropyl-2-cyanoethyl chlorophosphoramidite. The phosphitylated protein is then coupled to a nucleic acid linker molecule that is designed to be biodegradable, for example a cytidine-deoxythymidine, cytidine-deoxyuridine, adenosine-deoxythymidine, or adenosine-deoxyuridine dimer that contains conjugated PEG molecule as described in Figure 18. Other biodegradable nucleic acid linkers can be similarly used, for example other dimers, trimers, tetramers etc. that are designed to be biodegradable.

Example 10: Galactosamine ribozyme conjugate targeting HBV

A nuclease-resistance ribozyme directed against the Heptatitis B viral RNA (HBV) (HepBzyme™) is in early stages of preclinical development. HepBzyme, which targets site 273 of the Hepatitis B viral RNA, has produced statistically significant decreases in serum HBV levels in a HBV transgenic mouse model in a dose-dependent manner (30 and 100 mg/kg/day). In an effort to improve hepatic uptake by targeting the asialoglycoprotein receptor, a series of 5 branched galactosamine residues were attached via phosphate linkages to the 5'-terminus of HepBzyme (Gal-HepBzyme). The affect of the galactosamine conjugation on HepBzyme was assessed by quantitation of ^{32}P-labeled HepBzyme and Gal-HepBzyme in plasma, liver and kidney of mice following a single SC bolus administration of 30 mg/kg. The plasma disposition of the intact ribozyme was similar between Gal-HepBzyme and HepBzyme. An approximate three-fold increase in the maximum observed concentration of intact ribozyme in liver (C_{max}) was observed in liver for Gal-HepBzyme (6.1 ± 1.8 ng/mg) vs. HepBzyme (2.2 ± 0.8 ng/mg) ($p < 0.05$). The area under the curve (AUCall) for Gal-HepBzyme was also increased by approximately two-fold. This was accompanied by a substantial decrease (approximately

40%) in the AUC_{all} for intact ribozyme in kidney. In addition to the significant increase in C_{max} observed for intact Gal-HepBzyme in the liver, there was an increase in the total number of ribozyme equivalents, which may be suggestive of increased affinity of both the intact ribozyme and metabolites for asialoglycoprotein receptor and galactose-specific receptors in the liver. These data demonstrate that conjugation of a ribozyme with galactosamine produces a compound with a more favorable disposition profile, and illustrates the utility of conjugated ribozymes with improved *in vivo* pharmacokinetics and biodistribution.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Other embodiments are within the following claims.

TABLE I

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-OH of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintenance of the active structure.
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [i,ii].
- Complete kinetic framework established for one ribozyme [iii,iv,v,vi].
- Studies of ribozyme folding and substrate docking underway [vii,viii,ix].
- Chemical modification investigation of important residues well established [x,xi].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the Tetrahymena group I intron has been used to repair a "defective" β-galactosidase message by the ligation of new β-galactosidase sequences onto the defective message [xii].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [xiii].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3'-OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [xiv, xv]
- Important phosphate and 2' OH contacts recently identified [xvi, xvii]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [xviii, xix].
- Sequence requirements not fully determined.
- Reaction mechanism: 2'-OH of an internal adenosine generates cleavage products with 3'-OH and a "lariat" RNA containing a 3'-5' and a 2'-5' branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [xx, xxi] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [xxii].
- Important 2' OH contacts beginning to be identified [xxiii]
- Kinetic framework under development [xxiv]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [xxv].
- Sequence requirements not fully determined.

- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme
(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures [[xxvi], [xxvii]]
- Minimal ligation activity demonstrated (for engineering through *in vitro* selection) [[xxviii]]
- Complete kinetic framework established for two or more ribozymes [[xxix]].
- Chemical modification investigation of important residues well established [[xxx]].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [[xxxi], [xxxii], [xxxiii], [xxxiv]]

- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through *in vitro* selection [xxxv]
- Complete kinetic framework established for one ribozyme [xxxvi].
- Chemical modification investigation of important residues begun [xxxvii, xxxviii].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [xxxix].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [xl].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [xli]

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Table II:

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 µmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 µL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 µL	NA	NA	NA

- **Wait time does not include contact time during delivery.**

Table 3: Peptides for Conjugation

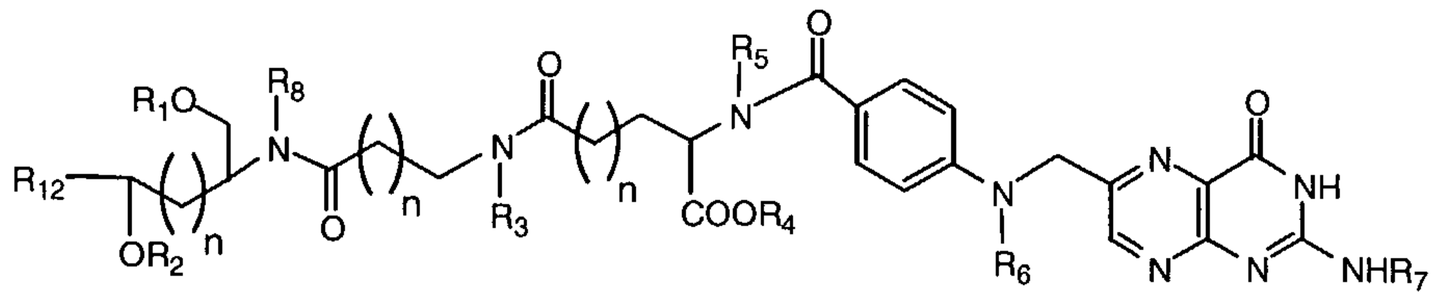
Peptide	Sequence	SEQ ID NO
ANTENNAPEDIA	RQI KIW FQN RRM KWK K amide	14
Kaposi fibroblast growth factor	AAV ALL PAV LLA LLA P + VQR KRQ KLMP	15
caiman crocodylus Ig(5) light chain	MGL GLH LLV LAA ALQ GA	16
HIVenvelope glycoprotein gp41	GAL FLG FLG AAG STM GA + PKS KRK 5 (NLS of the SV40)	17
HIV-1 Tat	RKK RRQ RRR	18
Influenza hemagglutinin envelop glycoprotein	GLFEAIAGFIENGWEGMIDGGGYC	19
RGD peptide	X-RGD-X where X is any amino acid or peptide	20
transportan A	GWT LNS AGY LLG KIN LKA LAA LAK KIL	21
Somatostatin (tyr-3-octreotate)	(S)*F*C Y*W*K TCT	22
Pre-S-peptide	(S)DH QLN PAF	23

(S) optional Serine for coupling

Italic = optional D isomer for stability

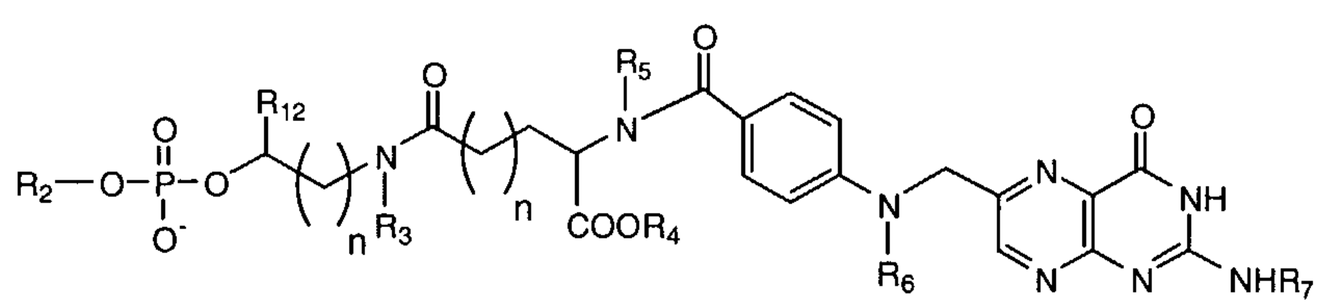
Claims

1. A compound having the Formula 1:



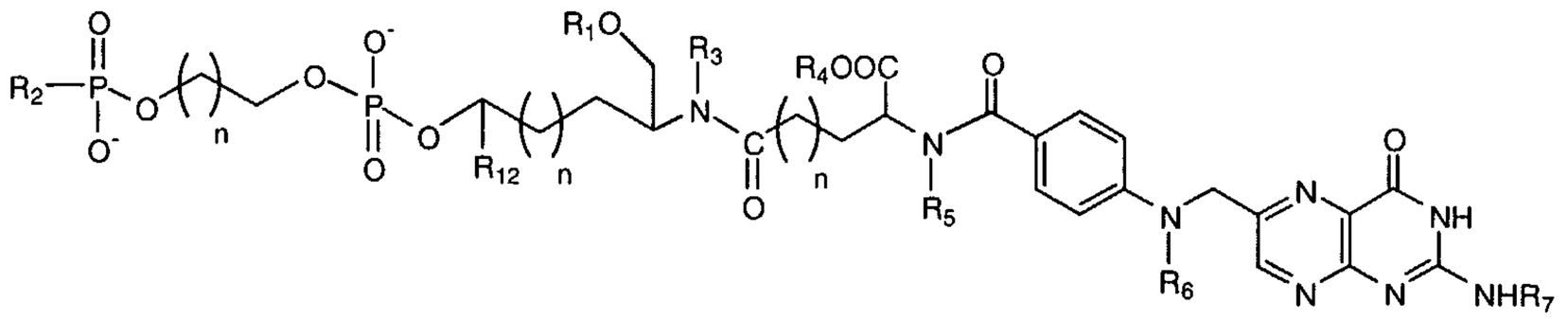
wherein each R_1, R_3, R_4 ,R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

2. A compound having the Formula 2:



wherein each R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

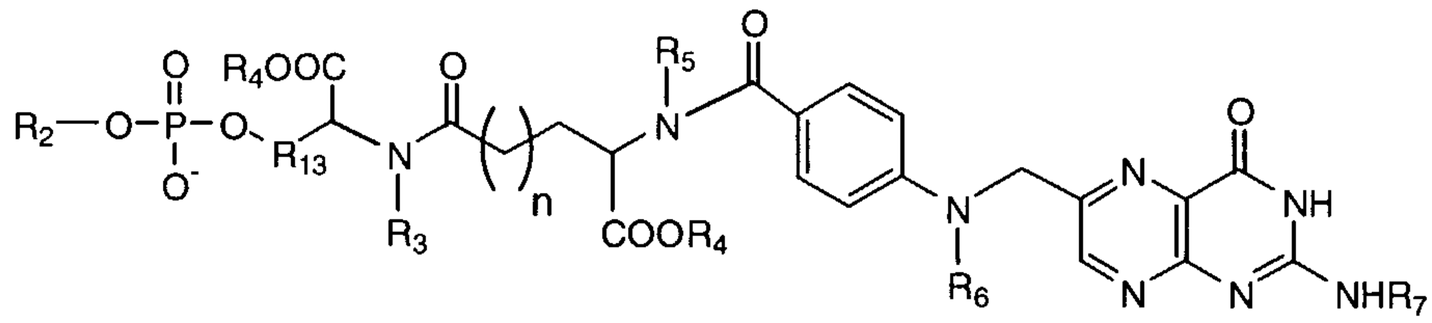
3. A compound having the Formula 3:



wherein each R_1, R_3, R_4 ,R_5 R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain

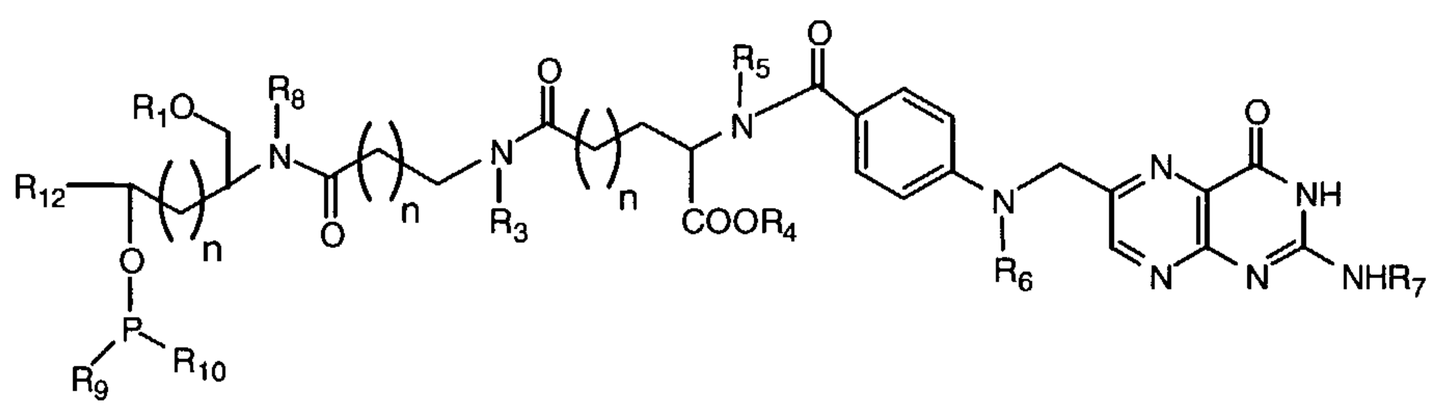
alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, or nucleic acid.

4. A compound having the Formula 4:



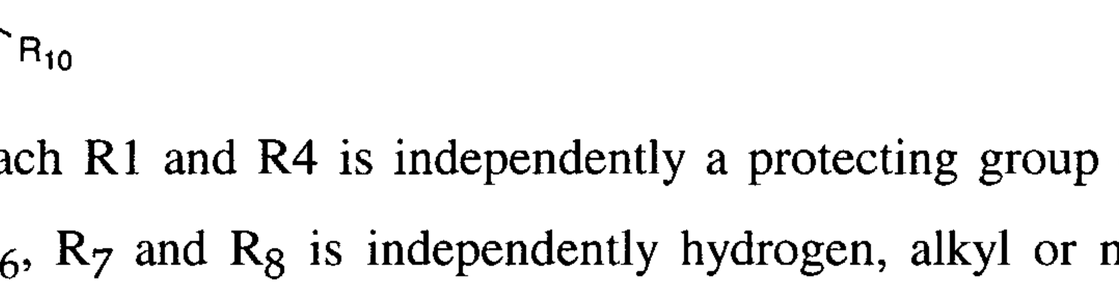
wherein each R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, and R_{13} is an amino acid side chain.

5. A compound having the Formula 5:



wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

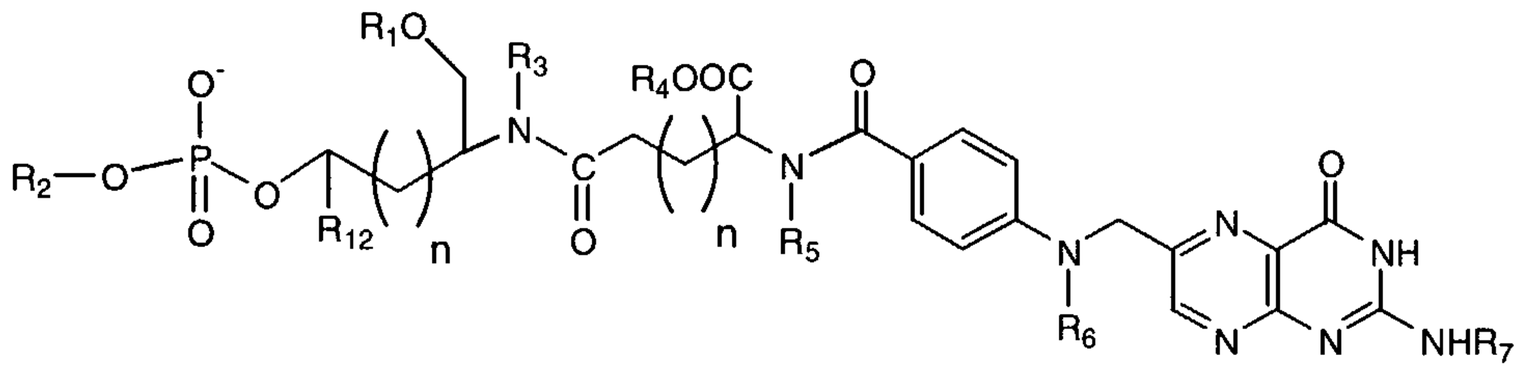
6. A compound having the Formula 6:



wherein each R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, R_2 is a phosphorus containing

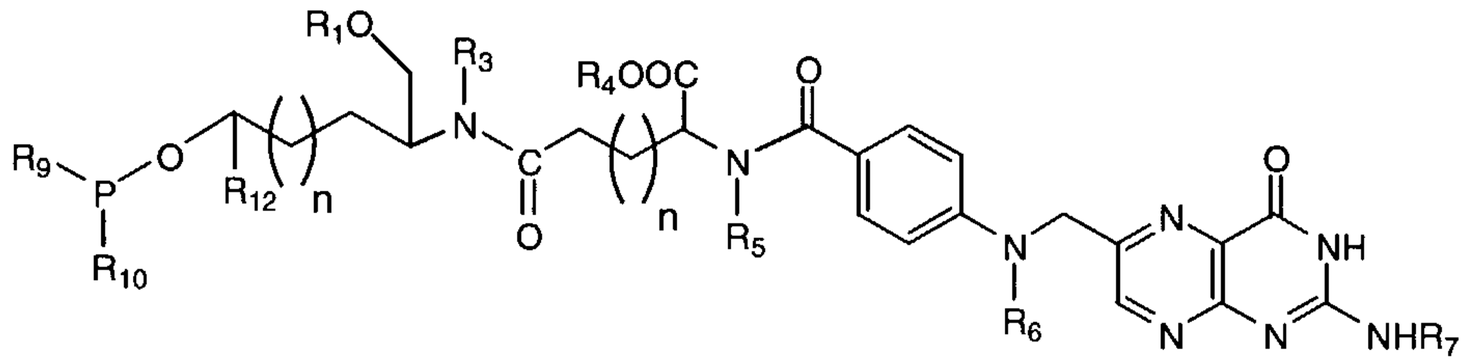
group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, each "n" is independently an integer from 0 to about 200, and L is a degradable linker.

7. A compound having the Formula 7:



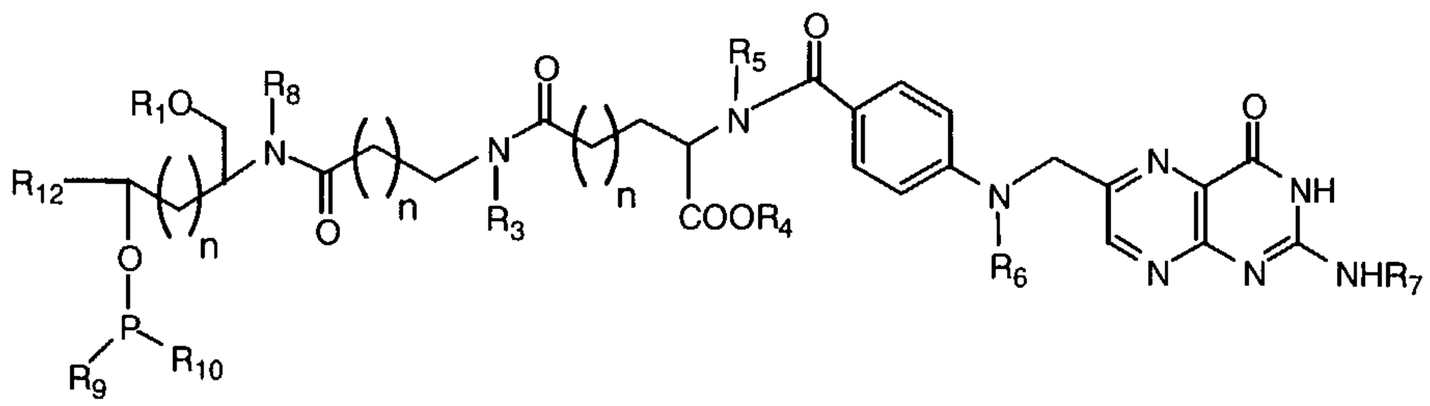
wherein each R_1, R_3, R_4 ,R_5, R_6 and R_7 is independently hydrogen, alkyl , substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

8. A compound having the Formula 8:



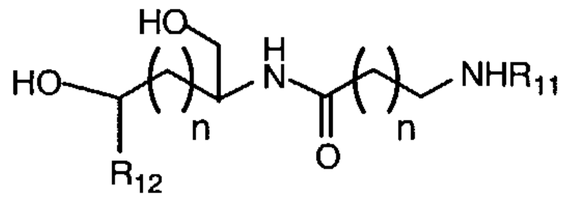
wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

9. A method for synthesizing a compound having Formula 5:



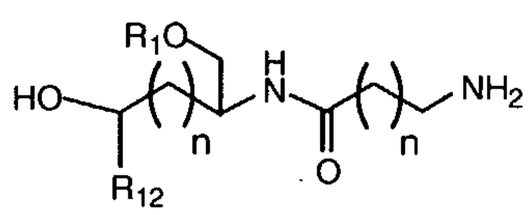
wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, comprising;

(a) coupling a bis-hydroxy aminoalkyl derivative with a N-protected aminoalkanoic acid to yield a compound of Formula 9;



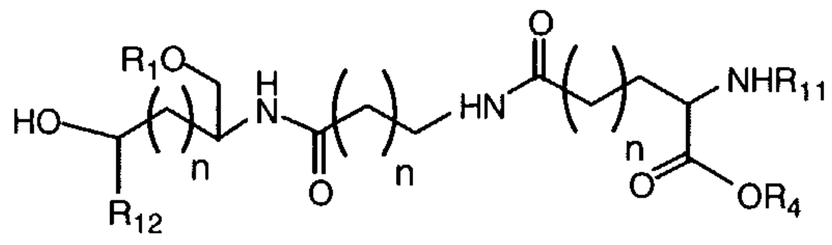
wherein R_{11} is an amino protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200;

(b) introducing primary hydroxy protection followed by amino deprotection to yield a compound of Formula 10;



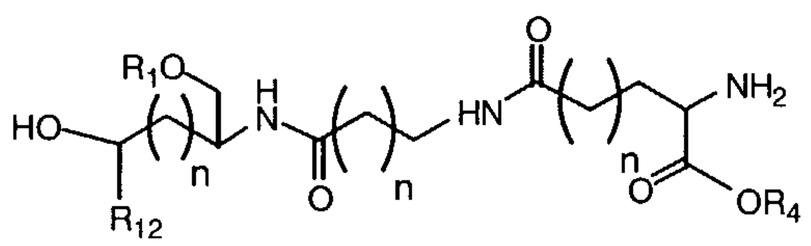
wherein R_1 is a protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200;

(c) coupling the deprotected amine with a protected amino acid to yield a compound of Formula 11;



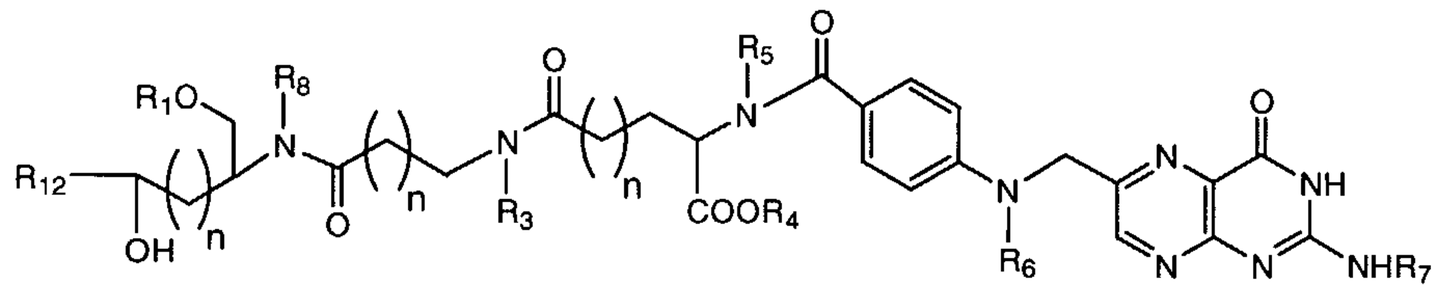
wherein each R1 and R4 is independently a protecting group or hydrogen, each "n" is independently an integer from 0 to about 200, R_{11} is an amino protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl;

(d) deprotecting the amine of the conjugated glutamic acid to yield a compound of Formula 12;



wherein each R1 and R4 is independently a protecting group or hydrogen, each "n" is independently an integer from 0 to about 200, R_{11} is an amino protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl;

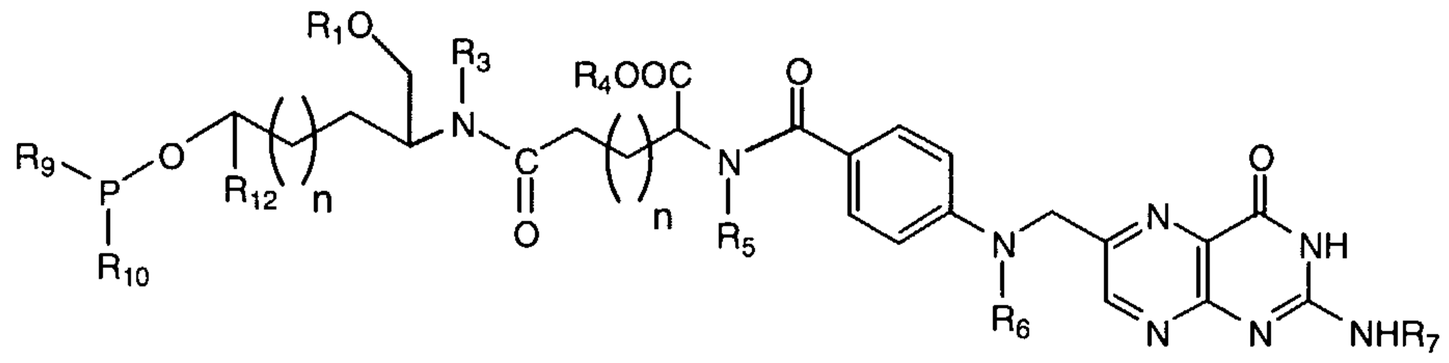
(e) coupling the deprotected amine with an amino protected pteroic acid to yield a compound of Formula 13;



wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and

(f) introducing a phosphorus containing group at the secondary hydroxyl to yield a compound of Formula 5.

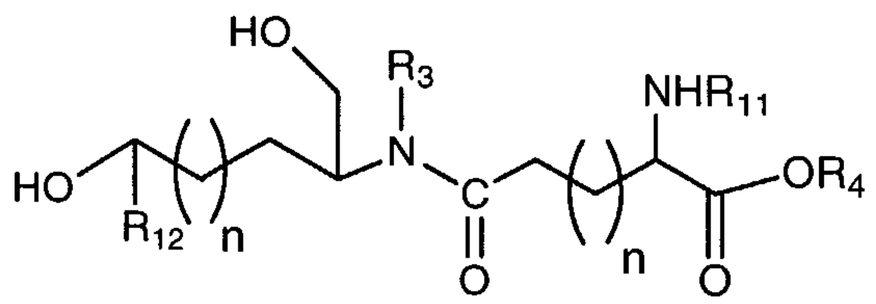
10. A method for synthesizing a compound having Formula 8:



wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each

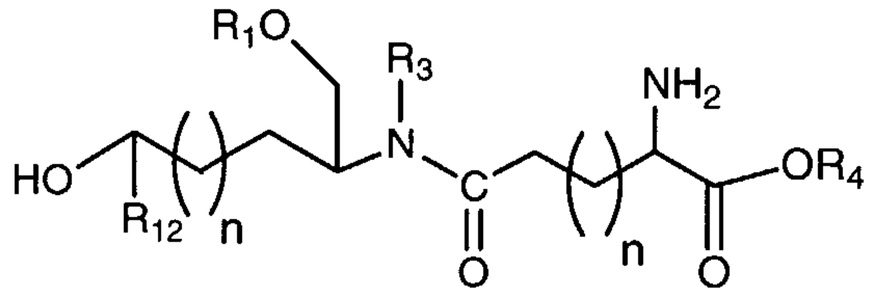
"n" is independently an integer from 0 to about 200, each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, comprising;

(a) coupling a bis-hydroxy aminoalkyl derivative with a protected amino acid to yield a compound of Formula 14;



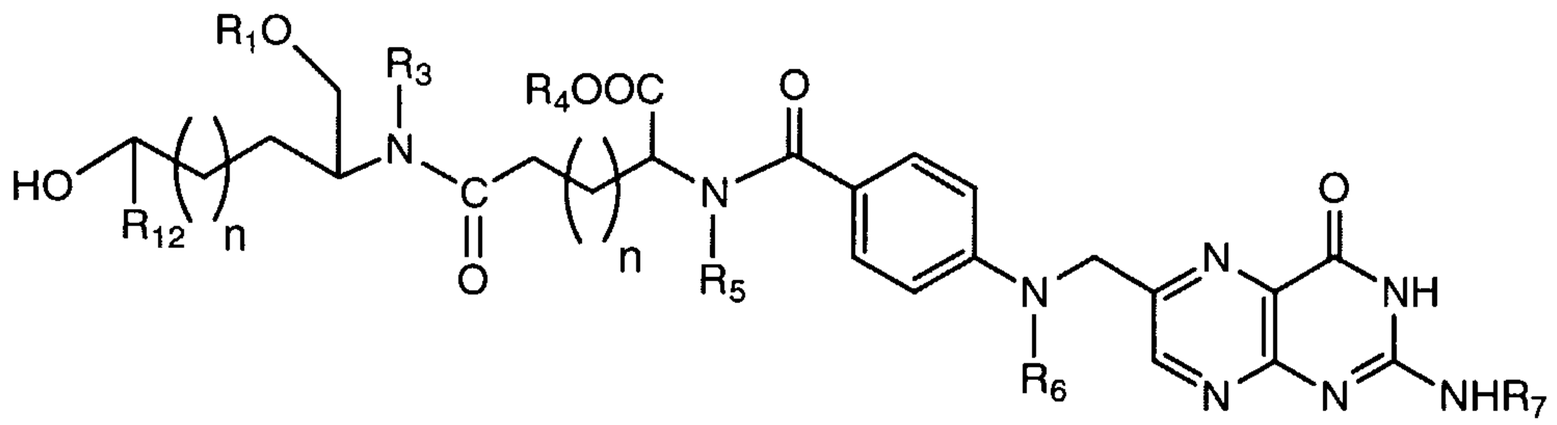
wherein R_{11} is an amino protecting group, each "n" is independently an integer from 0 to about 200, R_4 is independently a protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl;

(b) introducing primary hydroxy protection followed by amino deprotection to yield a compound of Formula 15;



wherein each R_1 and R_4 is independently a protecting group or hydrogen, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200;

(c) coupling the deprotected amine with an amino protected pteroic acid to yield a compound of Formula 16;



wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and

(f) introducing a phosphorus containing group at the secondary hydroxyl to yield a compound of Formula 18.

11. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a phosphorus containing group.

12. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleoside.

13. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleotide.

14. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a small molecule.

15. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleic acid.

16. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a solid support comprising a linker.

17. The compound of claim 12, wherein said nucleoside is a nucleoside with anticancer activity.

18. The compound of claim 12, wherein said nucleoside is a nucleoside with antiviral activity.

19. The compound of claim 12, wherein said nucleoside is fludarabine.

20. The compound of claim 12, wherein said nucleoside is lamivudine (3TC).

21. The compound of claim 12, wherein said nucleoside is 5-fluro uridine.

22. The compound of claim 12, wherein said nucleoside is AZT.

23. The compound of claim 12, wherein said nucleoside is ara-adenosine or ara-adenosine monophosphate.

24. The compound of claim 12, wherein said nucleoside is a dideoxy nucleoside analog.

25. The compound of claim 12, wherein said nucleoside is carbodeoxyguanosine.

26. The compound of claim 12, wherein said nucleoside is ribavirin.

27. The compound of claim 12, wherein said nucleoside is fialuridine.

28. The compound of claim 12, wherein said nucleoside is lobucavir.

29. The compound of claim 12, wherein said nucleoside is a pyrophosphate nucleoside analog.

30. The compound of claim 12, wherein said nucleoside is an acyclic nucleoside analog.

31. The compound of claim 12, wherein said nucleoside is acyclovir.

32. The compound of claim 12, wherein said nucleoside is gangciclovir.

33. The compound of claim 12, wherein said nucleoside is penciclovir.

34. The compound of claim 12, wherein said nucleoside is famciclovir.

35. The compound of claim 12, wherein said nucleoside is an L-nucleoside analog.

36. The compound of claim 12, wherein said nucleoside is FTC.

37. The compound of claim 12, wherein said nucleoside is L-FMAU.

38. The compound of claim 12, wherein said nucleoside is L-ddC or L-FddC.

39. The compound of claim 12, wherein said nucleoside is L-d4C or L-Fd4C.

40. The compound of claim 12, wherein said nucleoside is an L-dideoxypurine nucleoside analog.

41. The compound of claim 12, wherein said nucleoside is cytallene.

42. The compound of claim 12, wherein said nucleoside is bis-POM PMEA (GS-840).

43. The compound of claim 12, wherein said nucleoside is BMS-200,475.

44. The compound of claim 4, wherein R_{13} comprises an alkylamine.

45. The compound of claim 4, wherein R_{13} comprises an alkanol.

46. The compound of claim 4, wherein R_{13} comprises -CH_2O-.

47. The compound of claim 4, wherein R_{13} comprises –$CH(CH_2)CH_2O$-.

48. The compound of claim 6, wherein L is serine.

49. The compound of claim 6, wherein L is threonine.

50. The compound of claim 6, wherein L is a photolabile linkage.

51. The compound of any of claims 5, 8, 9, or 10, wherein R_9 comprises a phosphorus protecting group

52. The compound of claim 51, wherein said phosphorus protecting group is -OCH_2CH_2CN (oxyethylcyano).

53. The compound of any of claims 5 or 8, wherein R_{10} comprises a nitrogen containing group.

54. The compound of claim 53, wherein said nitrogen containing group is –$N(R_{14})$ wherein R_{14} is a straight or branched chain alkyl having form about 1 to 10 carbons.

55. The compound of any of claims 5 or 8, wherein R_{10} comprises a heterocycloalkyl or heterocycloalkenyl ring containing from about 4 to 7 atoms, and having up to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

56. The compound of any of claims 1, 5 or 8, wherein R_1 is an acid labile protecting group.

57. The compound of any of claims 1, 5 or 8, wherein R_1 is a trityl or substituted trityl group.

58. The compound of claim 57, wherein said substituted trityl group is a dimethoxytrityl or mono-methoxytrityl group.

59. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_4 is *tert*-butyl, Fm (fluorenyl-methoxy), or allyl.

60. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_6 is TFA (trifluoracetyl).

61. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_3, R_5 R_7 and R_8 are hydrogen.

62. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is isobutyryl.

63. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is dimethylformamide.

64. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is hydrogen.

65. The compound of any of claims 1, 2, 3, 5, 7 or 8, wherein R_{12} is methyl.

66. The compound of any of claims 1, 2, 3, 5, 7 or 8, wherein R_{12} is ethyl.

67. The compound of any of claim 15, wherein said nucleic acid is an enzymatic nucleic acid.

68. The compound of claim 67, wherein said enzymatic nucleic acid is a hammerhead.

69. The compound of claim 67, wherein said enzymatic nucleic acid is an Inozyme.

70. The compound of claim 67, wherein said enzymatic nucleic acid is a DNAzyme.

71. The compound of claim 67, wherein said enzymatic nucleic acid is a G-cleaver.

72. The compound of claim 67, wherein said enzymatic nucleic acid is a Zinzyme.

73. The compound of claim 67, wherein said enzymatic nucleic acid is an Amberzyme.

74. The compound of claim 67, wherein said enzymatic nucleic acid is an allozyme.

75. The compound of any of claim 15, wherein said nucleic acid is an antisense nucleic acid.

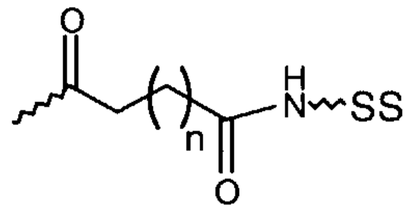
76. The compound of any of claim 15, wherein said nucleic acid is a 2-5A nucleic acid chimera.

77. The compound of any of claim 15, wherein said nucleic acid is a decoy nucleic acid.

78. The compound of claim 13, wherein said nucleotide is a nucleotide with anticancer activity.

79. The compound of claim 13, wherein said nucleotide is a nucleotide with antiviral activity.

80. The compound of claim 16, wherein said solid support comprising a linker is of Formula 17:



wherein SS is a solid support, and each "n" is independently an integer from 1 to 200.

81. The compound of claim 80, wherein said solid support is controlled pore glass (CPG).

82. The compound of claim 80, wherein said solid support is polystyrene.

83. The compound of claim 16, wherein said compound is used in the synthesis of a nucleic acid.

84. A pharmaceutical composition comprising the compound of claim 1 in a pharmaceutically acceptable carrier.

85. A pharmaceutical composition comprising the compound of claim 2 in a pharmaceutically acceptable carrier.

86. A pharmaceutical composition comprising the compound of claim 3 in a pharmaceutically acceptable carrier.

87. A pharmaceutical composition comprising the compound of claim 4 in a pharmaceutically acceptable carrier.

88. A pharmaceutical composition comprising the compound of claim 6 in a pharmaceutically acceptable carrier.

89. A pharmaceutical composition comprising the compound of claim 7 in a pharmaceutically acceptable carrier.

90. A method of treating cancer in a patient, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 84-89, under conditions suitable for said treatment.

91. The method of claim 90, further comprising the use of one or more other drug therapies under conditions suitable for said treatment.

92. The method of claim 90, wherein said cancer is breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancers.

93. A method of treating a patient infected with a virus, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 84-89, under conditions suitable for said treatment.

94. The method of claim 93, further comprising the use of one or more other drug therapies under conditions suitable for said treatment.

95. The method of claim 93, wherein said virus is HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, west nile virus, Ebola virus, foot and mouth virus, and papilloma virus.

96. A kit for detecting the presence of a nucleic acid in a sample, comprising the compound of claim 15.

97. A kit for detecting the presence of a target molecule in a sample, comprising the compound of claim 15.

98. A kit for detecting the presence of a nucleic acid in a cancer cell, comprising the compound of any of claim 74.

99. A kit for detecting the presence of a nucleic acid in a virus infected cell, comprising the compound of claim 74.

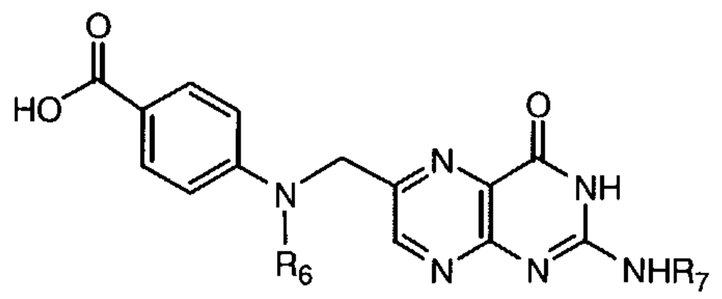
100. The compound of any of claims 2, 3, 4, or 7, wherein said compound contains a modified phosphate.

101. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein said phosphorus containing group is a phosphoramidite, phosphodiester, phosphoramidate,

phosphorothioate, phosphorodithioate, alkylphosphonate, arylphosphonate, monophosphate, diphosphate, triphosphate, or pyrophosphate.

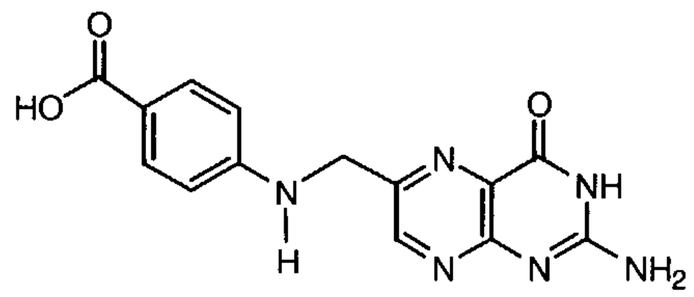
102. The compound of claim 12, wherein said nucleoside is carbovir or abacavir.

103. A method for synthesizing a compound having Formula 18:

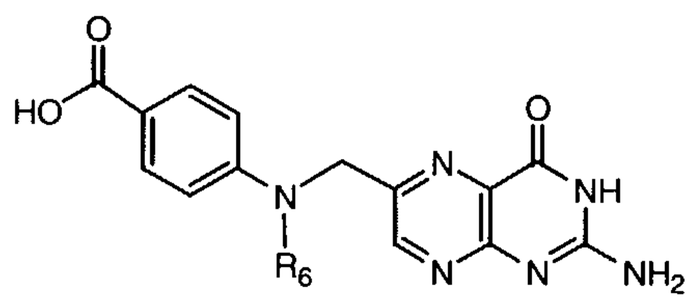


wherein each R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, comprising;

(a) treating folic acid with a carboxypeptidase to yield a compound of Formula 19;



(b) introducing protection of the secondary amine to yield a compound of Formula 20;



wherein R_6 is a nitrogen protecting group; and

(c) introducing protection of the primary amine to yield a compound of Formula 18.

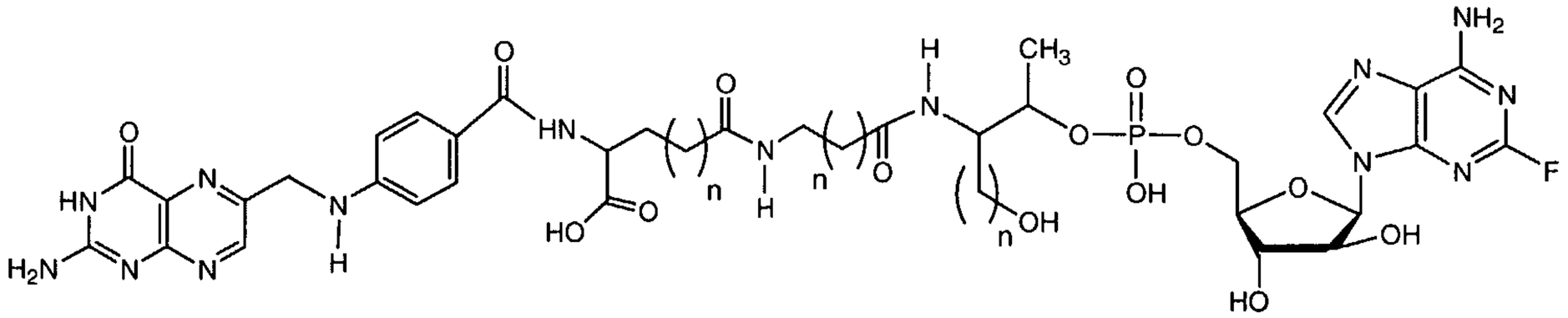
104. The method of claim 103, wherein R_6 is trifluoroacetyl (TFA).

105. The method of claim 103, wherein R_7 is isobutyryl (iBu).

106. The method of claim 9, wherein said amino protected pteroic acid is a compound of Formula 18.

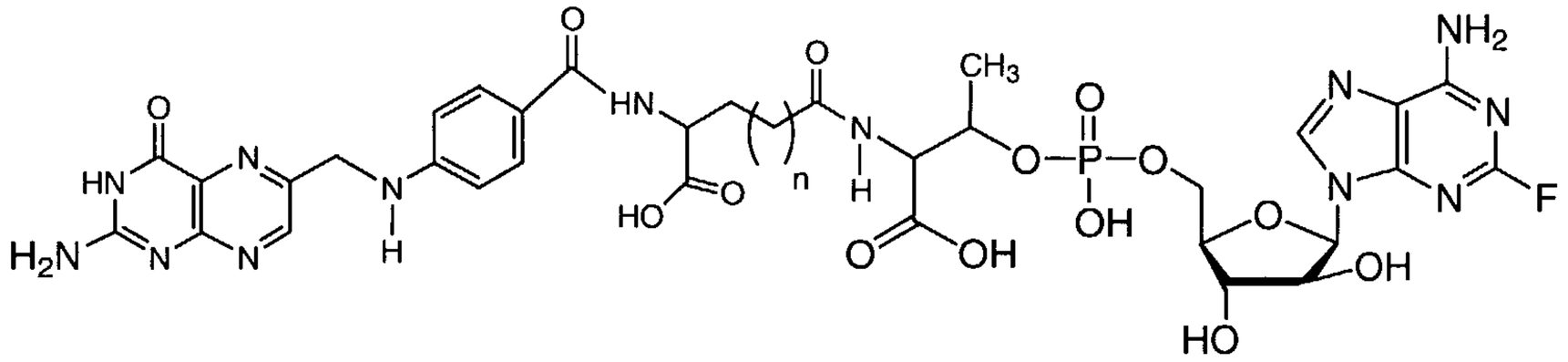
107. The method of claim 10, wherein said amino protected pteroic acid is a compound of Formula 18.

108. A compound of claim 1, having Formula 21:



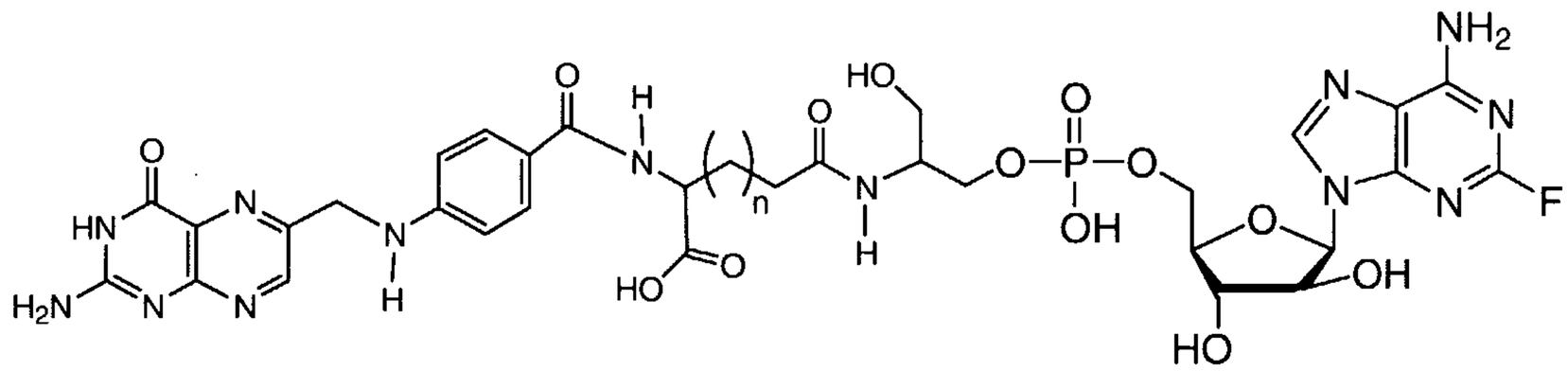
wherein each "n" is independently an integer from 0 to about 200.

109. A compound of claim 4, having Formula 22:



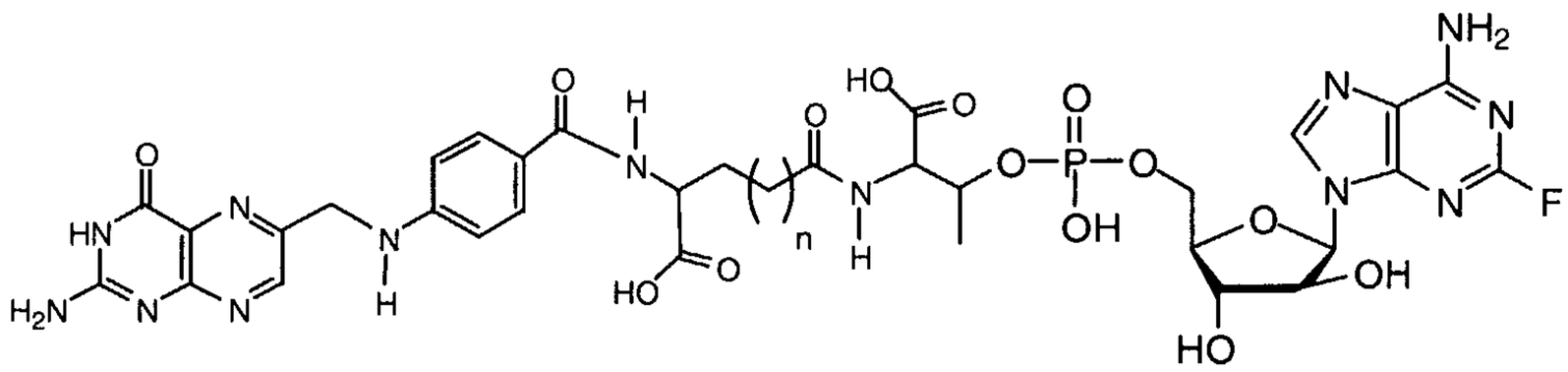
wherein each "n" is independently an integer from 0 to about 200.

110. A compound of claim 7, having Formula 23:



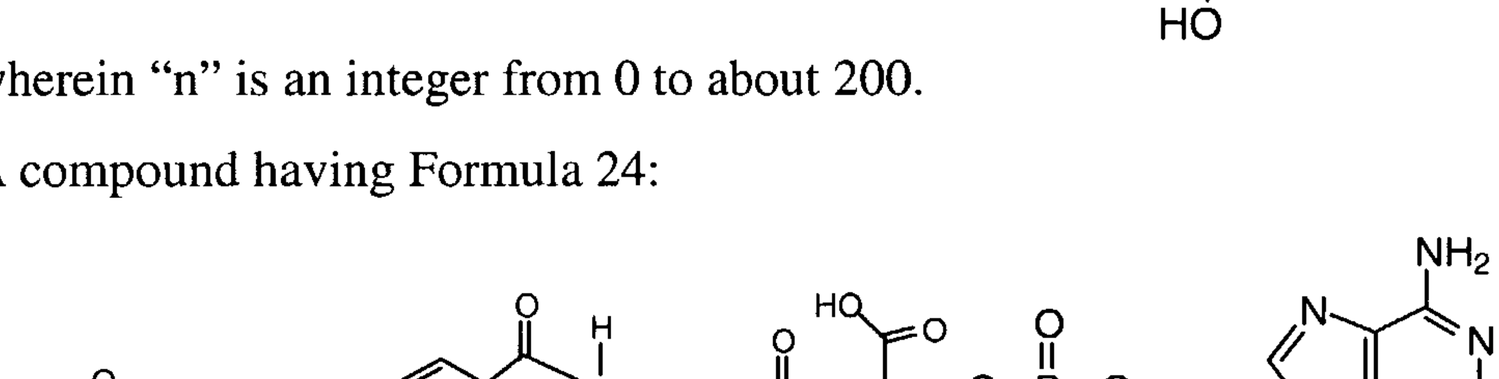
wherein "n" is an integer from 0 to about 200.

111. A compound having Formula 24:

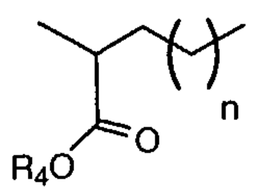


wherein "n" is an integer from 0 to about 200.

112. A compound having Formula 25:



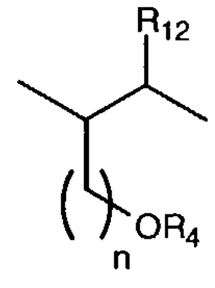
wherein each R_5 and R_7 is independently hydrogen, alkyl or a nitrogen protecting group, each R_{15}, R_{16}, R_{17}, and R_{18} is independently O, S, alkyl, substituted alkyl, aryl, substituted aryl, or halogen, X_1 is $-CH(X_1')$ or a group of Formula 38:



wherein R_4 is a protecting group and "n" is an integer from 0 to about 200;

X_1' is the protected or unprotected side chain of a naturally occurring or non-naturally-occurring amino acid, X_2 is an amide, alkyl, or carbonyl containing linker or a bond, and X_3 is a degradable linker which is optionally absent.

113. The compound of claim 112, wherein X_3 is a group of Formula 26:



wherein R_4 is hydrogen or a protecting group, "n" is an integer from 0 to about 200 and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl.

114. The compound of claim 113, wherein R_4 is hydrogen and R_{12} is methyl or hyrdogen.

115. A pharmaceutical composition comprising the compound of claim 108 in a pharmaceutically acceptable carrier.

116. A pharmaceutical composition comprising the compound of claim 109 in a pharmaceutically acceptable carrier.

117. A pharmaceutical composition comprising the compound of claim 110 in a pharmaceutically acceptable carrier.

118. A pharmaceutical composition comprising the compound of claim 111 in a pharmaceutically acceptable carrier.

119. A pharmaceutical composition comprising the compound of claim 112 in a pharmaceutically acceptable carrier.

120. A method of treating a cancer patient, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 115-119, under conditions suitable for said treatment.

121. The method of claim 120, further comprising the use of one or more other therapies under conditions suitable for said treatment.

122. The method of claim 120, wherein said cancer is breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancers.

123. The compound of claim 2, wherein R_{12} is a alkylhyrdroxyl.

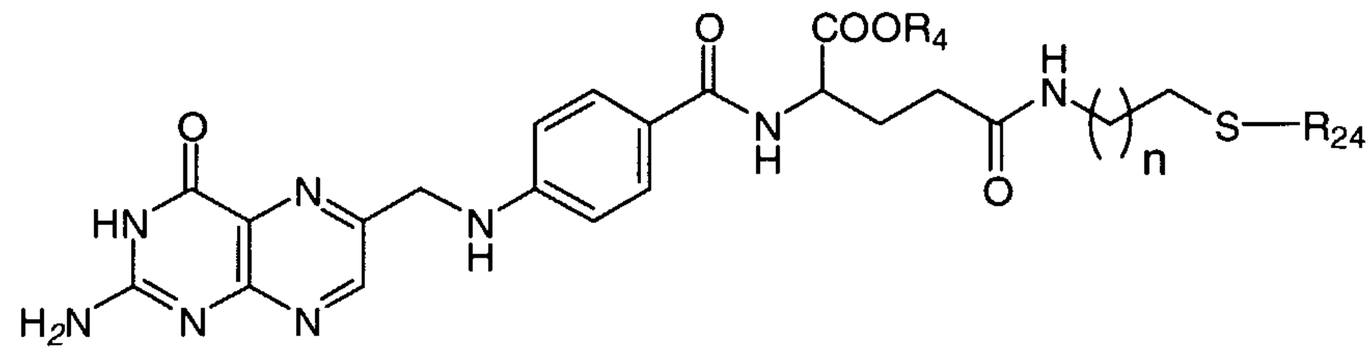
124. The compound of claim 123, wherein said alkylhydroxyl is $-(CH_2)_nOH$.

125. The compound of claim 124, wherein said n is an integer from 1-10.

126. The kit of claim 96, wherein said sample is from a cancer cell.

127. The kit of claim 96, wherein said sample is from a virus infected cell.

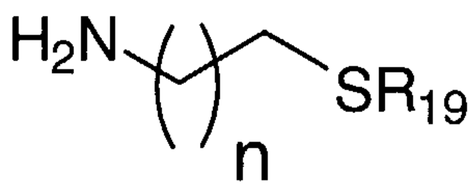
128. A compound having Formula 27:



wherein "n" is an integer from about 0 to about 20 and R_4 is H or a cationic salt.

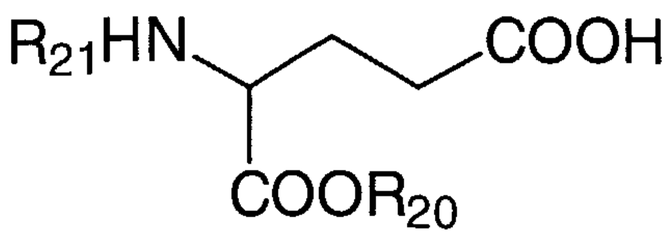
129. A method for synthesizing a compound having Formula 27 comprising:

(a) Selective tritylation of the thiol of cysteamine under conditions suitable to yield a compound having Formula 28:

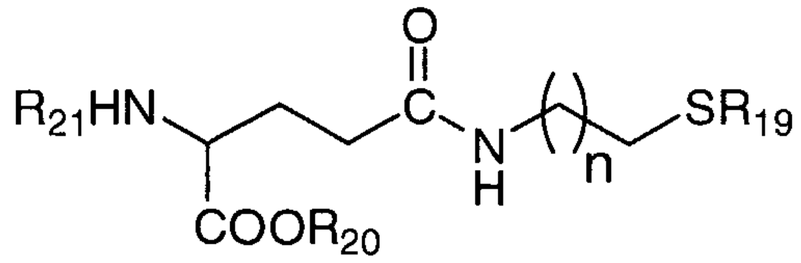


wherein "n" is an integer from about 0 to about 20 and R_{19} is a thiol protecting group;

(b) peptide coupling of the product of (a) with a compound having Formula 29:

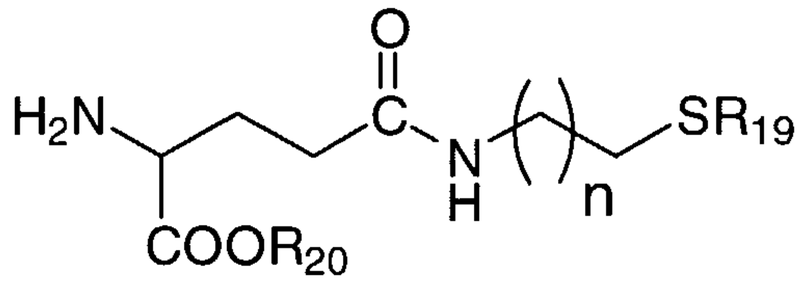


wherein R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group, under conditions suitable to yield a compound having Formula 30:



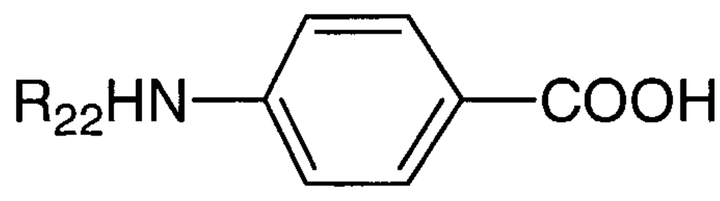
wherein "n" is an integer from about 0 to about 20, R_{19} is a thiol protecting group, R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group;

(c) removing the amino protecting group R_{21} of the product of (b) under conditions suitable to yield a compound having Formula 31:

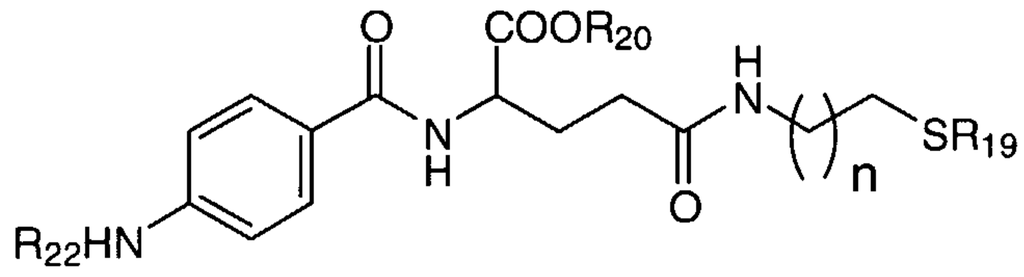


wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b);

(d) condensation of the product of (c) with a compound having Formula 32:

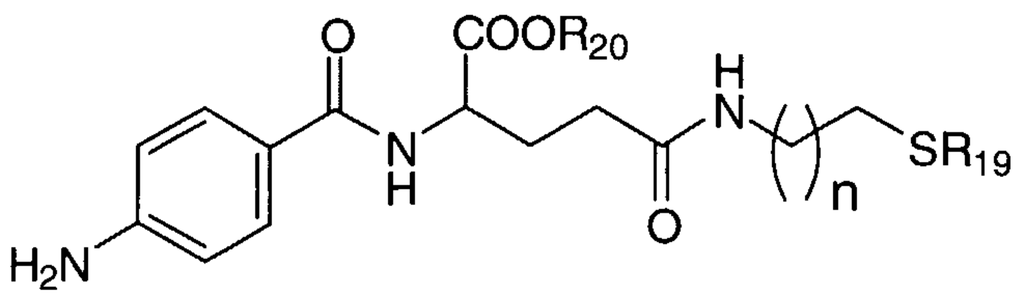


wherein R_{22} is an amino protecting group, under conditions suitable to yield a compound having Formula 33:



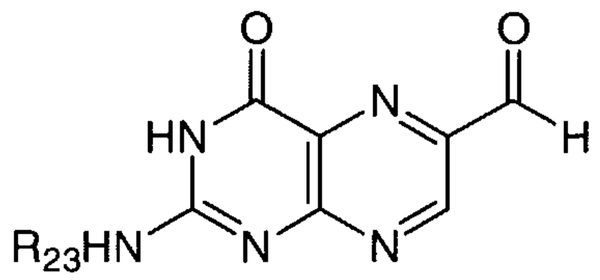
wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b) and R_{22} is as described in (d);

(e) selective cleave of R_{22} from the product of (d) under conditions suitable to yield a compound having Formula 34:

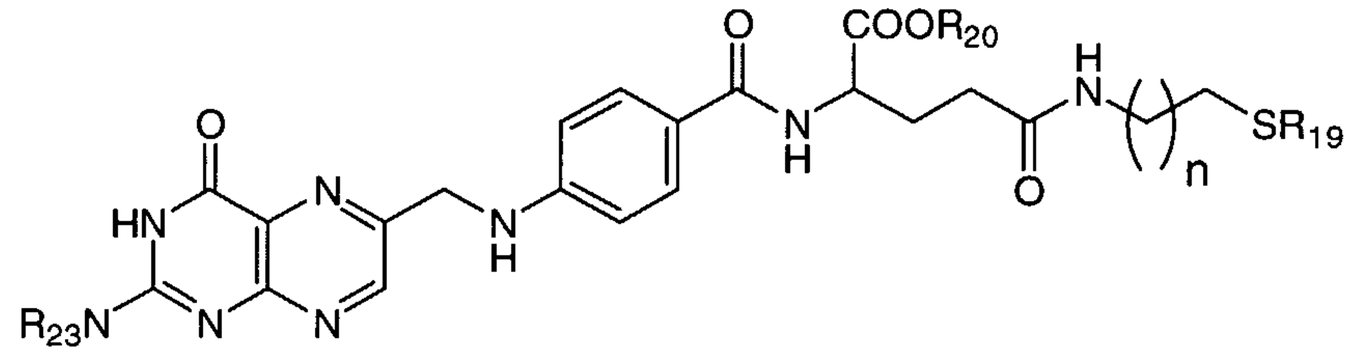


wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b);

(f) coupling the product of (e) with a compound having Formula 35:

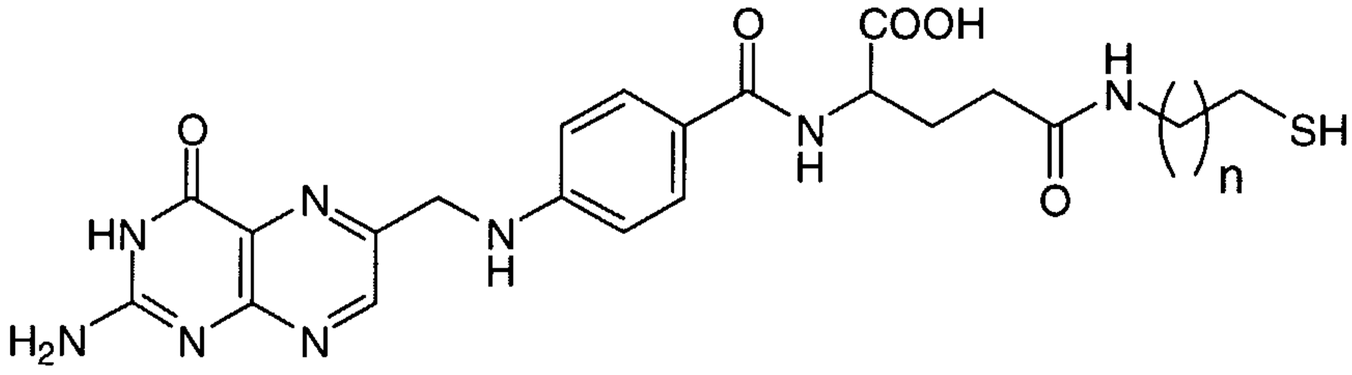


wherein R_{23} is an amino protecting group under conditions suitable to yield a compound having Formula 36:



wherein "n" is an integer from about 0 to about 20; and R_{23} is an amino protecting group, and R_{19} and R_{20} are as described in (b);

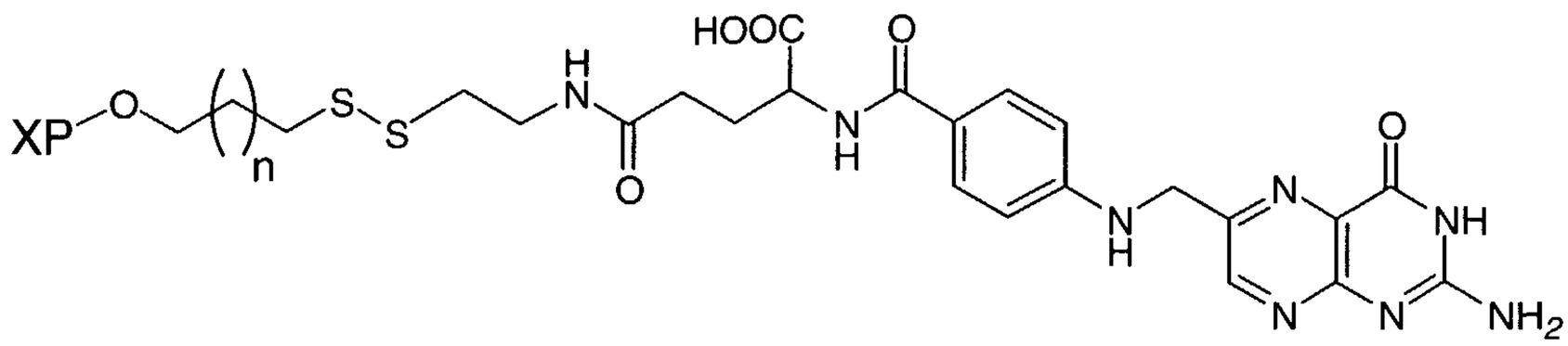
(g) deprotecting the product of (f) under conditions suitable to yield a compound having Formula 37.



wherein "n" is an integer from about 0 to about 20; and

(h) introducing a disulphide-based leaving group to the product of (g) under conditions suitable to yield a compound having Formula 27.

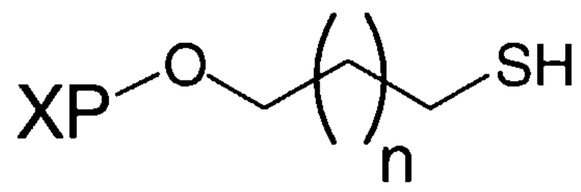
130. A compound having Formula 39:



wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group.

131. A method for synthesizing a compound having Formula 39, comprising:

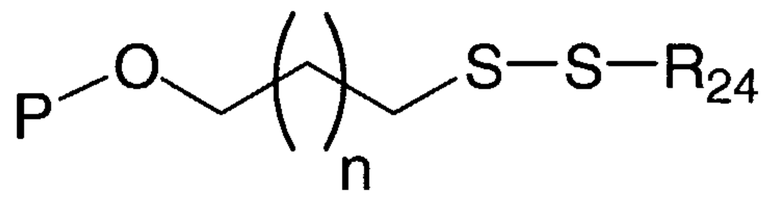
(a) Coupling a thiol containing linker to a nucleic acid, polynucleotide or oligonucleotide under conditions suitable to yield a compound having Formula 40:



wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group; and

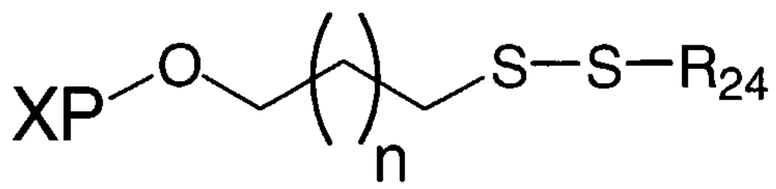
(b) coupling the product of (a) with a compound having Formula 27 under conditions suitable to yield a compound having Formula 39.

132. The method of claim 131, wherein said thiol containing linker is a compound having Formula 41:



wherein "n" is an integer from about 0 to about 20, P is a phosphorus containing group, and R24 is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

133. The method of claim 131, wherein said conditions suitable to yield a compound having Formula 40 comprises reduction of the disulfide bond of a compound having Formula 42:



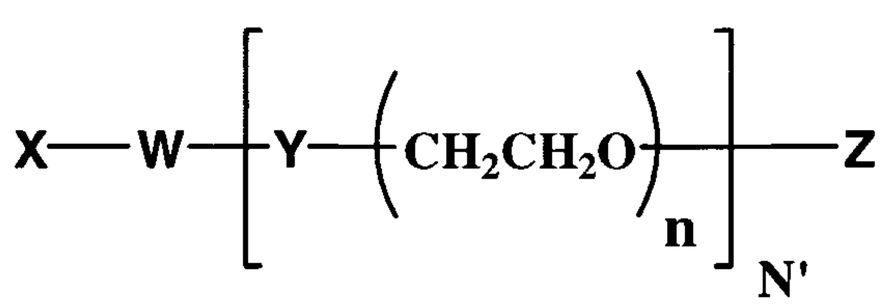
wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, P is a phosphorus containing group, and R_{24} is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

134. A pharmaceutical composition comprising the compound of claim 128 in a pharmaceutically acceptable carrier.

135. A pharmaceutical composition comprising the compound of claim 130 in a pharmaceutically acceptable carrier.

136. A method of treating a cancer patient, comprising contacting cells of said patient with the pharmaceutical composition of any claim 134 or claim 135, under conditions suitable for said treatment.

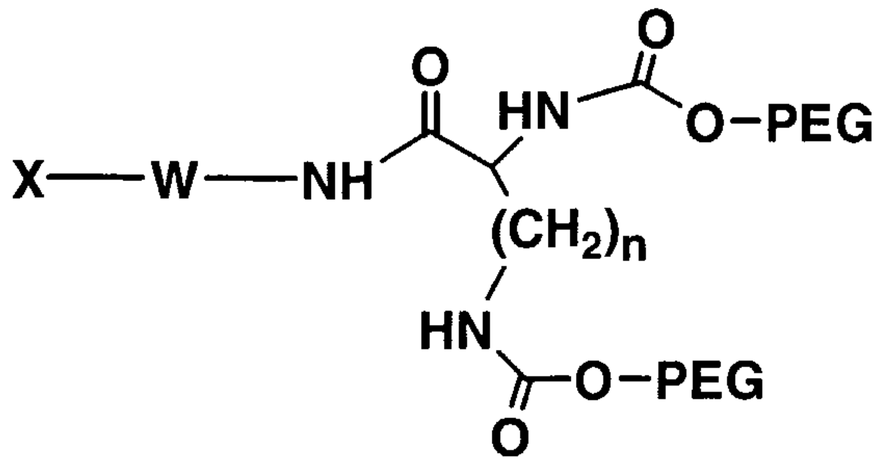
137. A compound having Formula 43:



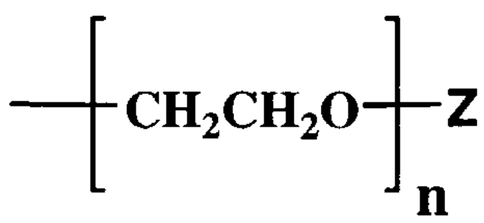
43

wherein X comprises a biologically active molecule; W comprises a degradable nucleic acid linker; Y comprises a linker molecule or amino acid that can be present or absent; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; n is an integer from about 1 to about 100; and N' is an integer from about 1 to about 20.

138. A compound having Formula 44:



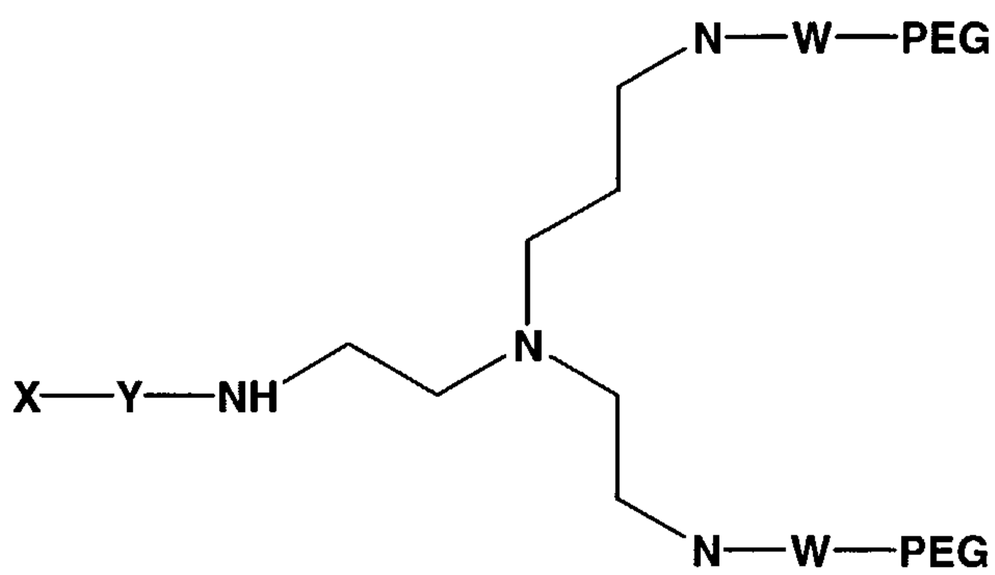
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; n is an integer from about 1 to about 50, and PEG represents a compound having Formula 45:



45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

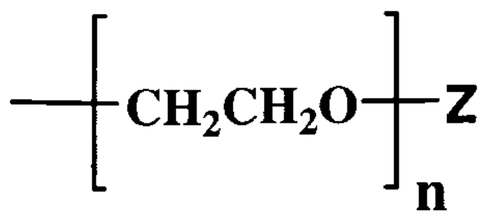
139. A compound having Formula 46:



46

wherein X comprises a biologically active molecule; each W independently comprises linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule or chemical linkage that

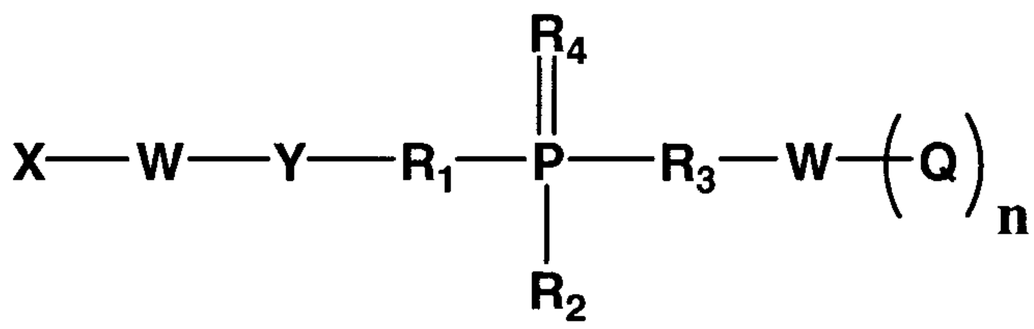
can be present or absent; and PEG represents a compound having Formula 45:



45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

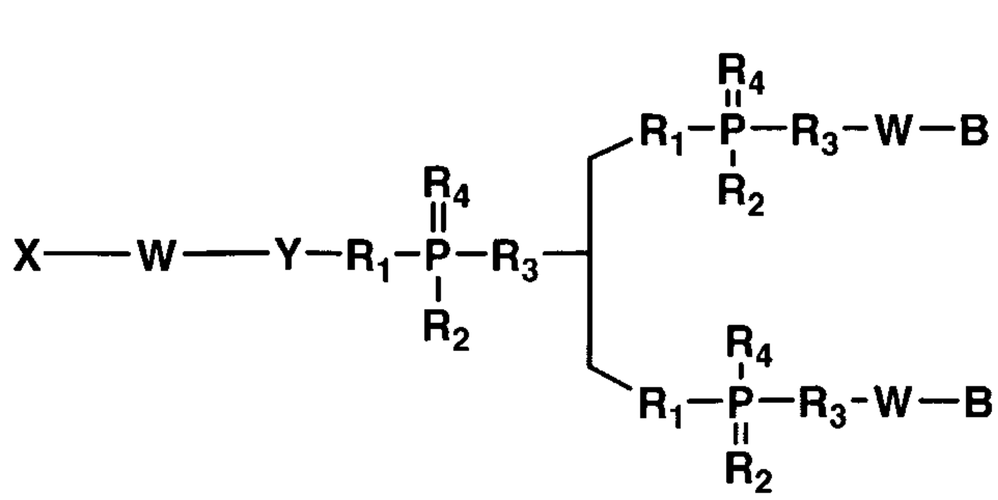
140. A compound having Formula 47:



47

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be the same or different and can be present or absent, Y comprises a linker molecule that can be present or absent; each Q independently comprises a hydrophobic group or phospholipid; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and n is an integer from about 1 to about 10.

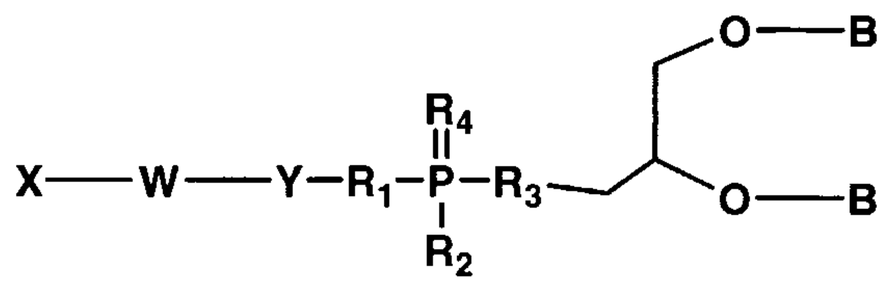
141. A compound having Formula 48:



48

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and B represents a lipophilic group.

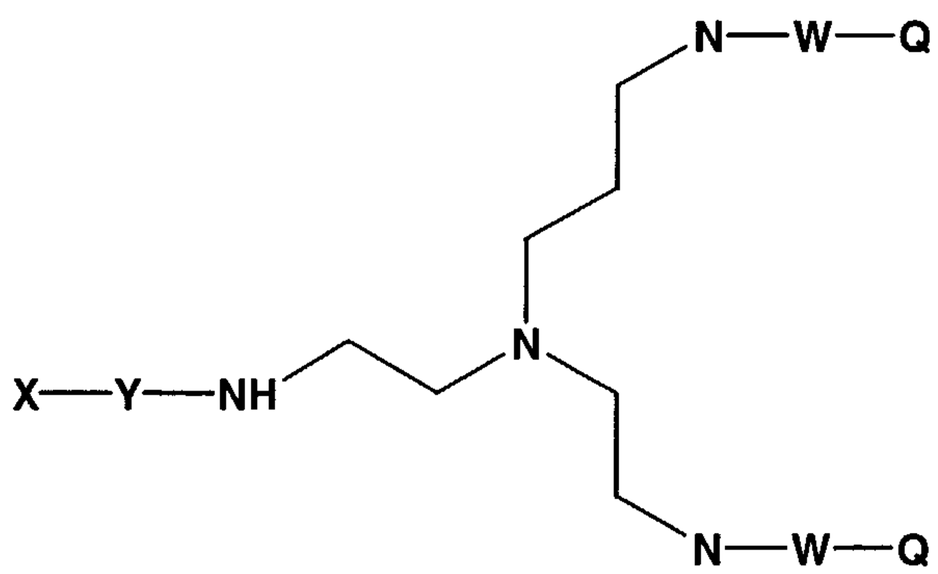
142. A compound having Formula 49:



49

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and B represents a lipophilic group.

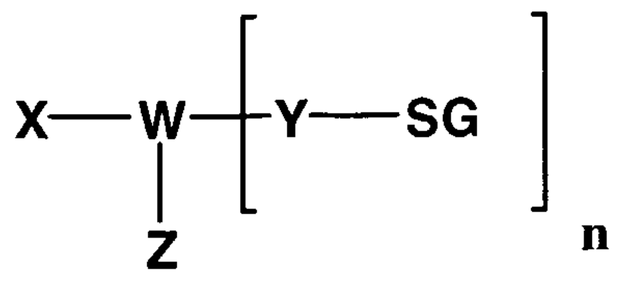
143. A compound having Formula 50:



50

wherein X comprises a biologically active molecule; W comprises linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule or chemical linkage that can be present or absent; and each Q independently comprises a hydrophobic group or phospholipid.

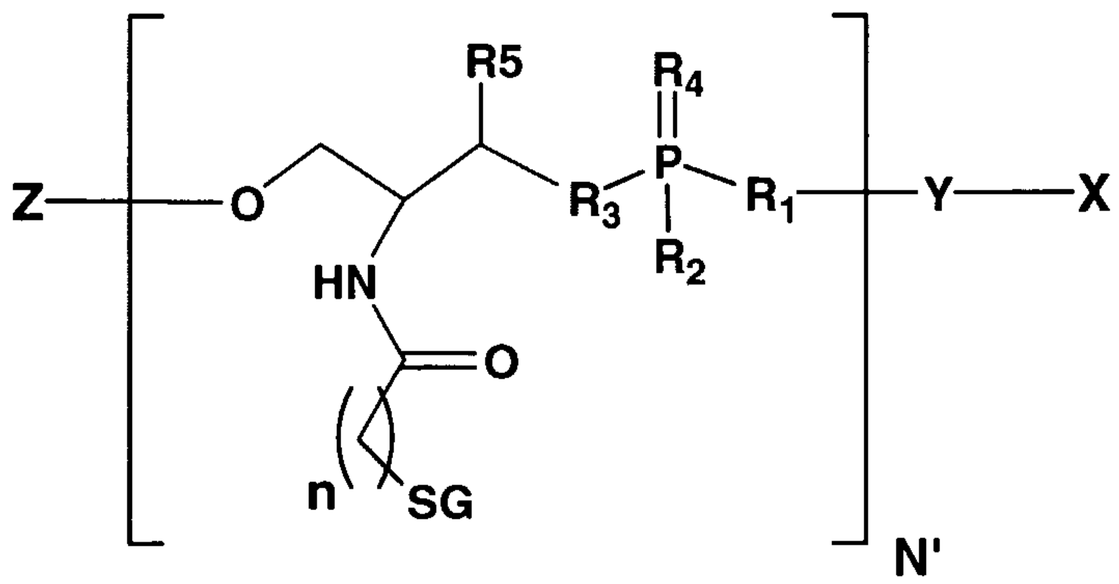
144. A compound having Formula 51:



51

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; Y comprises a linker molecule or amino acid that can be present or absent; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar and n is an integer from about 1 to about 20.

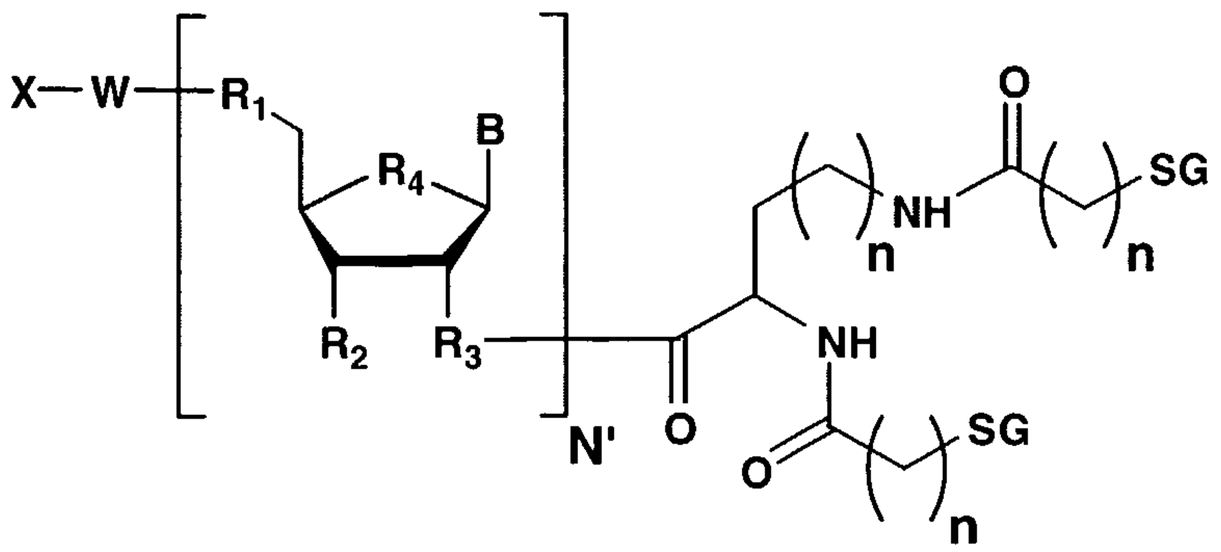
145. A compound having Formula 52:



52

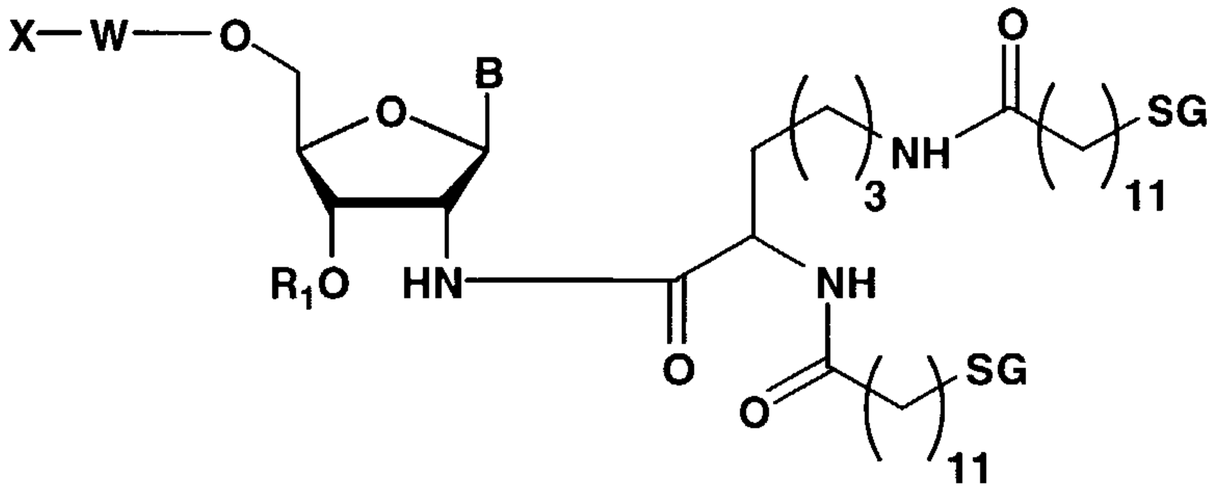
wherein X comprises a biologically active molecule; Y comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, R3, R4, and R5 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar, n is an integer from about 1 to about 20; and N' is an integer from about 1 to about 20.

146. A compound having Formula 53:



wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises N or O-N, each R4 independently comprises O, CH2, S, sulfone, or sulfoxy; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 10.

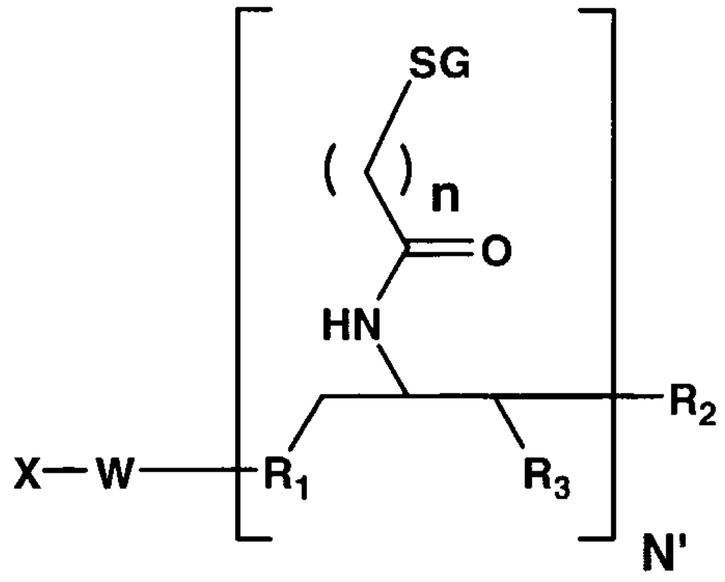
147. A compound having Formula 54:



wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide,

enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; and SG comprises a sugar.

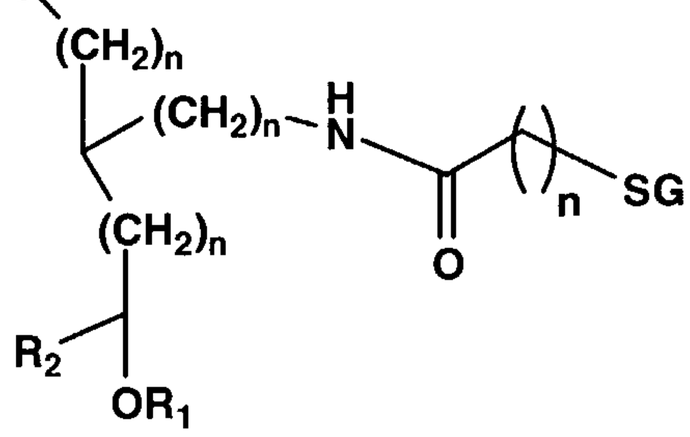
148. A compound having Formula 55:



55

wherein each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100.

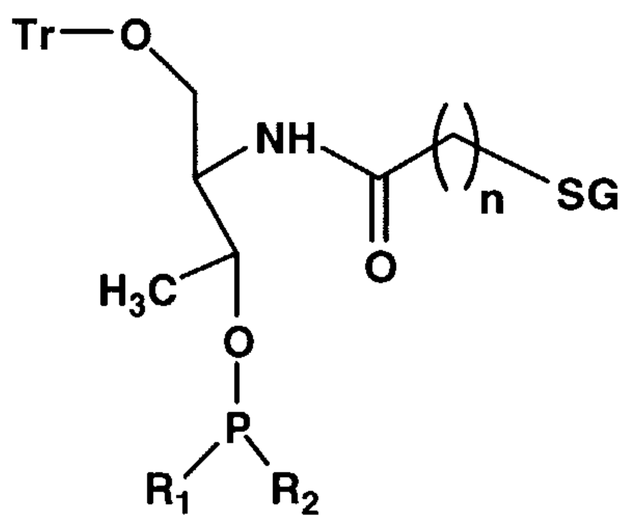
149. A compound having Formula 56:



56

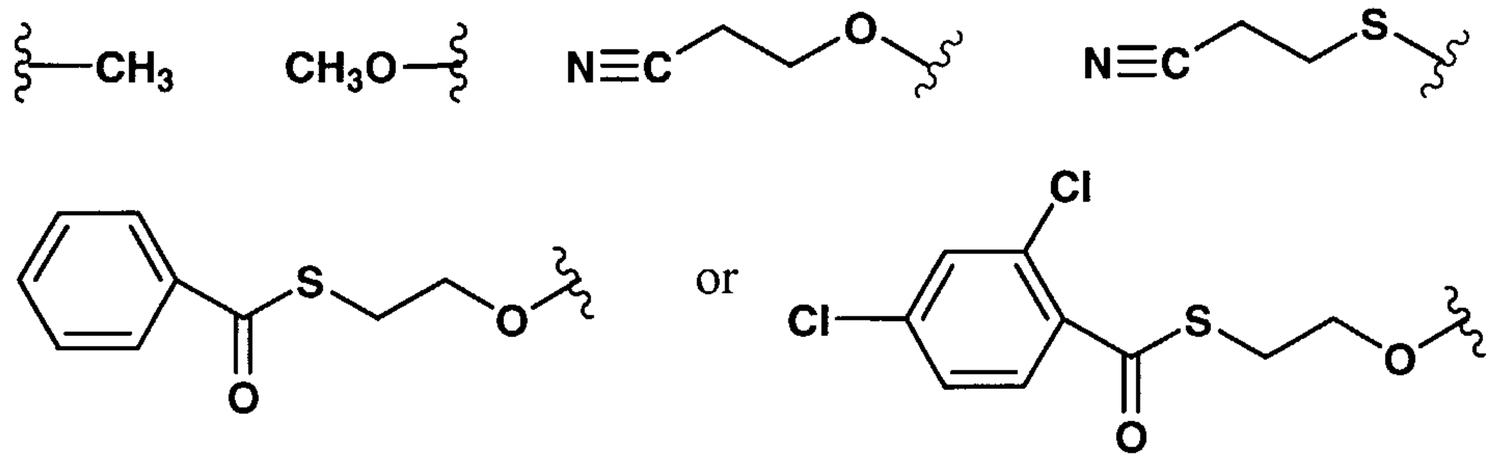
wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, and each n is independently an integer from about 0 to about 20.

150. A compound having Formula 57:

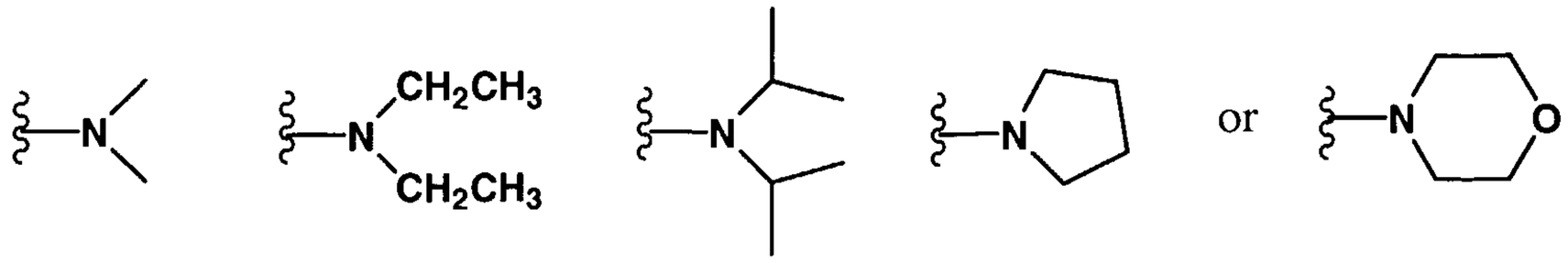


57

wherein R1 can include the groups:

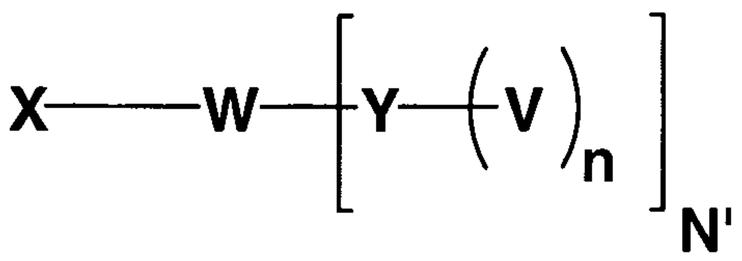


and wherein R2 can include the groups:



and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; SG comprises a sugar, and n is an integer from about 1 to about 20.

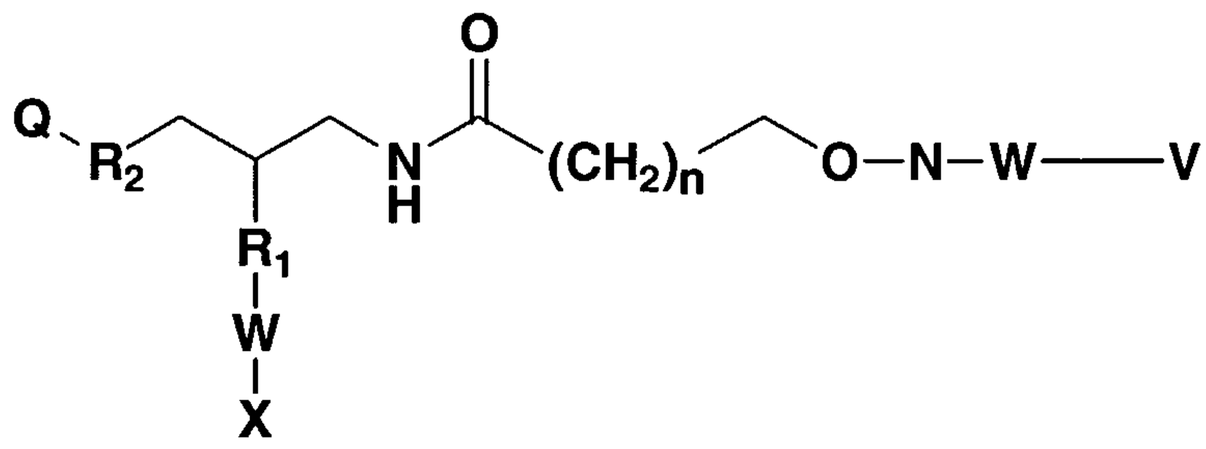
151. A compound having Formula 58:



58

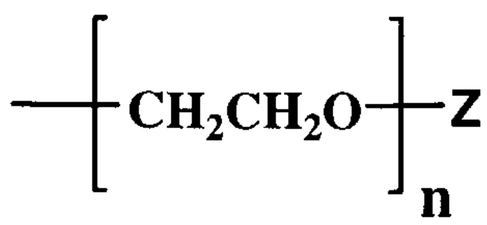
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; Y comprises a linker molecule or amino acid that can be present or absent; V comprises a protein or peptide; each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100.

152. A compound having Formula 59:



59

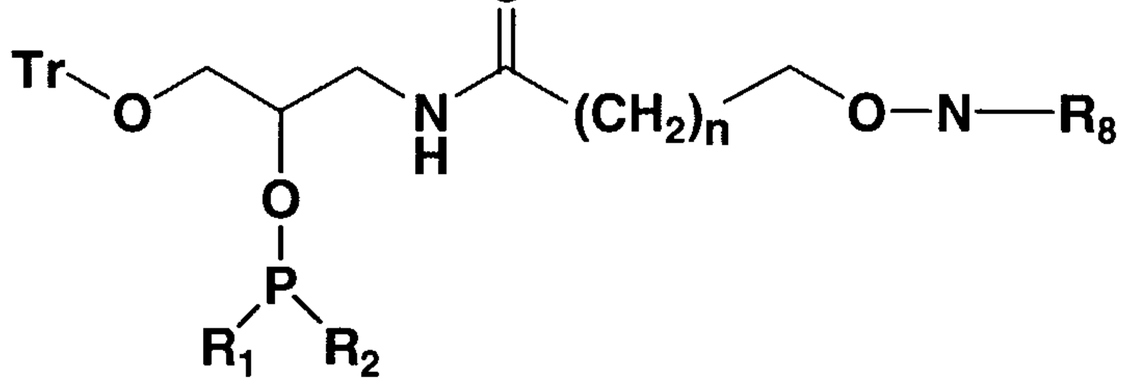
wherein each R1 independently comprises O, S, N, substituted N, or a phosphorus containing group; each R2 independently comprises O, S, or N; X comprises H, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, or enzymatic nucleic acid or other biologically active molecule; n is an integer from about 1 to about 50, Q comprises H or a removable protecting group which can be optionally absent, each W independently comprises a linker molecule or chemical linkage that can be present or absent, and V comprises a protein or peptide or a compound having Formula 45



45

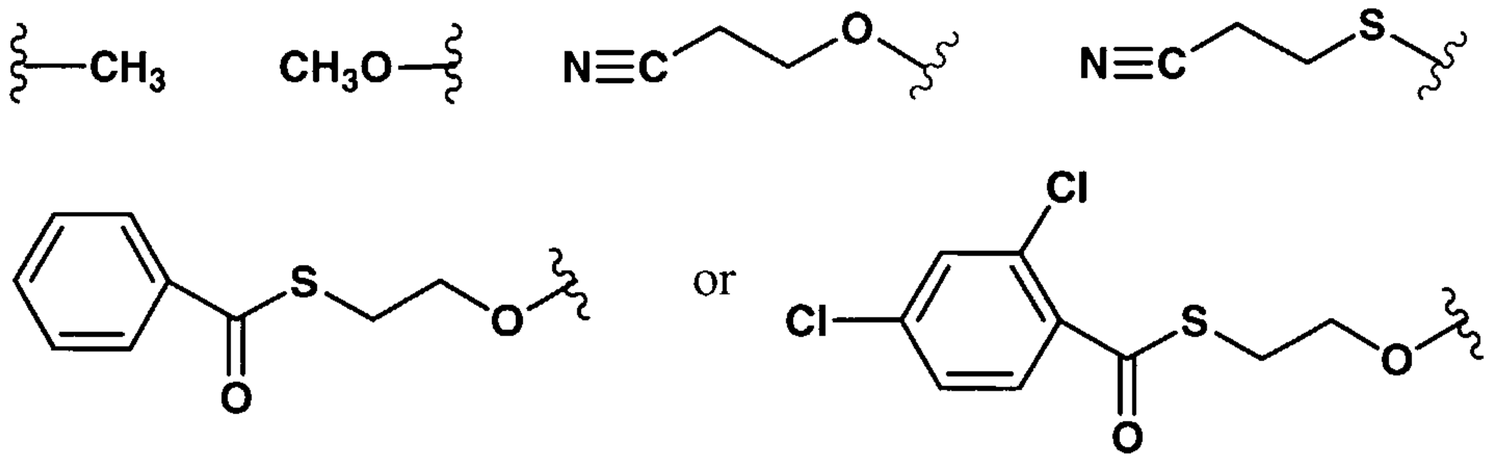
wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100.

153. A compound having Formula 60:

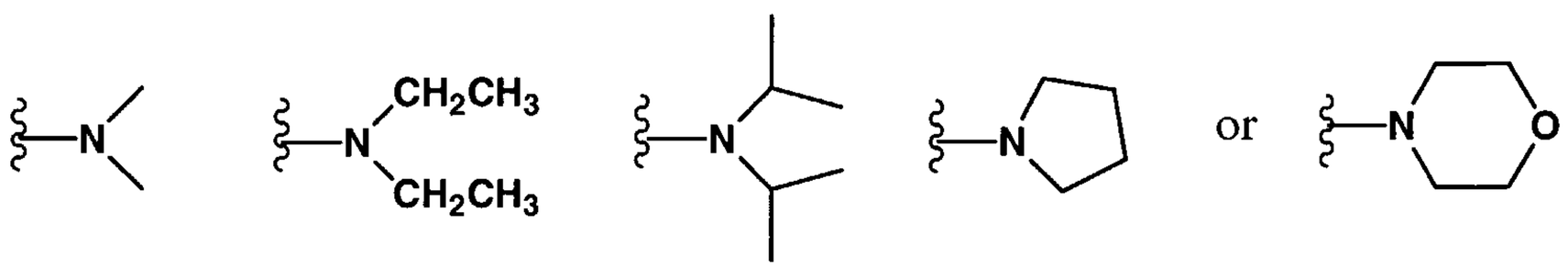


60

wherein R1 can include the groups:

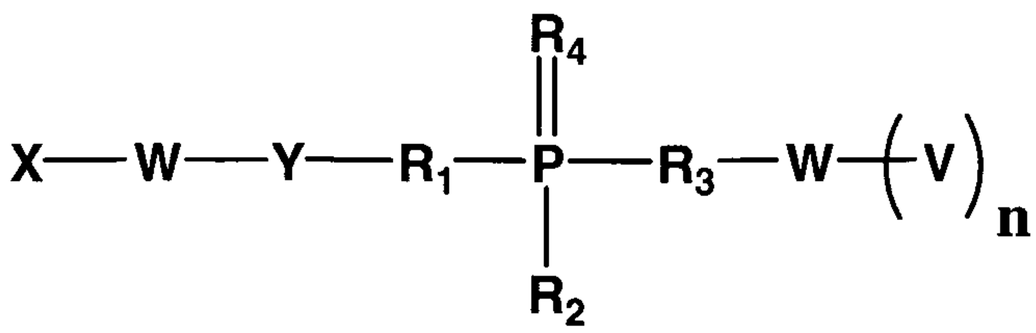


and wherein R2 can include the groups:



and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; n is an integer from about 1 to about 50; and R8 is a nitrogen protecting group.

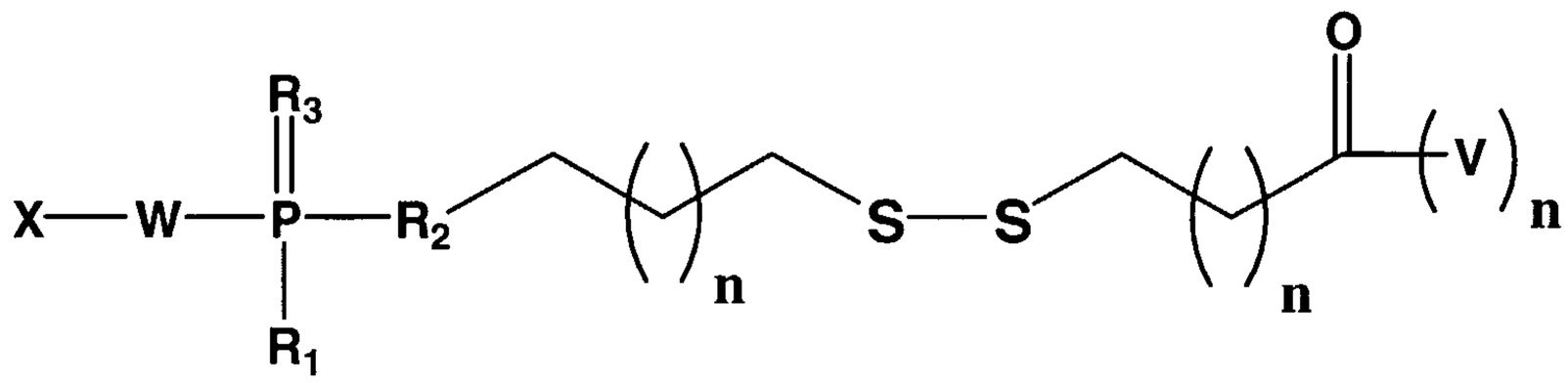
154. A compound having Formula 61:



61

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be the same or different and can be present or absent, Y comprises a linker molecule that can be present or absent; each 5 independently comprises a protein or peptide; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and n is an integer from about 1 to about 10.

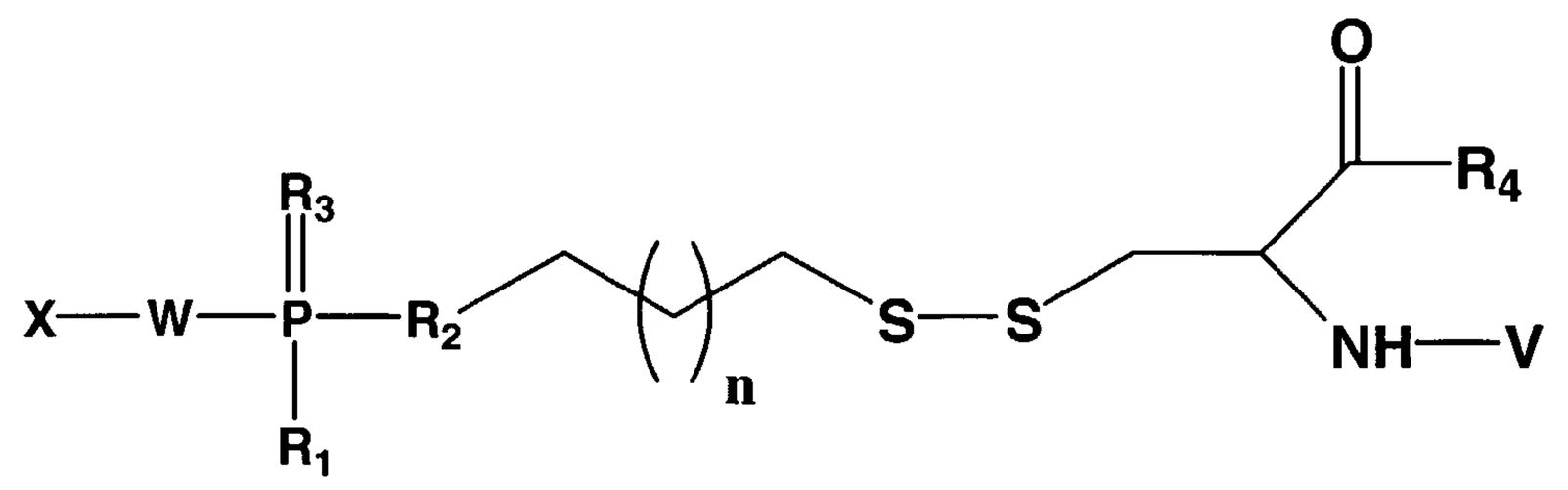
155. A compound having Formula 62:



62

wherein X comprises a biologically active molecule; each 5 independently comprises a protein or peptide; W comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, and R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, and each n is independently an integer from about 1 to about 10.

156. A compound having Formula 63:

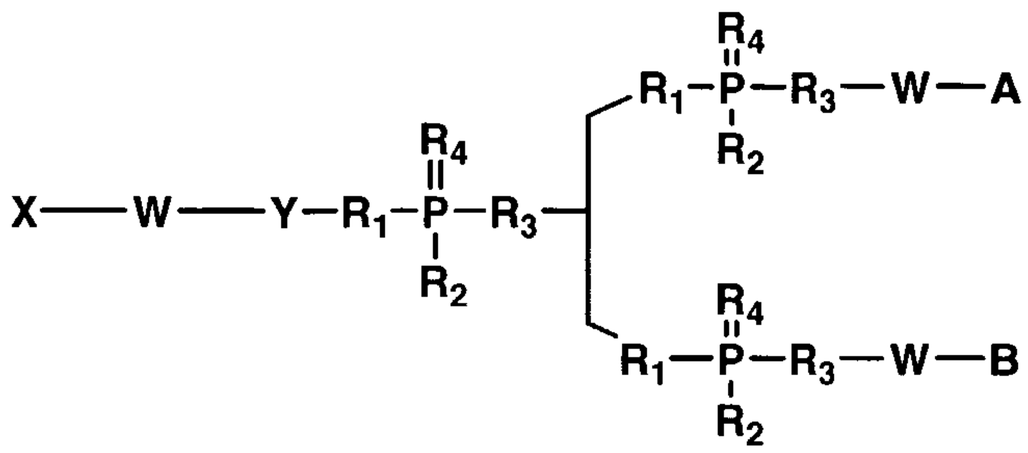


63

wherein X comprises a biologically active molecule; V comprises a protein or peptide; W comprises a linker molecule or chemical linkage that can be

present or absent; each R1, R2, R3 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, R4 represents an ester, amide, or protecting group, and each n is independently an integer from about 1 to about 10.

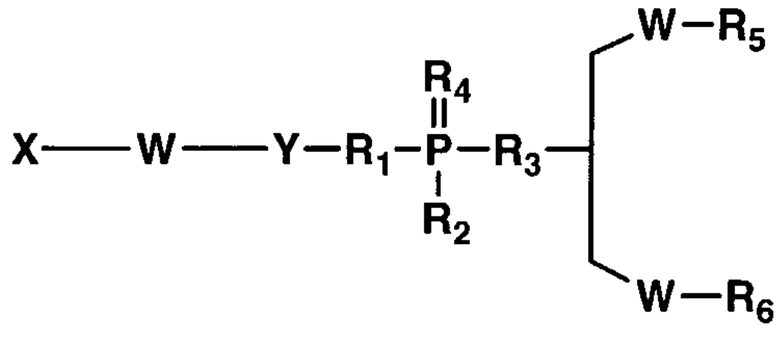
157. A compound having Formula 64:



64

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, A comprises a nitrogen containing group, and B comprises a lipophilic group.

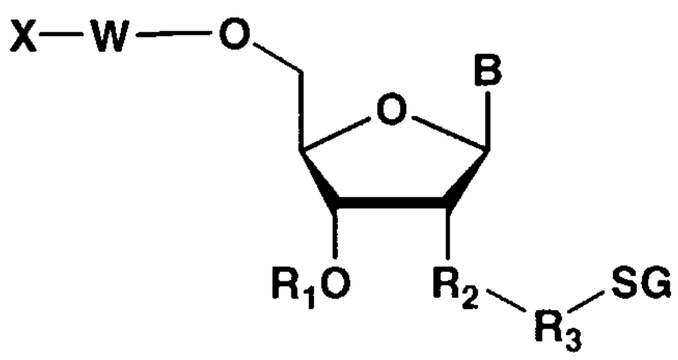
158. A compound having Formula 65:



65

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, RV comprises the lipid or phospholipid component of any of Formulae 47-50, and R6 comprises a nitrogen containing group.

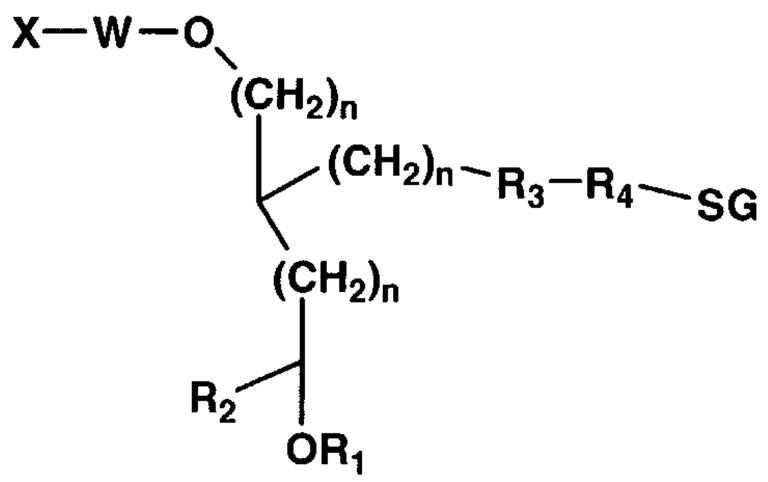
159. In another embodiment, the invention features a compound having Formula 92:



92

wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; R2 comprises O, NH, S, CO, COO, ON=C, or alkyl; R3 comprises alkyl, akloxy, or an aminoacyl side chain; and SG comprises a sugar.

160. A compound having Formula 66:

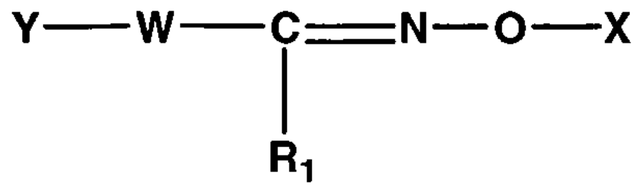


66

wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; R3 comprises O, NH, S, CO, COO, ON=C, or alkyl; R4

comprises alkyl, akloxy, or an aminoacyl side chain; and SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and each n is independently an integer from about 0 to about 20.

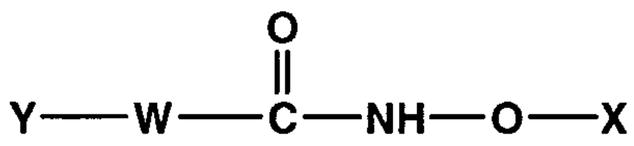
161. A compound having Formula 87:



87

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; W comprises a linker molecule or chemical linkage that can be present or absent; and Y comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody; R1 comprises H, alkyl, or substituted alkyl.

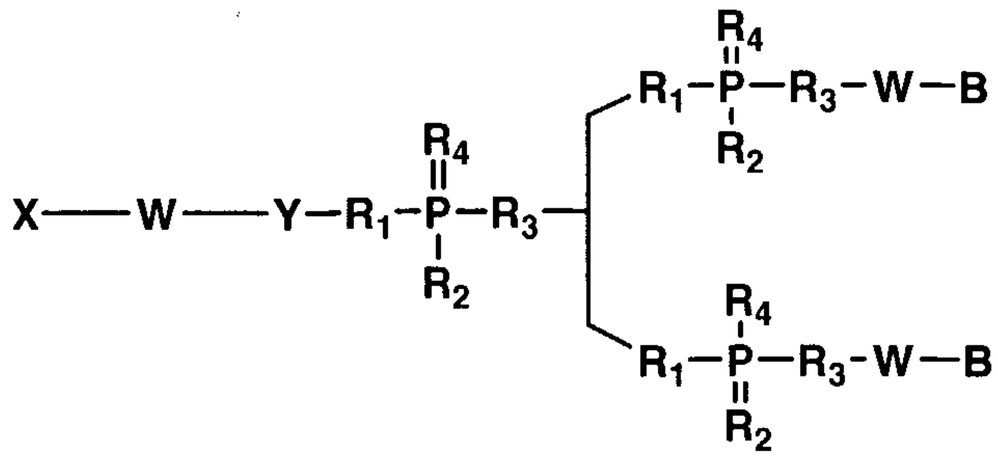
162. A compound having Formula 88:



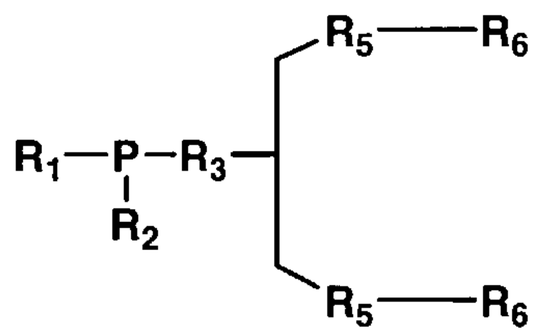
88

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a biologically active molecule.

163. A method for the synthesis of a compound having Formula 48:

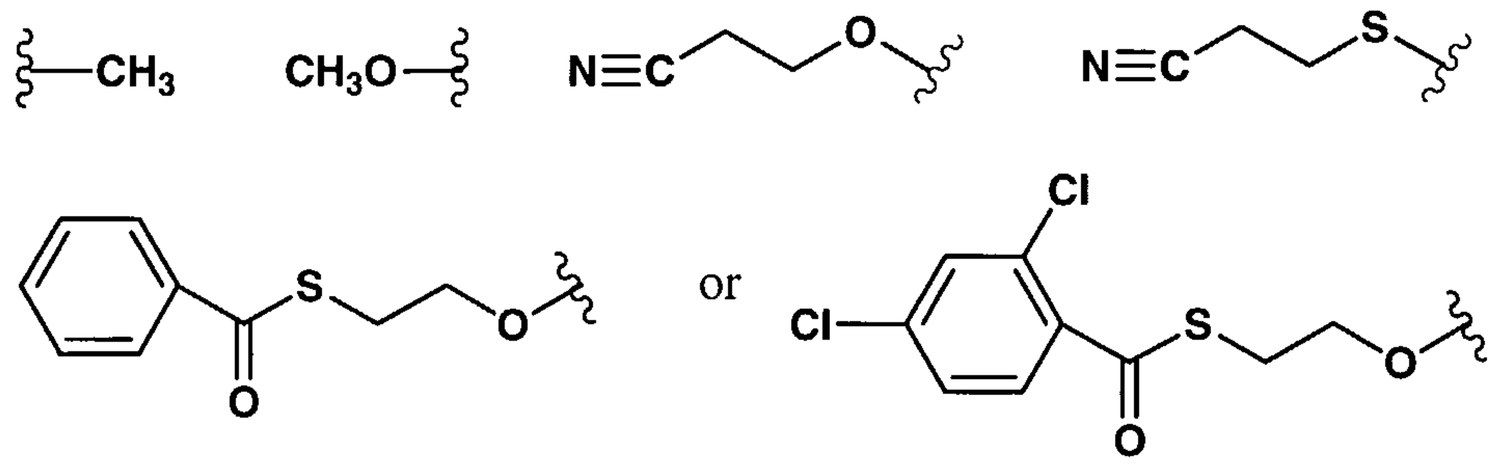


wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; and each B independently represents a lipophilic group, comprising: (a) introducing a compound having Formula 66:

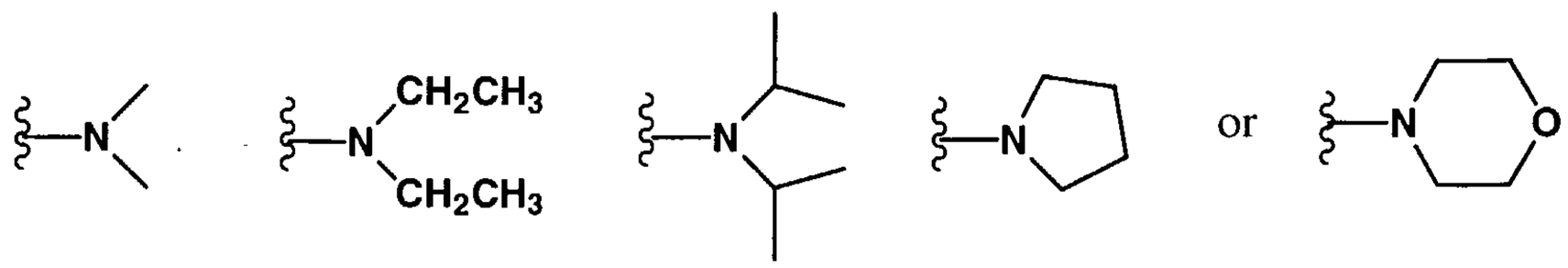


66

wherein R1 is defined as in Formula 48 and can include the groups:



and wherein R2 is defined as in Formula 48 and can include the groups:

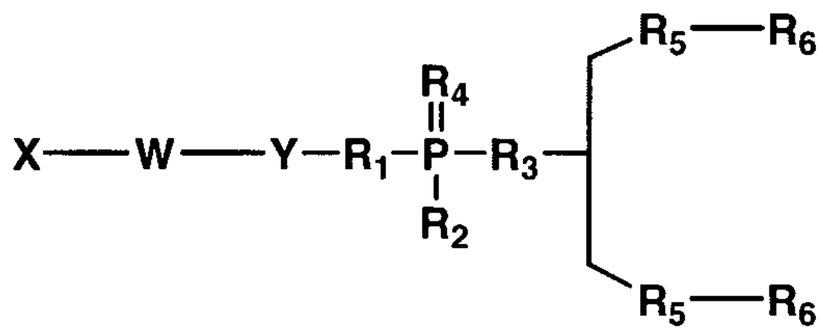


and wherein each R5 independently comprises O, N, or S and each R6 independently comprises a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group, to a compound having Formula 67:

X——W——Y

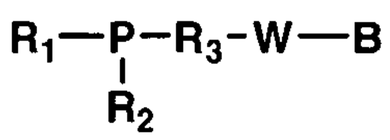
67

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 68:



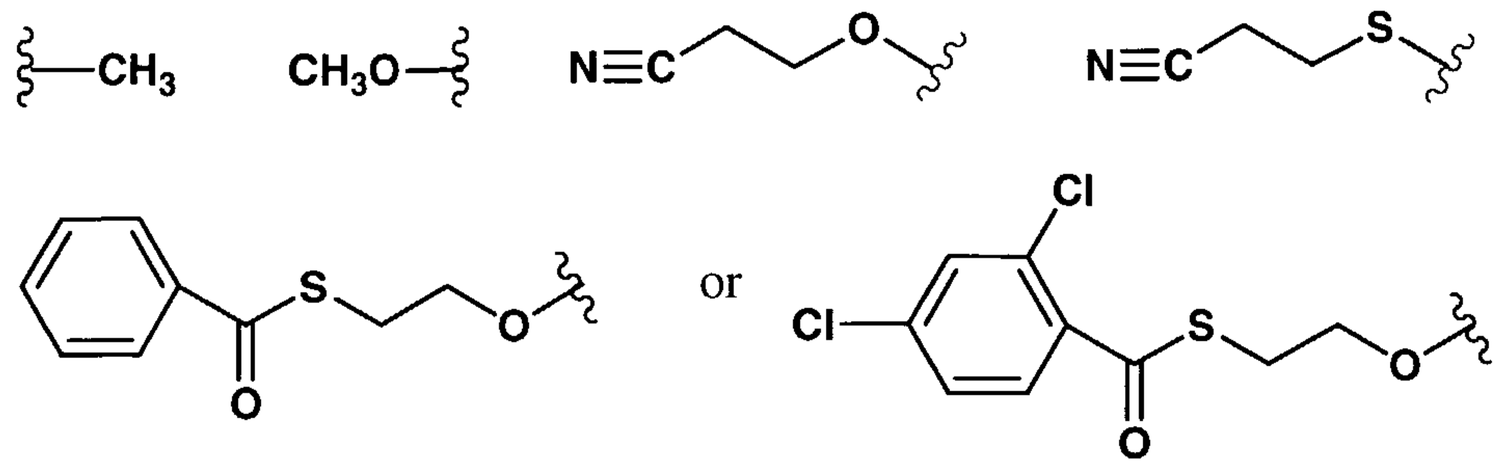
68

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; and each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N comprising, each R5 independently comprises O, S, or N; and each R6 is independently a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group; (b) removing R6 from the compound having Formula 26 and (c) introducing a compound having Formula 69:

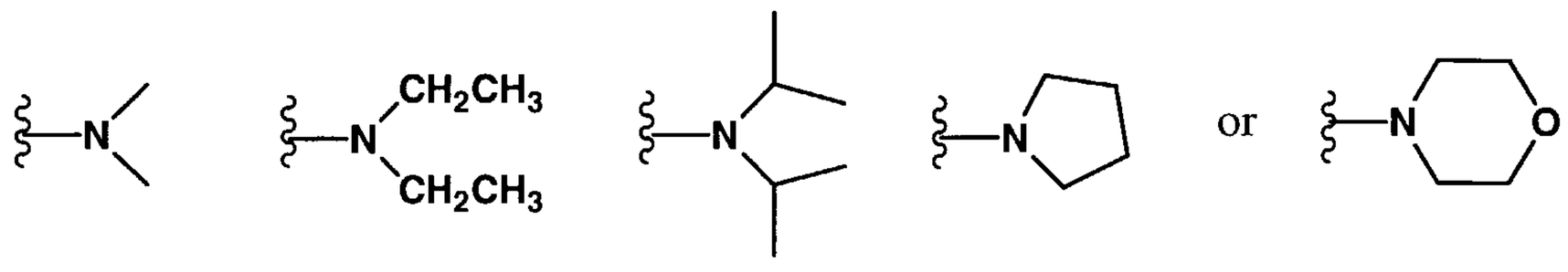


69

wherein R1 is defined as in Formula 48 and can include the groups:

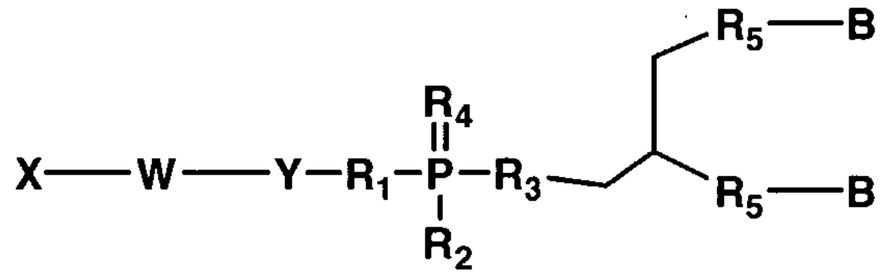


and wherein R2 is defined as in Formula 48 and can include the groups:



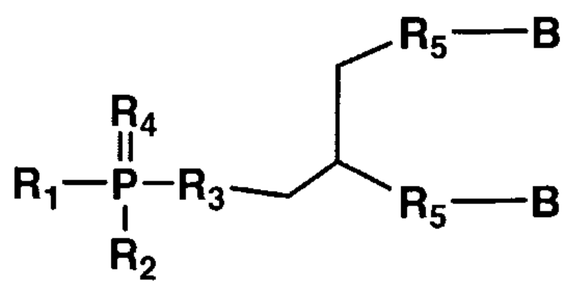
and wherein W and B are defined as in Formula 48, to the compound having Formula 68 under conditions suitable for the formation of a compound having Formula 48.

164. A method for the synthesis of a compound having Formula 49:



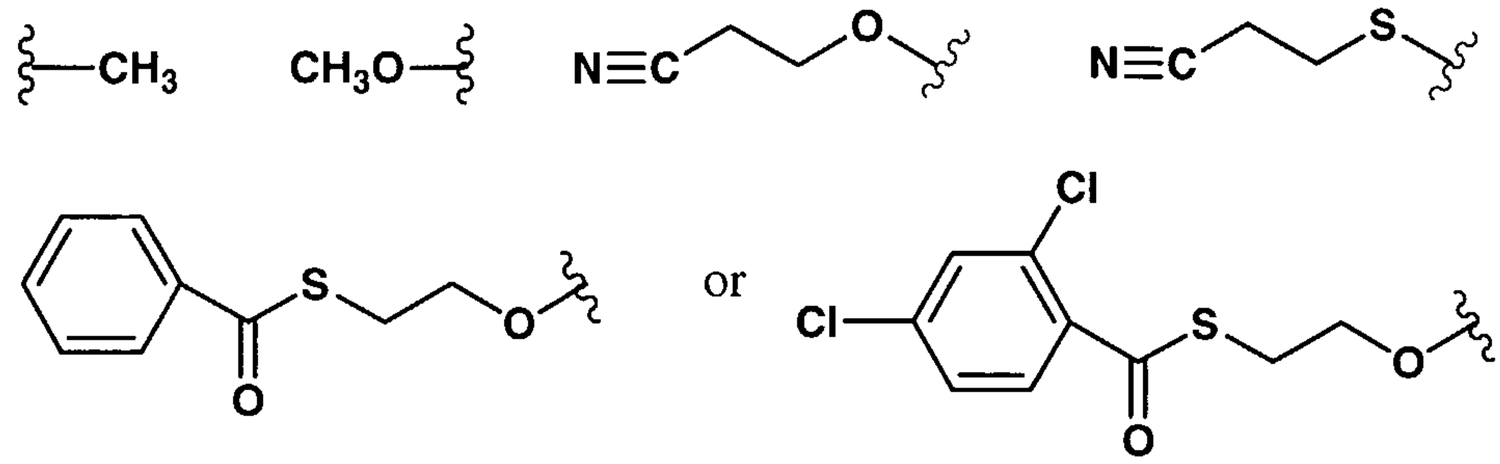
49

wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; each R5 independently comprises O, S, or N; and each B independently comprises a lipophilic group, comprising: (a) coupling a compound having Formula 70:

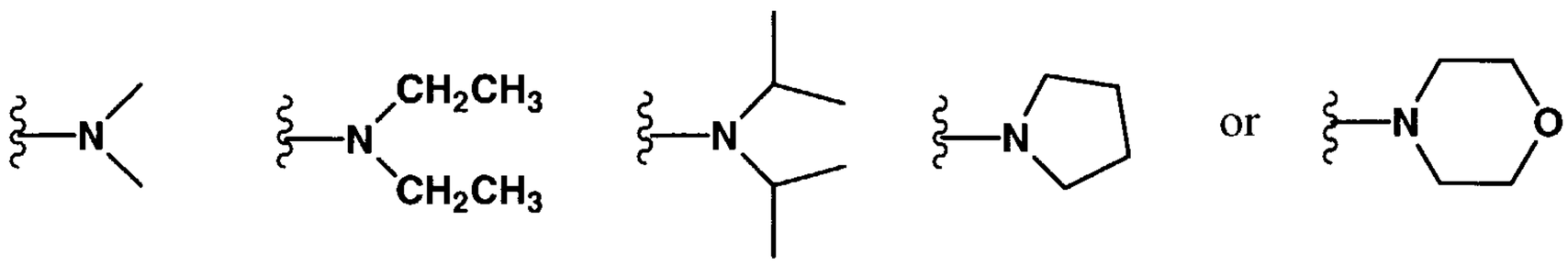


70

wherein R1 is defined as in Formula 49 and can include the groups:



and wherein R2 is defined as in Formula 49 and can include the groups:



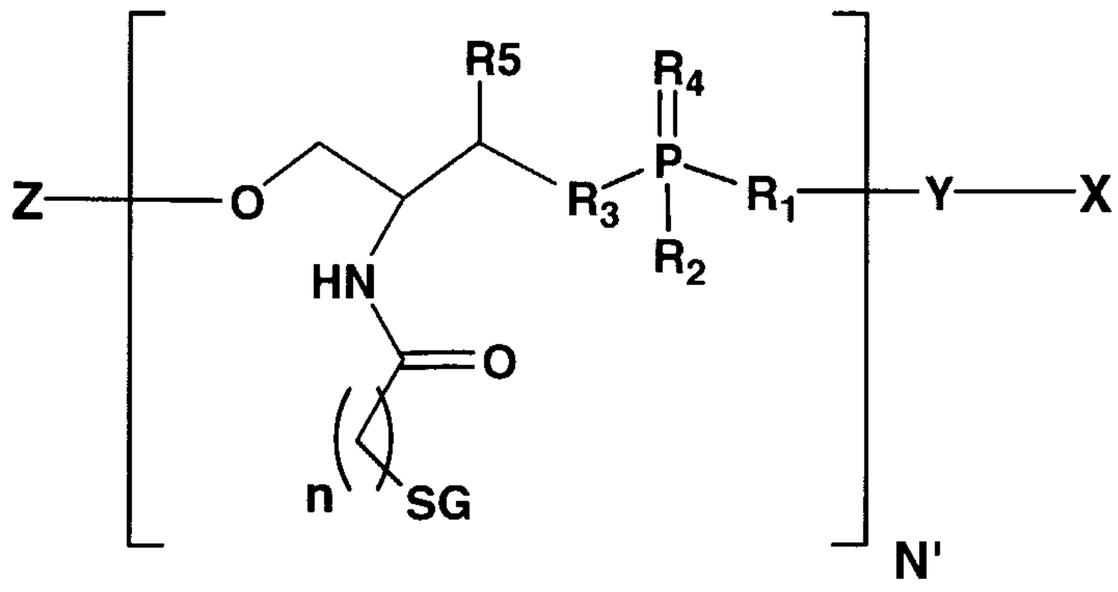
and wherein each R5 independently comprises O, S, or N, and wherein each B independently comprises a lipophilic group, with a compound having Formula 67:



67

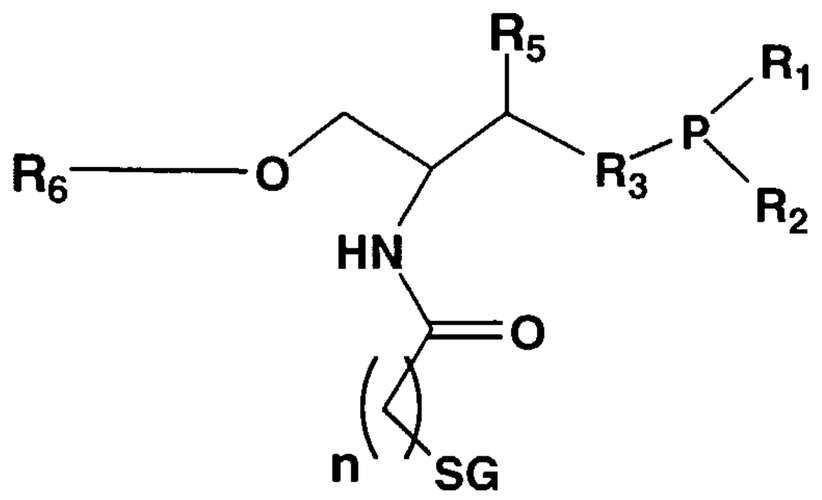
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 49.

165. A method for the synthesis of a compound having Formula 52:



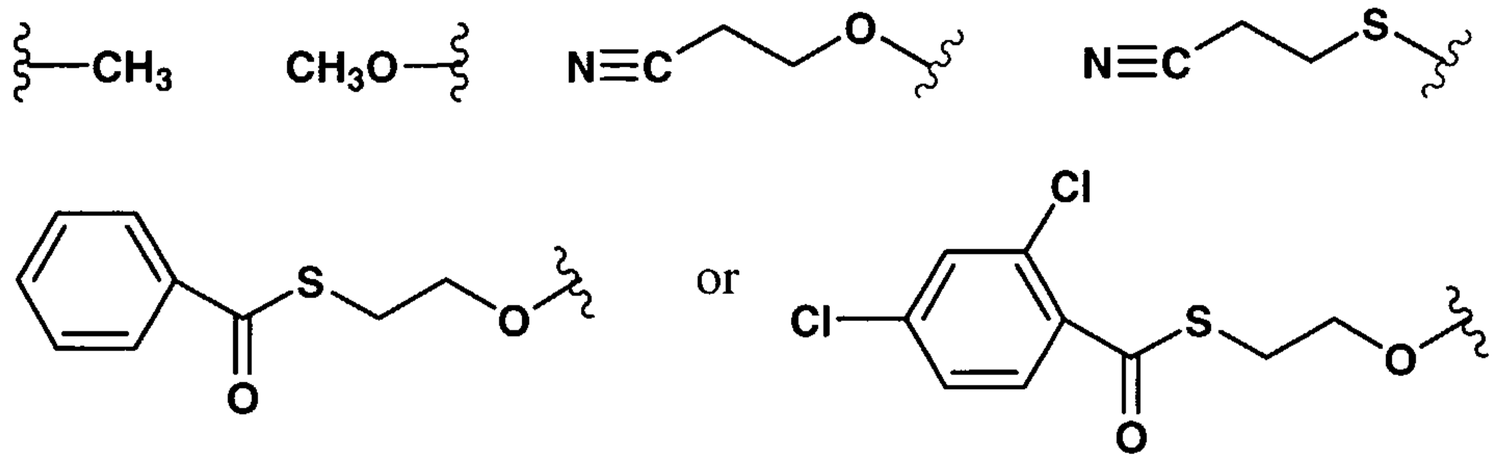
52

wherein X comprises a biologically active molecule; Y comprises a linker molecule or chemical linkage that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N; Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; SG comprises a sugar, n is an integer from about 1 to about 20; and N' is an integer from about 1 to about 20, comprising: (a) coupling a compound having Formula 71:

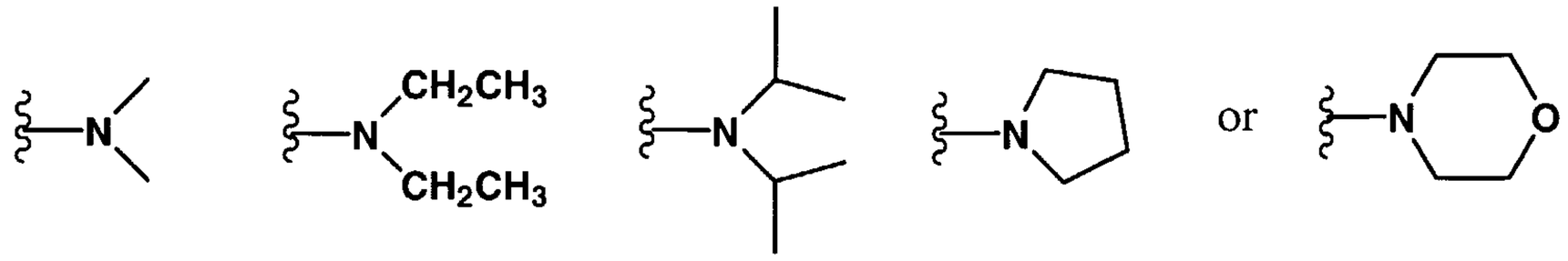


71

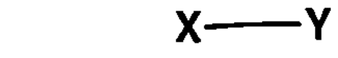
wherein R1, R2, R3, R5, SG, and n as is defined in Formula 10, and wherein R1 can include the groups:



and wherein R2 can include the groups:

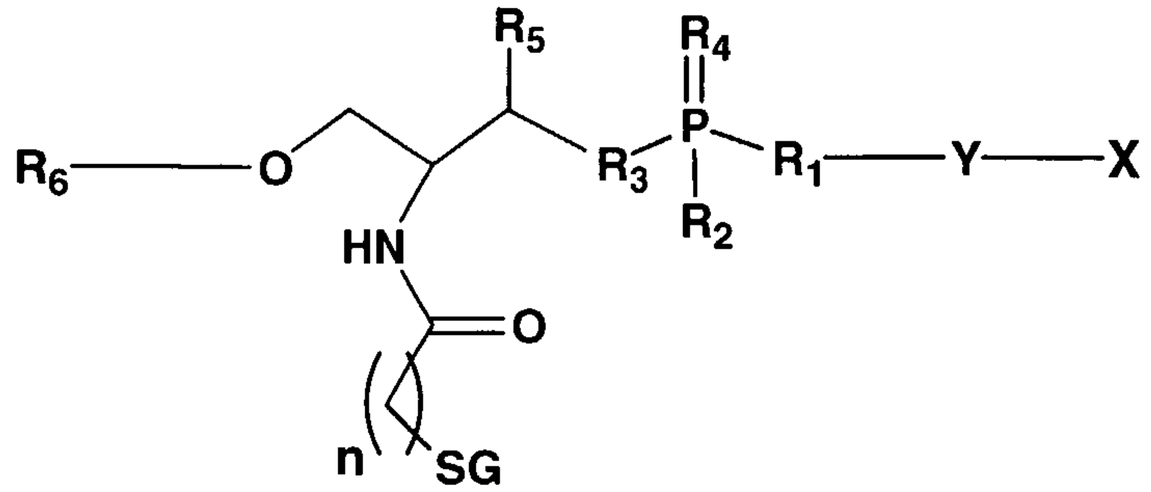


and R6 comprises a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl group; with a compound having Formula 72:



72

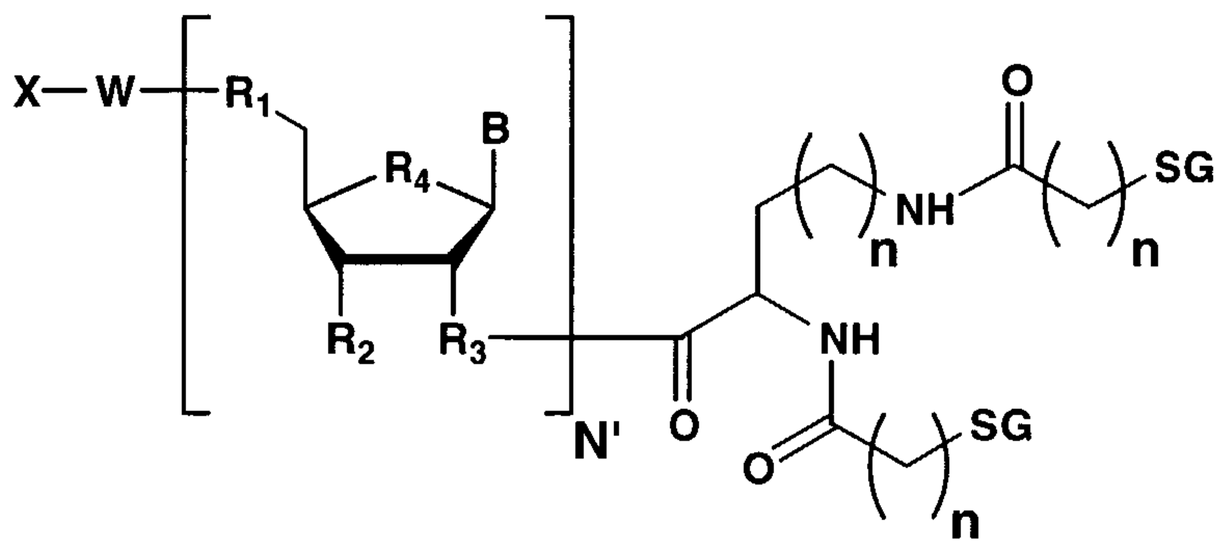
wherein X comprises a biologically active molecule and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 95:



95

(b) removing R6 from the compound having Formula 95 and (c) optionally coupling a nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label, or optionally; coupling a compound having Formula 71 under and optionally repeating (b) and (c) under conditions suitable for the formation of a compound having Formula 52.

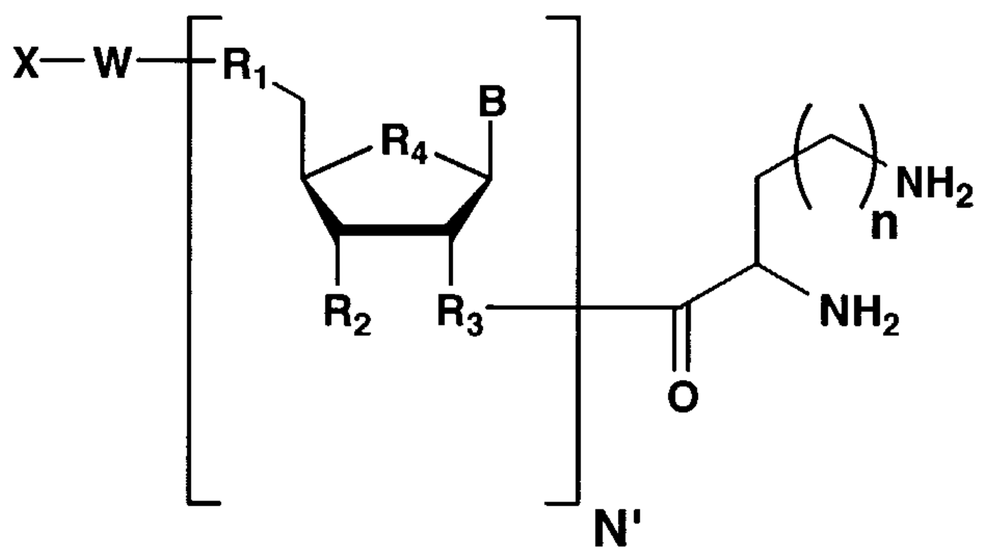
166. A method for synthesizing a compound having Formula 53:



53

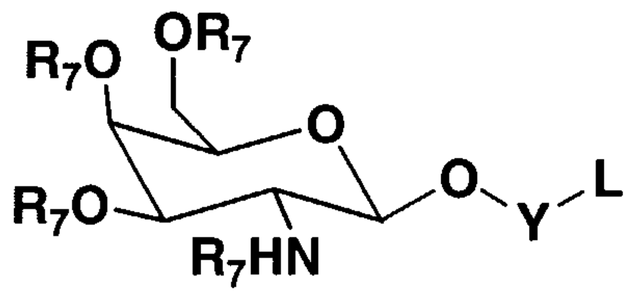
wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus

containing group; each R3 independently comprises N or O-N, each R4 independently comprises O, CH2, S, sulfone, or sulfoxy; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 10, comprising: coupling a compound having Formula 73:



73

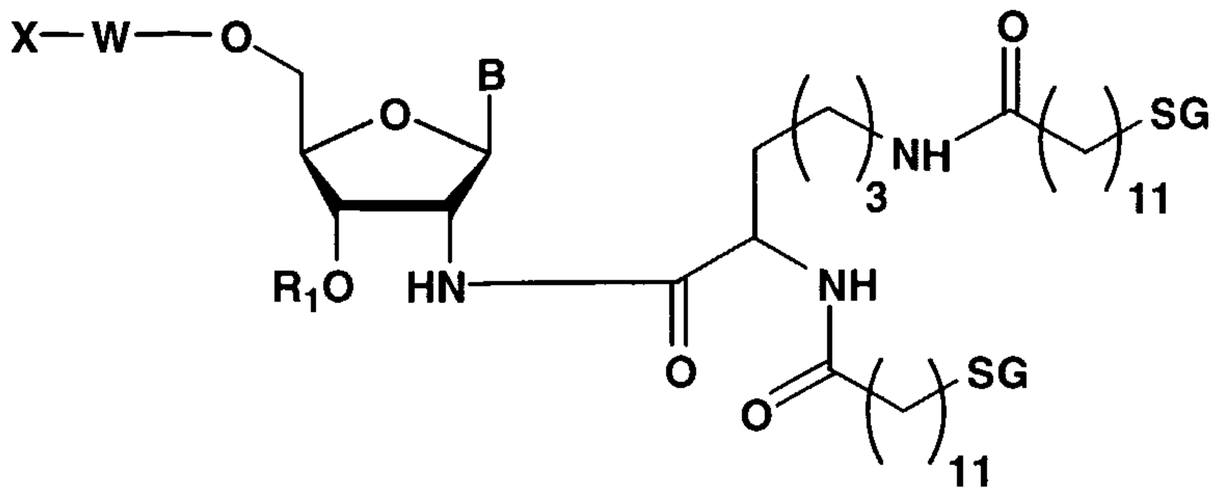
wherein R1, R2, R3, R4, X, W, B, N' and n are as defined in Formula 53, with a compound having Formula 74:



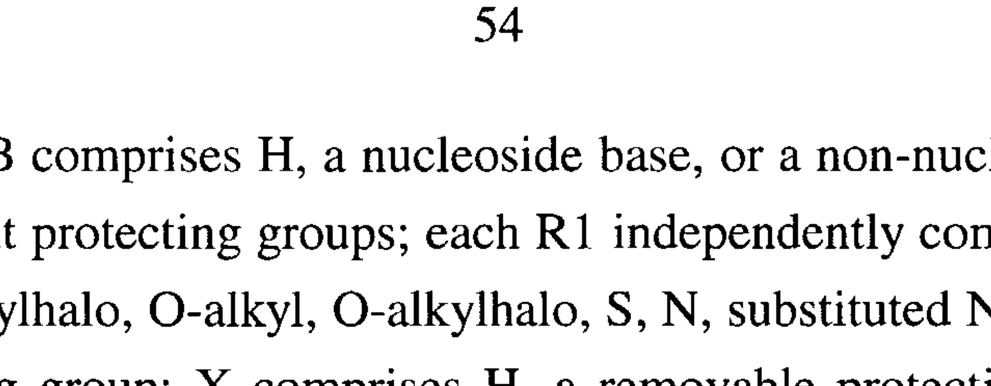
74

wherein Y comprises a linker molecule or chemical linkage that can be present or absent; L represents a reactive chemical group, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; under conditions suitable for the formation of a compound having Formula 53.

167. A method for the synthesis of a compound having Formula 54:

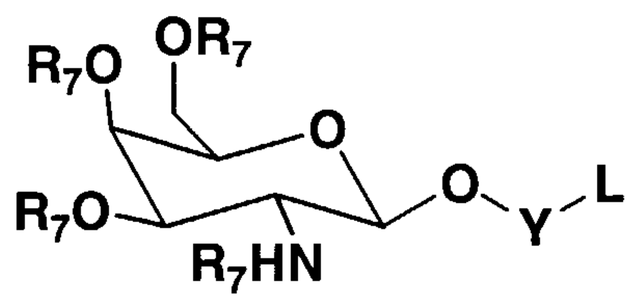


wherein B comprises H, a nucleoside base, or a non-nucleosidic base with or without protecting groups; each R1 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, comprising (a) coupling a compound having Formula 75:



75

wherein R1, R2, R3, R4, X, W, and B are as defined in Formula 53, with a compound having Formula 74:

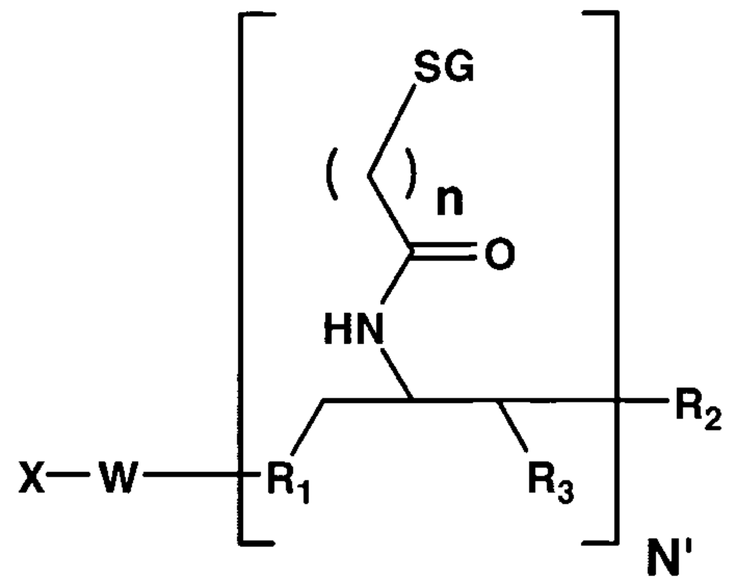


74

wherein Y comprises a C11 alkyl linker molecule; L represents a reactive chemical group, for example a NHS ester, and each R7 independently

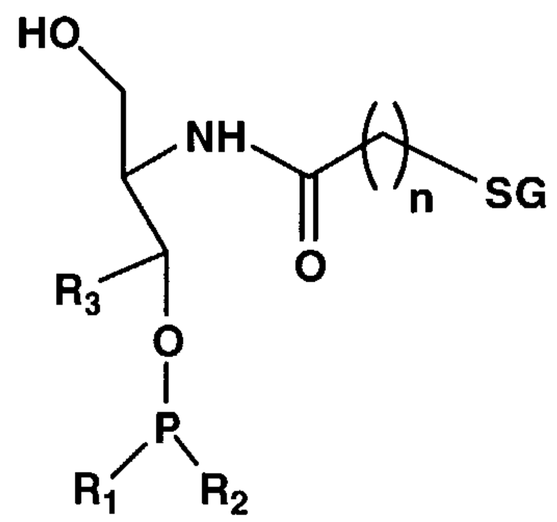
comprises an acyl group that can be present or absent, under conditions suitable for the formation of a compound having Formula 54.

168. A method for the synthesis of a compound having Formula 55:



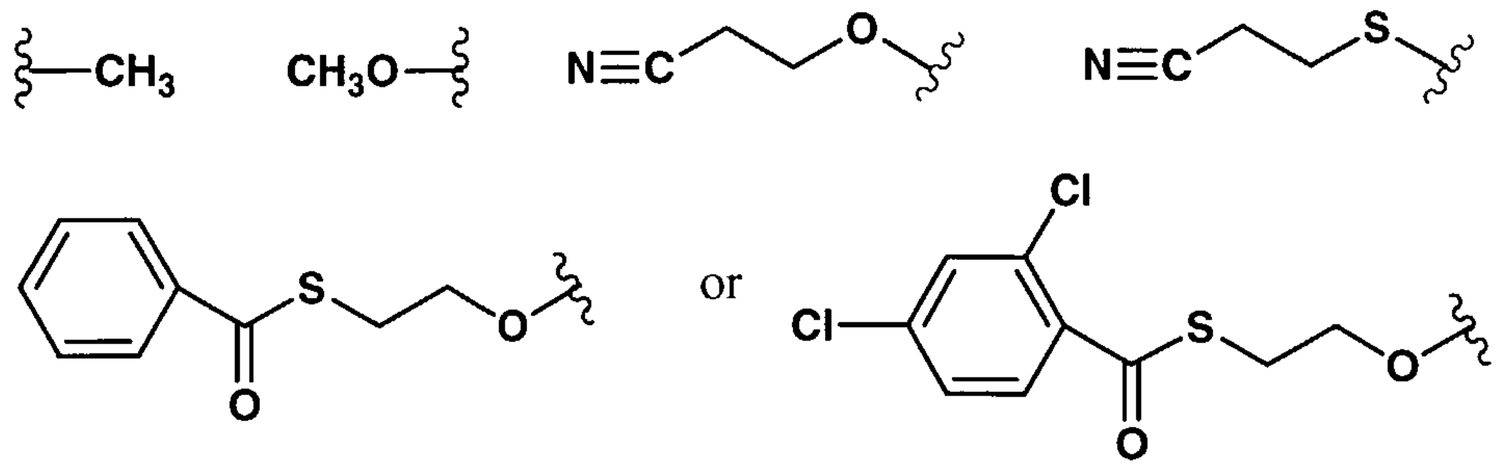
55

wherein each R1 independently comprises O, N, S, alkyl, or substituted N; each R2 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylhalo, S, N, substituted N, or a phosphorus containing group; each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; X comprises H, a removable protecting group, nucleotide, nucleoside, nucleic acid, oligonucleotide, or enzymatic nucleic acid or biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, each n is independently an integer from about 1 to about 50; and N' is an integer from about 1 to about 100, comprising: (a) coupling a compound having Formula 76:

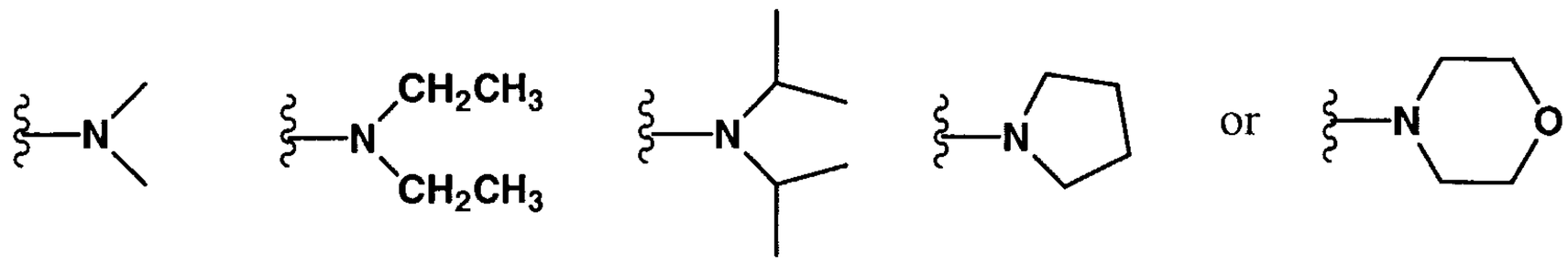


76

wherein R1 can include the groups:

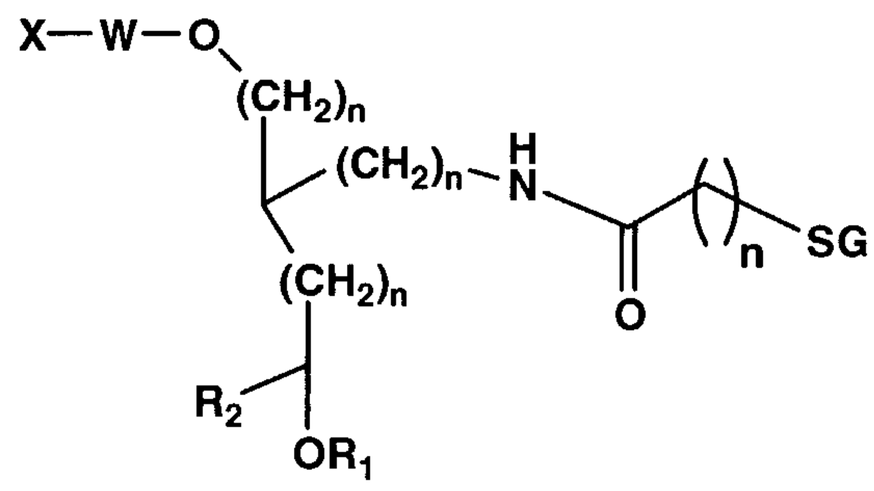


and wherein R2 can include the groups:



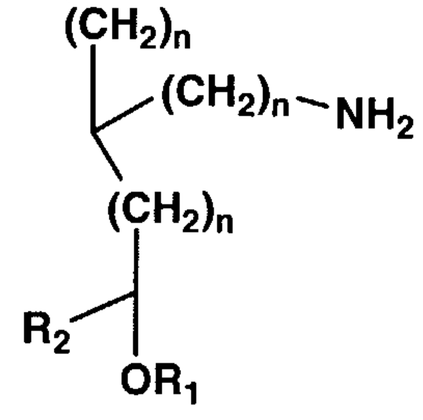
and wherein each R3 independently comprises H, OH, alkyl, substituted alkyl, or halo; SG comprises a sugar, for example galactose, galactosamine, N-acetyl-galactosamine, glucose, mannose, fructose, or fucose and the respective D or L, alpha or beta isomers, and n is an integer from about 1 to about 20, to a compound X-W, wherein X comprises a nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label, and W comprises a linker molecule or chemical linkage that can be present or absent; and (b) optionally repeating step (a) under conditions suitable for the formation of a compound having Formula 55.

169. A method for the synthesis of a compound having Formula 56:



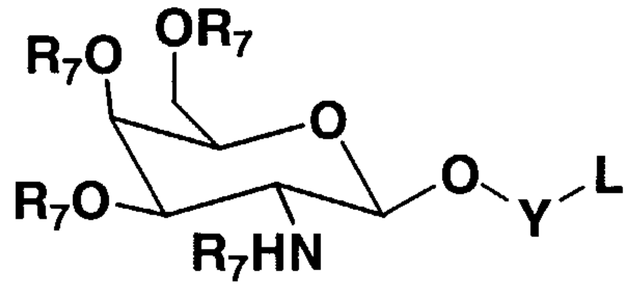
wherein R1 comprises H, alkyl, alkylhalo, N, substituted N, or a phosphorus containing group; R2 comprises H, O, OH, alkyl, alkylhalo, halo, S, N, substituted N, or a phosphorus containing group; X comprises H, a removable protecting group, amino, substituted amino, nucleotide,

nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, biologically active molecule or label; W comprises a linker molecule or chemical linkage that can be present or absent; SG comprises a sugar, and each n is independently an integer from about 0 to about 20, comprising: (a) coupling a compound having Formula 77:



77

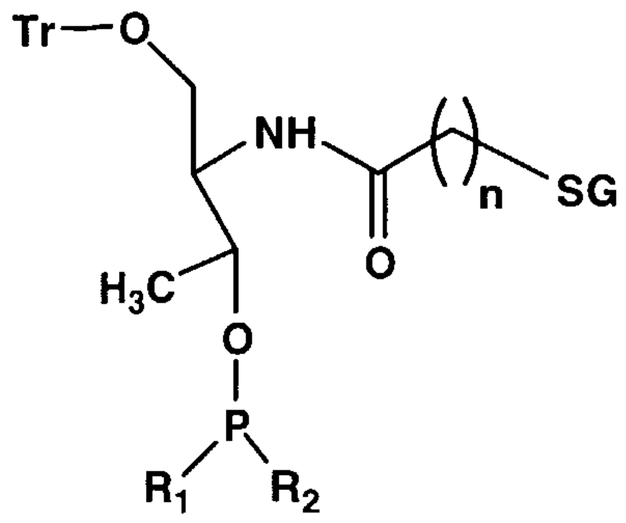
wherein each R1, X, W, and n are as defined in Formula 56, to a compound having Formula 74:



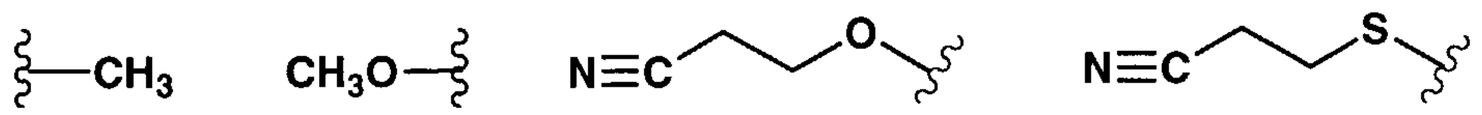
74

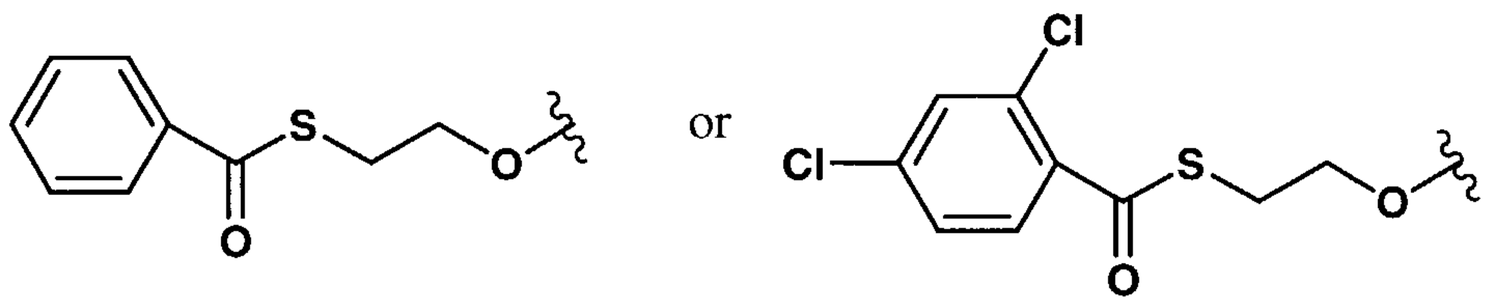
wherein Y comprises an alkyl linker molecule of length n, where n is an integer from about 1 to about 20; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; and (b) optionally coupling X-W, wherein X comprises a removable protecting group, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid, amino acid, peptide, protein, lipid, phospholipid, or label and W comprises a linker molecule or chemical linkage that can be present or absent, under conditions suitable for the formation of a compound having Formula 54.

170. A method for synthesizing a compound having Formula 57:

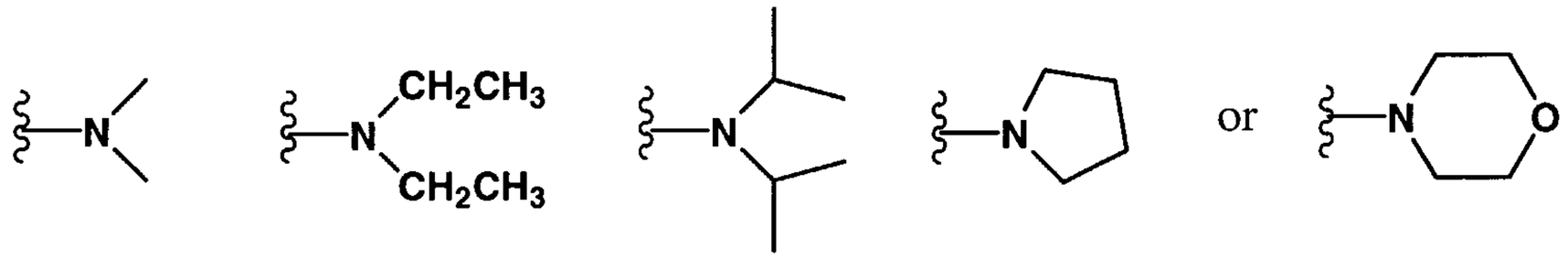


wherein R1 can include the groups:

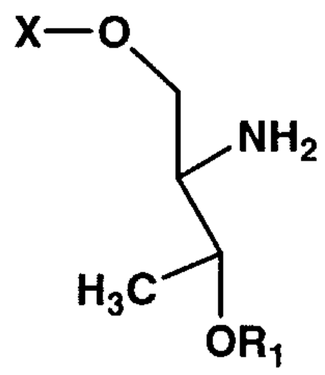




and wherein R2 can include the groups:

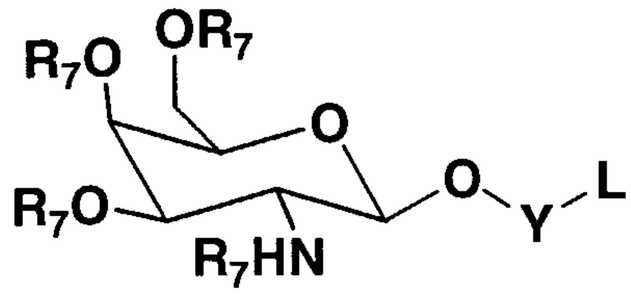


and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; SG comprises a sugar, and n is an integer from about 1 to about 20, comprising: (a) coupling a compound having Formula 77:



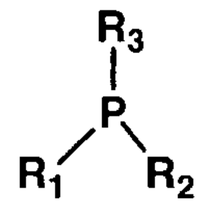
77

wherein R1 and X comprise H, to a compound having Formula 74:



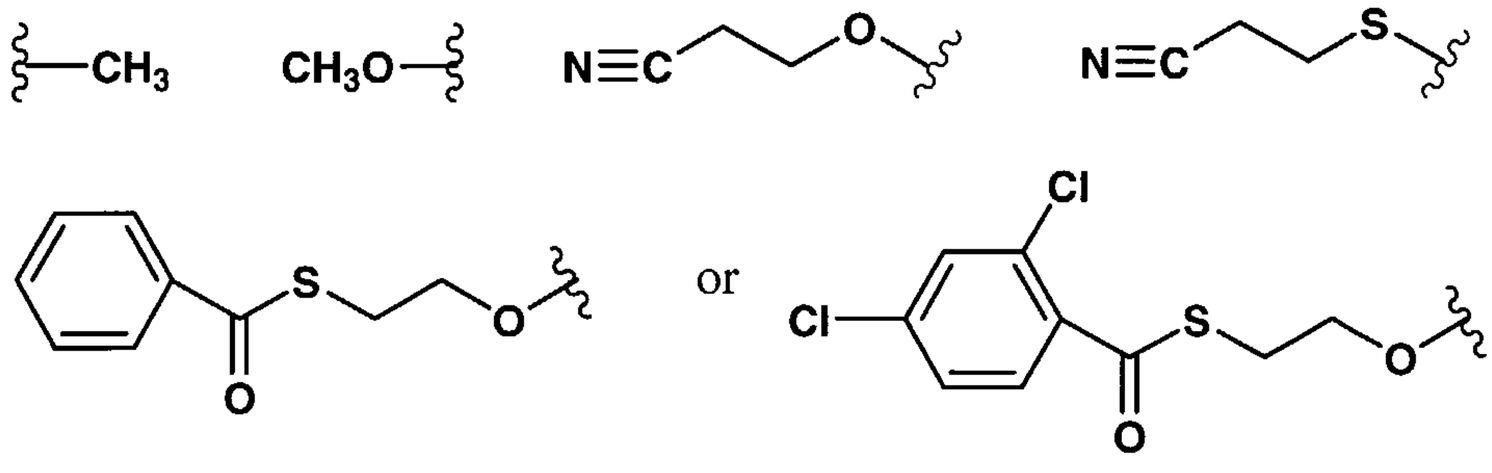
74

wherein Y comprises an alkyl linker molecule of length n, where n is an integer from about 1 to about 20; L represents a reactive chemical group, for example a NHS ester, and each R7 independently comprises an acyl group that can be present or absent, for example a acetyl group; and (b) introducing a trityl group, for example a dimethoxytrityl, monomethoxytrityl, or trityl group to the primary hydroxyl of the product of (a) and (c) introducing a phosphorus containing group having Formula 78:

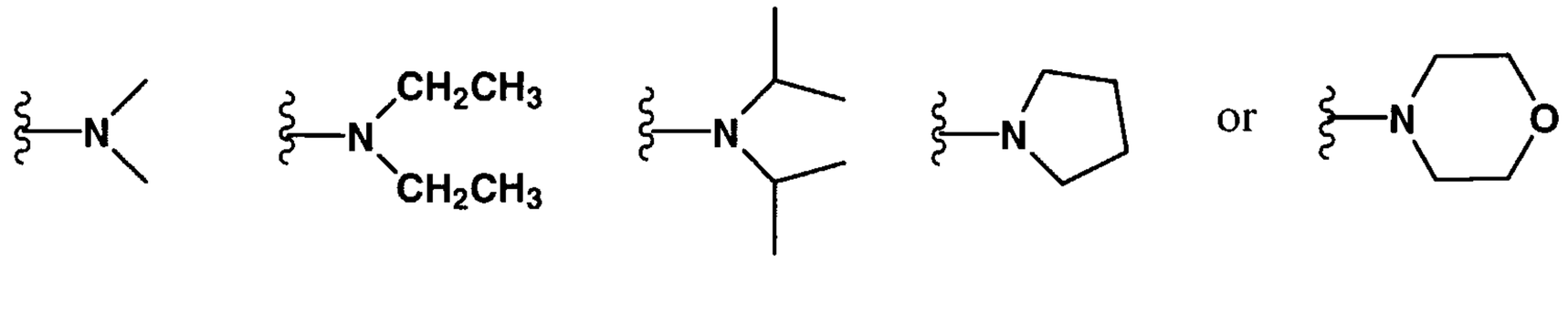


78

wherein R1 can include the groups:

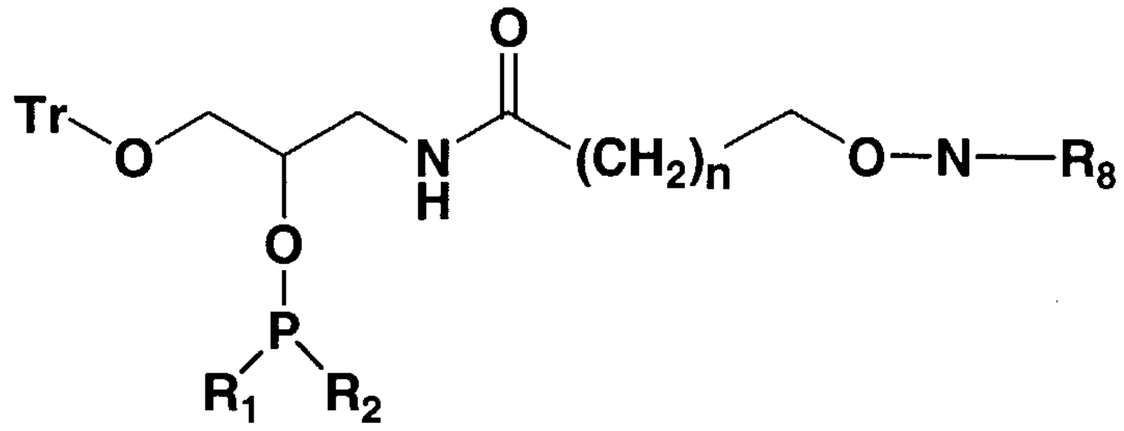


and wherein each R2 and R3 independently can include the groups:



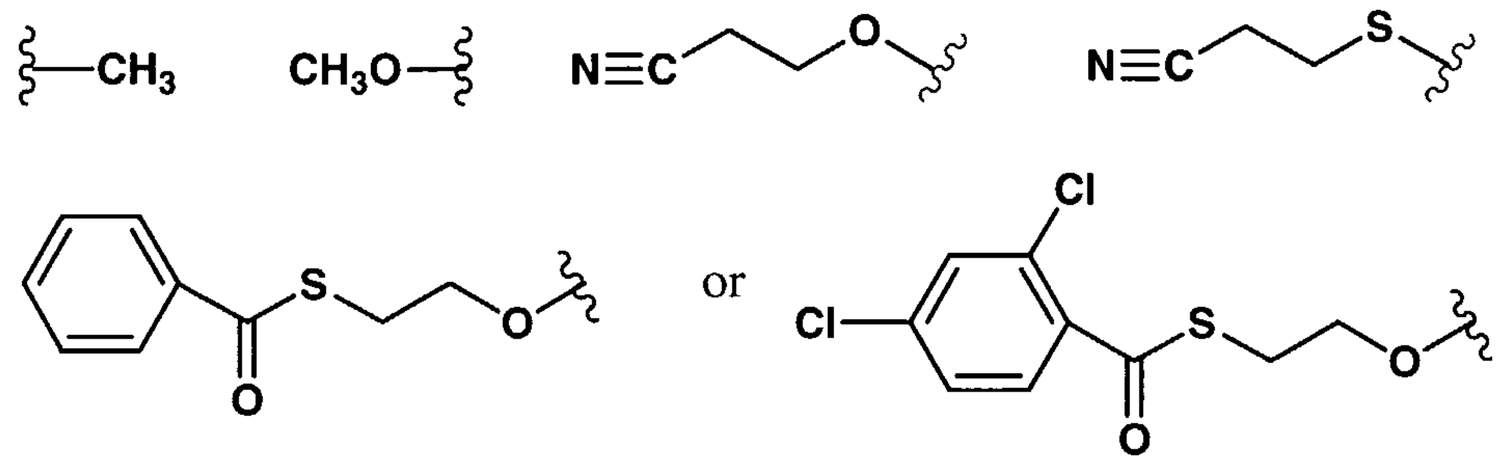
to the secondary hydroxyl of the product of (b) under conditions suitable for the formation of a compound having Formula 57.

171. In another embodiment, the invention features a method for synthesizing a compound having Formula 60:

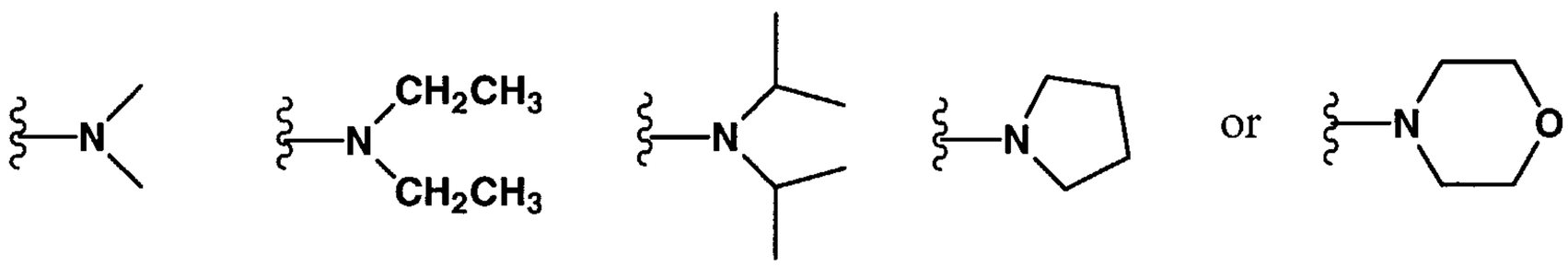


60

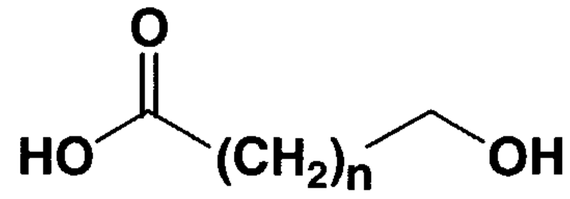
wherein R1 can include the groups:



and wherein R2 can include the groups:

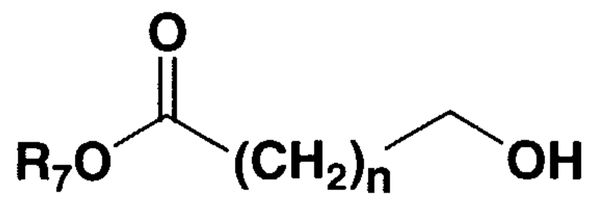


and wherein Tr is a removable protecting group, for example a trityl, monomethoxytrityl, or dimethoxytrityl; n is an integer from about 1 to about 50; and R8 is a nitrogen protecting group, comprising: (a) introducing carboxy protection to a compound having Formula 79:



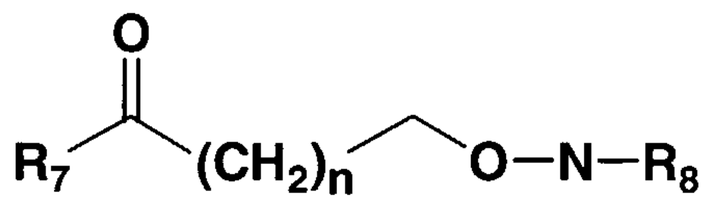
79

wherein n is an integer from about 1 to about 50, under conditions suitable for the formation of a compound having Formula 80:



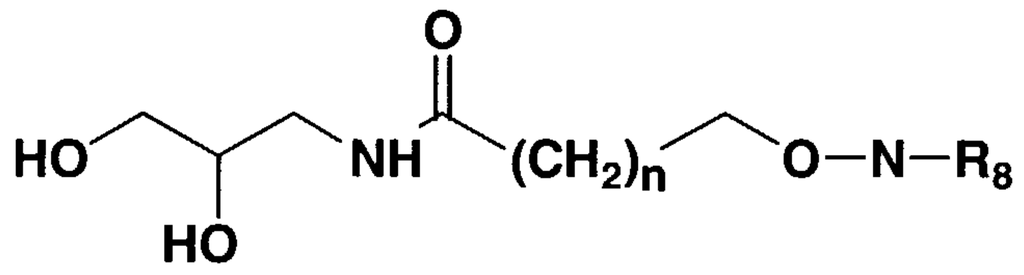
80

wherein n is an integer from about 1 to about 50 and R7 is a carboxylic acid protecting group, for example a benzyl group; (b) introducing a nitrogen containing group to the product of (a) under conditions suitable for the formation of a compound having Formula 81:



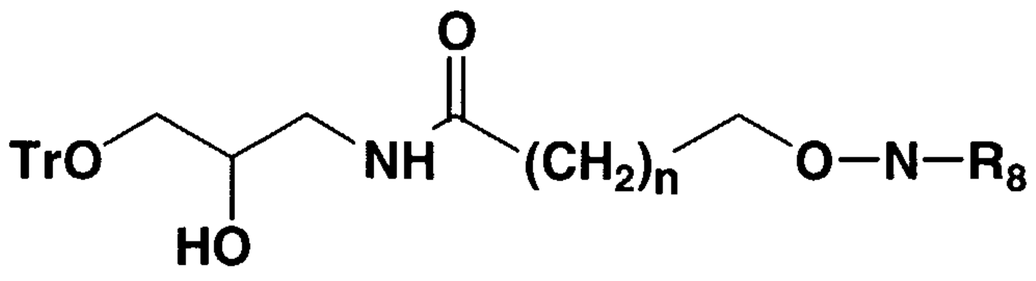
81

wherein n and R7 are as defined in Formula 80 and R8 is a nitrogen protecting group, for example a phthaloyl, trifluoroacetyl, FMOC, or monomethoxytrityl group; (c) removing the carboxylic acid protecting group from the product of (b) and introducing aminopropanediol under conditions suitable for the formation of a compound having Formula 82:



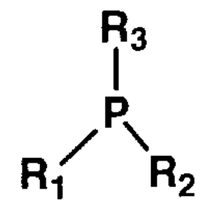
82

wherein n and R8 are as defined in Formula 81; (d) introducing a removable protecting group to the product of (c) under conditions suitable for the formation of a compound having Formula 83:



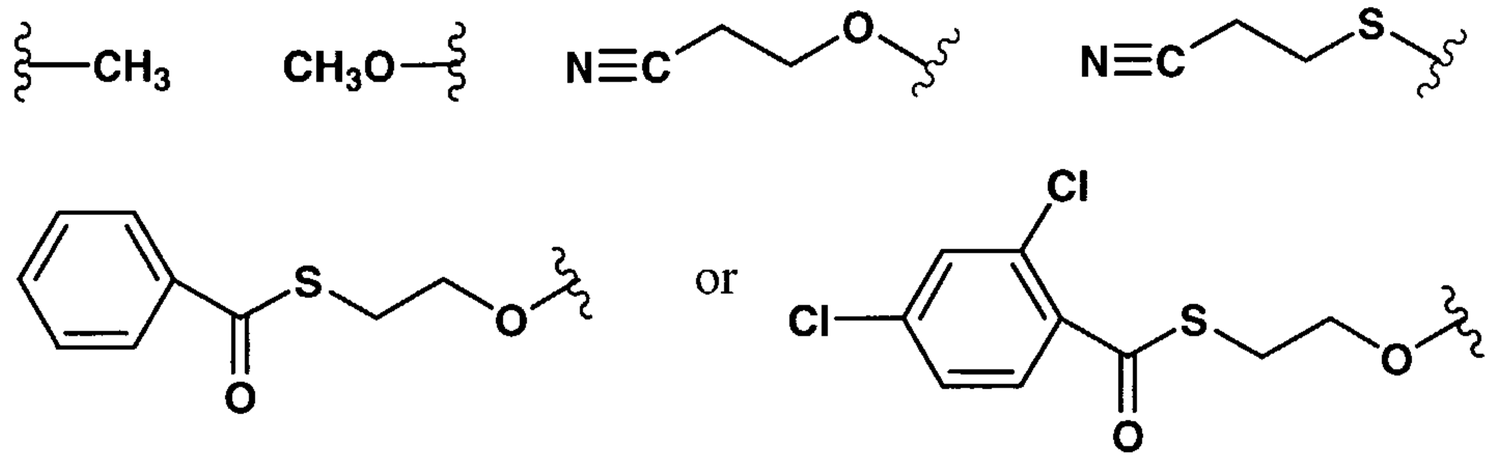
83

wherein Tr, n and R8 are as defined in Formula 60; and (e) introducing a phosphorus containing group having Formula 78:

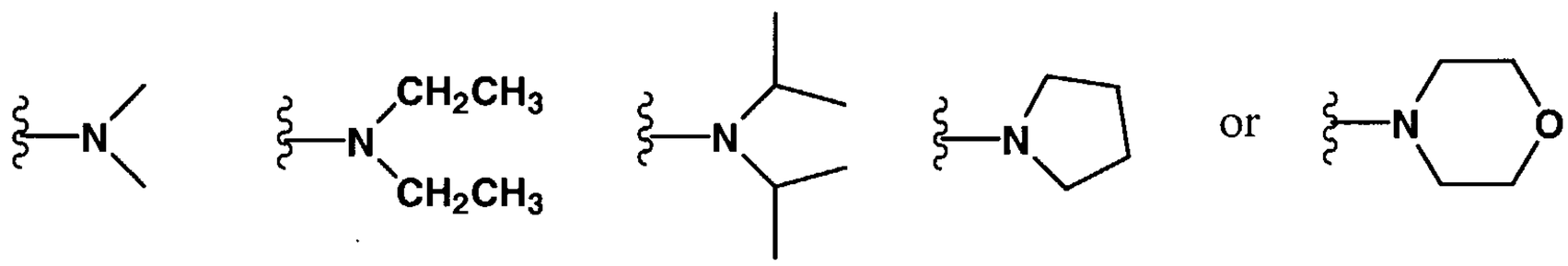


78

wherein R1 can include the groups:

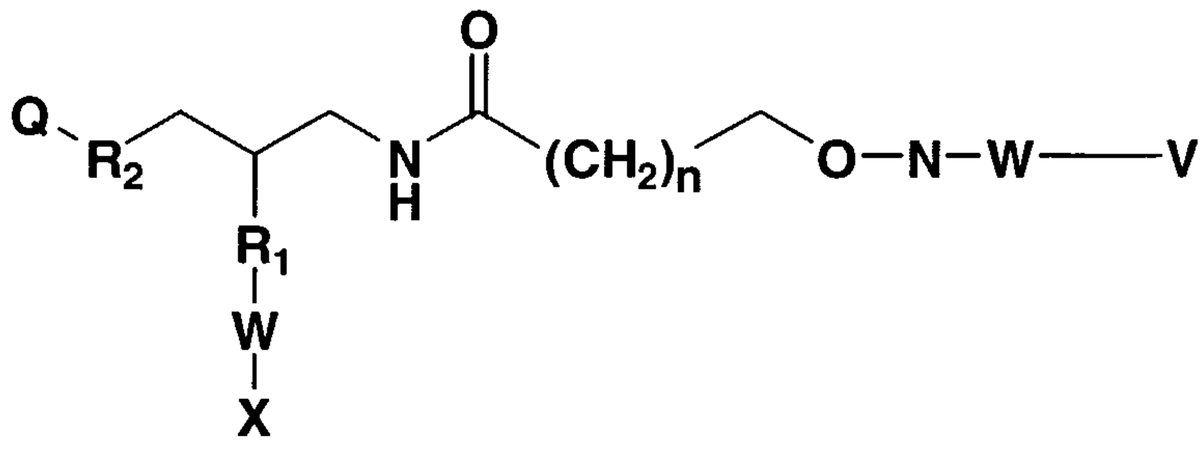


and wherein each R2 and R3 independently can include the groups:



to the product of (d) under conditions suitable for the formation of a compound having Formula 60.

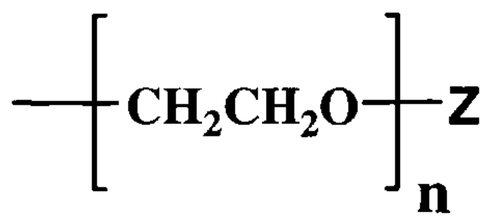
172. A method for the synthesis of a compound having Formula 59:



59

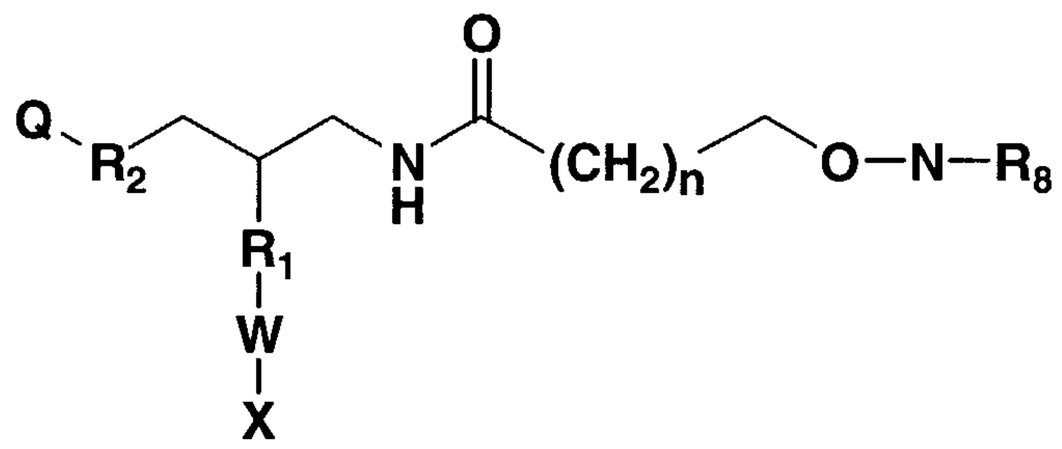
wherein each R1 independently comprises O, S, N, substituted N, or a phosphorus containing group; each R2 independently comprises O, S, or N; X comprises H, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, enzymatic nucleic acid or biologically active molecule; n is an integer from about 1 to about 50, Q comprises H or a removable protecting group which can be optionally absent, each W

independently comprises a linker molecule or chemical linkage that can be present or absent, and V comprises a protein or peptide or a compound having Formula 45:



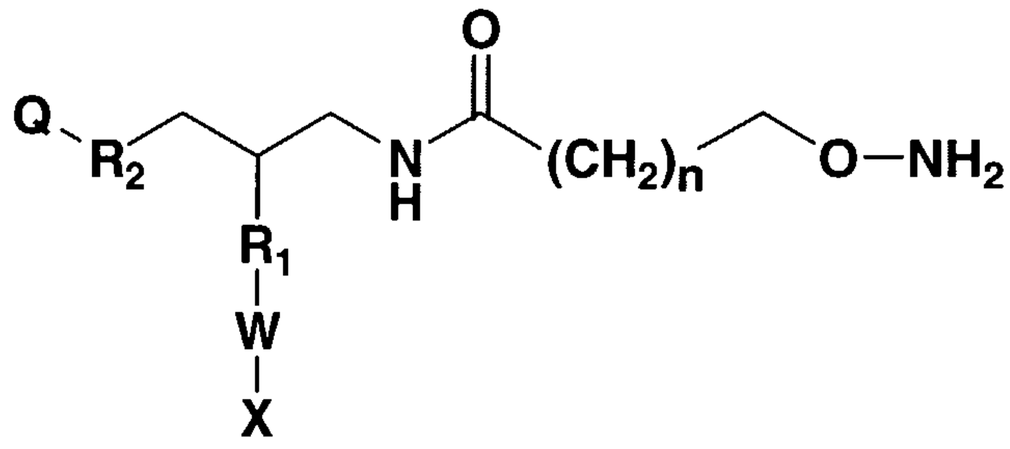
45

wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100, comprising: (a) removing R8 from a compound having Formula 84:



84

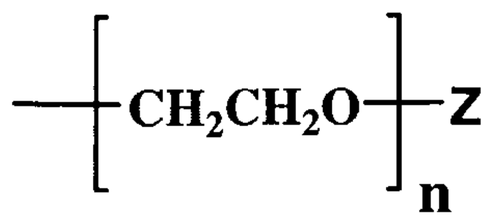
wherein Q, X, W, R1, R2, and n are as defined in Formula 59 and R8 is a nitrogen protecting group under conditions suitable for the formation of a compound having Formula 85:



85

wherein Q, X, W, R1, R2, and n are as defined in Formula 59; (b) introducing a group V to the product of (a) via the formation of an oxime

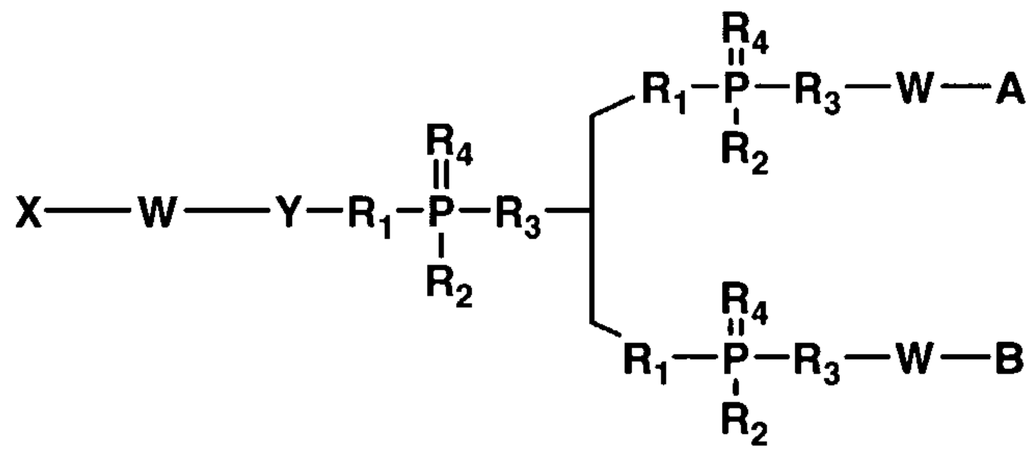
linkage, wherein V comprises a protein or peptide or a compound having Formula 45:



45

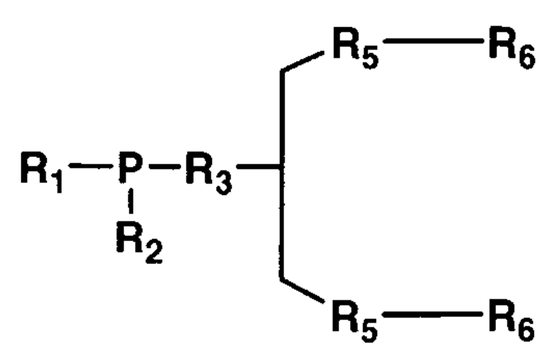
wherein Z comprises H, OH, O-alkyl, SH, S-alkyl, alkyl, substituted alkyl, aryl, substituted aryl, amino, substituted amino, nucleotide, nucleoside, nucleic acid, oligonucleotide, amino acid, peptide, protein, lipid, phospholipid, or label; and n is an integer from about 1 to about 100, under conditions suitable for the formation of a compound having Formula 59.

173. A method for synthesizing a compound having Formula 64:



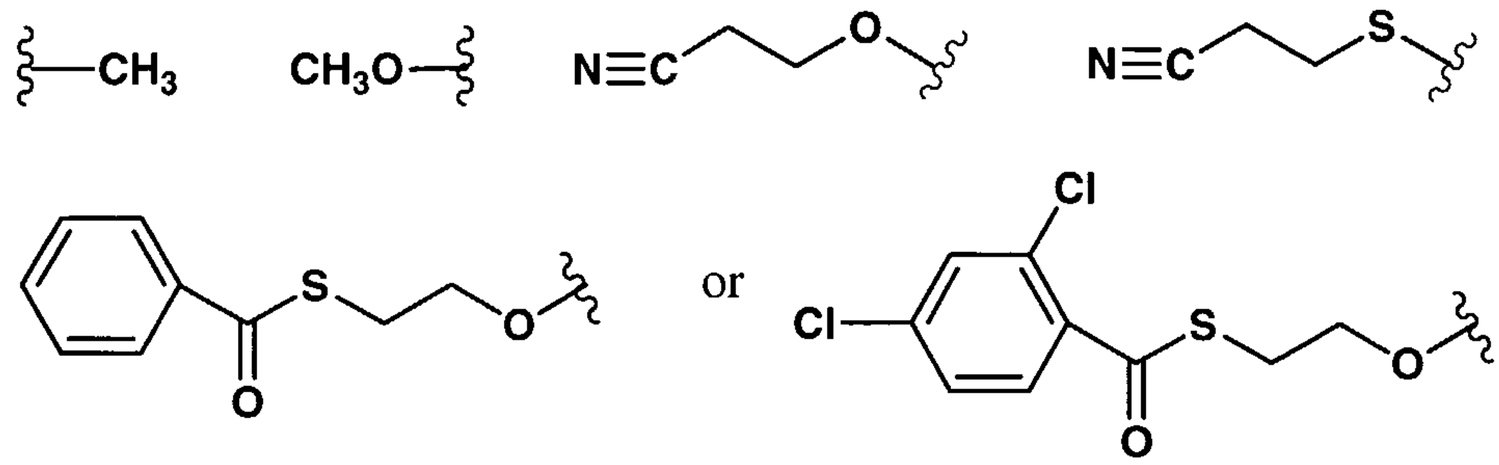
64

wherein X comprises a biologically active molecule; each W independently comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N, A comprises a nitrogen containing group, and B comprises a lipophilic group, comprising: (a) introducing a compound having Formula 66:

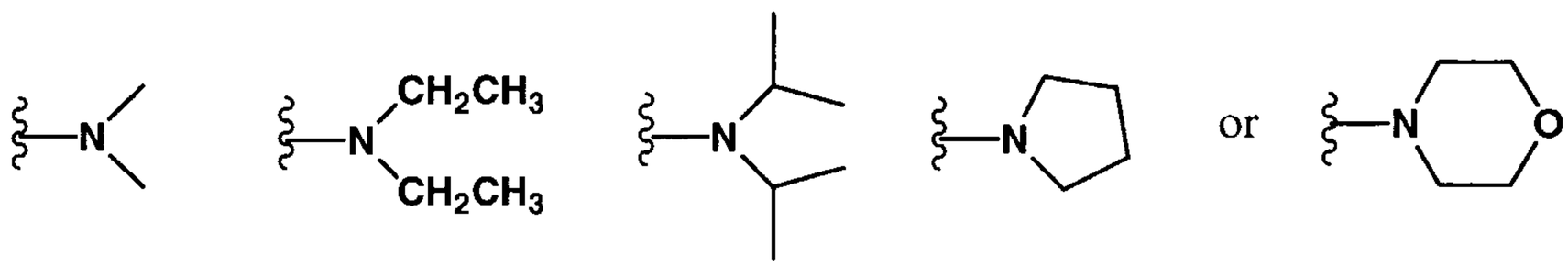


66

wherein R1 is defined as in Formula 64 and can include the groups:



and wherein R2 is defined as in Formula 64 and can include the groups:

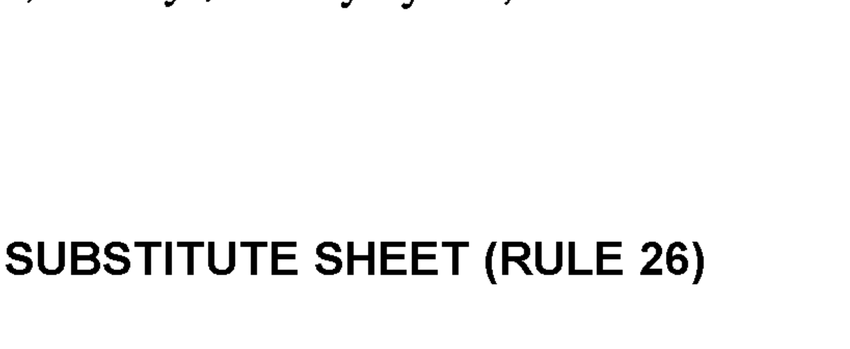


and wherein each R5 independently comprises O, N, or S and each R6 independently comprises a removable protecting group to a compound having Formula 67:



67

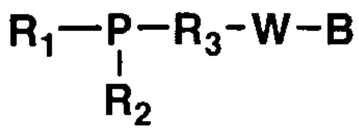
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a linker molecule that can be present or absent, under conditions suitable for the formation of a compound having Formula 68:



68

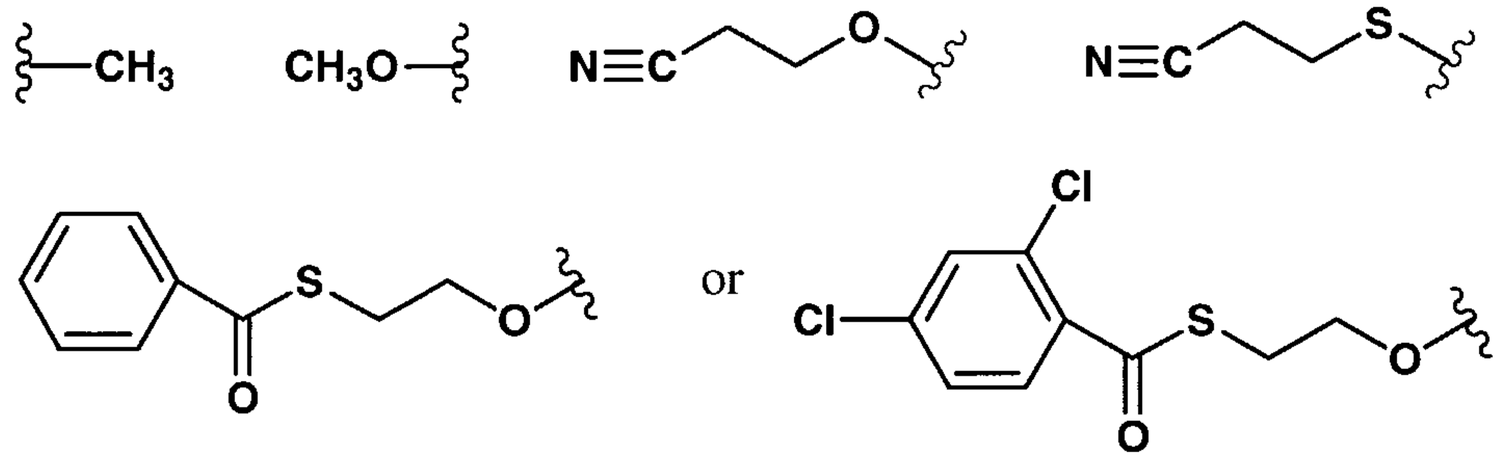
wherein X comprises a biologically active molecule; W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a linker molecule that can be present or absent; and each R1, R2, R3, and R4 independently comprises O, OH, H, alkyl, alkylhalo, O-alkyl, O-alkylcyano, S, S-alkyl, S-alkylcyano, N or substituted N comprising, each

R5 independently comprises O, S, or N; and each R6 is independently a removable protecting group; (b) removing R6 from the compound having Formula 26 and (c) introducing a compound having Formula 69:

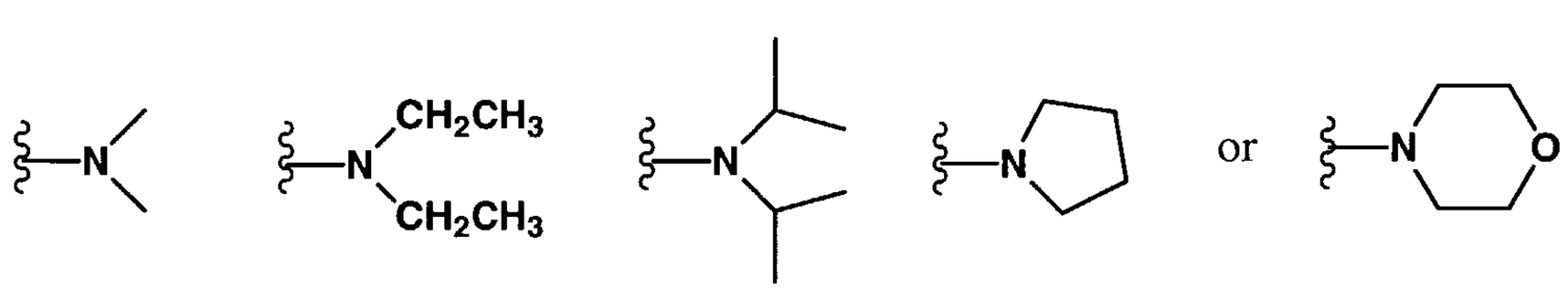


69

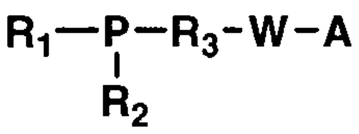
wherein R1 is defined as in Formula 64 and can include the groups:



and wherein R2 is defined as in Formula 64 and can include the groups:

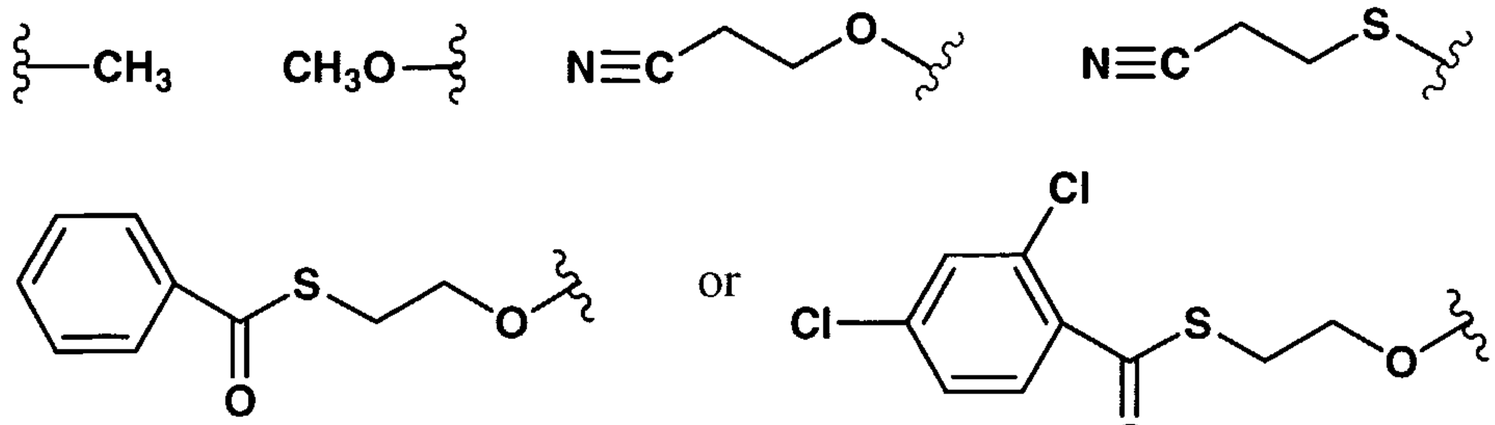


and wherein R3, W and B are defined as in Formula 64; and introducing a compound having Formula 69':

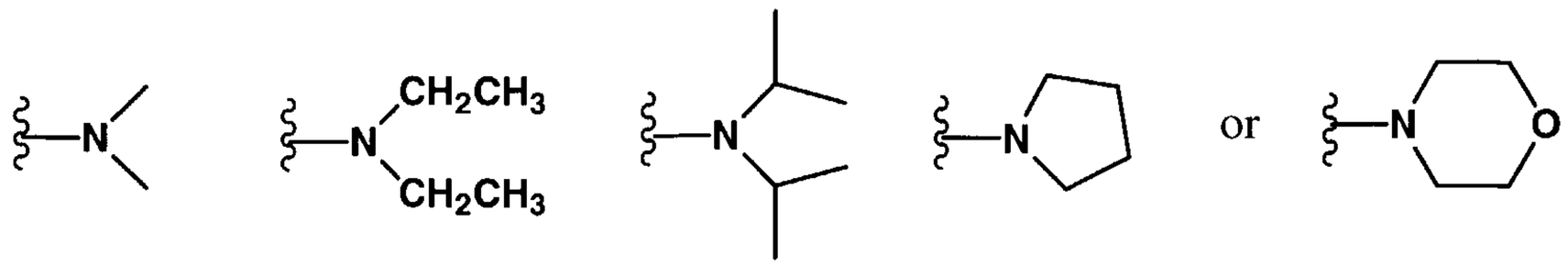


69'

wherein R1 is defined as in Formula 64 and can include the groups:



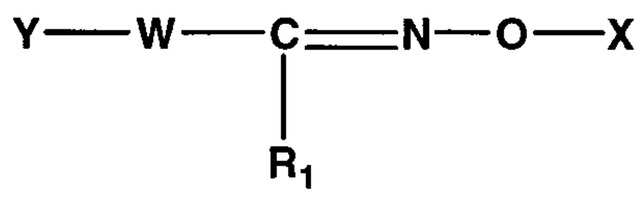
and wherein R2 is defined as in Formula 48 and can include the groups:



or

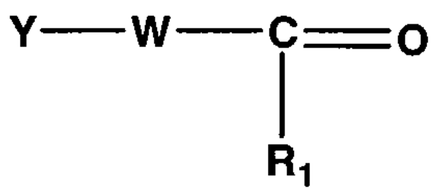
and wherein R3, W and A are defined as in Formula 64; to the compound having Formula 68 under conditions suitable for the formation of a compound having Formula 64.

174. In another embodiment, the invention features a method for the synthesis of a compound having Formula 87:



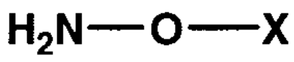
87

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, W comprises a linker molecule or chemical linkage that can be present or absent, Y comprises a biologically active molecule, and R1 comprises H, alkyl, or substituted alkyl, comprising (a) coupling a compound having Formula 89:



89

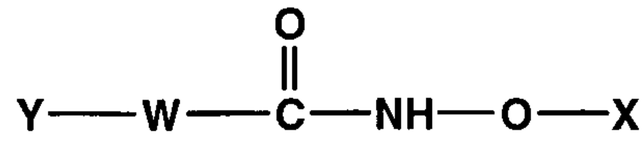
wherein Y, W and R are as defined in Formula 87, with a compound having Formula 90:



90

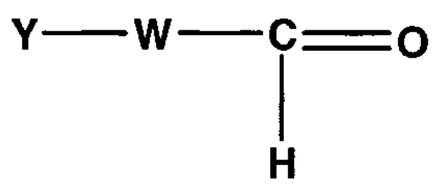
wherein X is as defined in Formula 87, under conditions suitable for the formation of a compound having Formula 87, for example by post-synthetic conjugation of a compound having Formula 89 with a compound having Formula 90, wherein X of compound 90 comprises an enzymatic nucleic acid molecule and Y of Formula 89 comprises a peptide.

175. In another embodiment, the invention features a method for the synthesis of a compound having Formula 88:



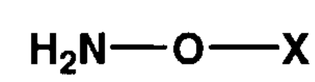
88

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, W comprises a linker molecule or chemical linkage that can be present or absent, and Y comprises a biologically active molecule, comprising (a) coupling a compound having Formula 91:



91

wherein Y and W is as defined in Formula 88, with a compound having Formula 90:



90

wherein X is as defined in Formula 88, under conditions suitable for the formation of a compound having Formula 88, by post-synthetic conjugation of a compound having Formula 91 with a compound having Formula 90, wherein X of compound 90 comprises an enzymatic nucleic acid molecule and Y of Formula 91 comprises a peptide.

176. In one embodiment, the invention features a compound having Formula 94:

94

wherein X comprises a protein, peptide, antibody, lipid, phospholipid, oligosaccharide, label, biologically active molecule, for example a vitamin such as folate, vitamin A, E, B6, B12, coenzyme, antibiotic, antiviral, nucleic acid, nucleotide, nucleoside, or oligonucleotide such as an

enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, or polymers such as polyethylene glycol; each Y independently comprises a linker or chemical linkage that can be present or absent, W comprises a biodegradable nucleic acid linker molecule, and Z comprises a biologically active molecule, for example an enzymatic nucleic acid, allozyme, antisense nucleic acid, siRNA, 2,5-A chimera, decoy, aptamer or triplex forming oligonucleotide, peptide, protein, or antibody.

177. The kit of claim 97, wherein said sample is from a cancer cell.

178. The kit of claim 97, wherein said sample is from a virus infected cell.

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

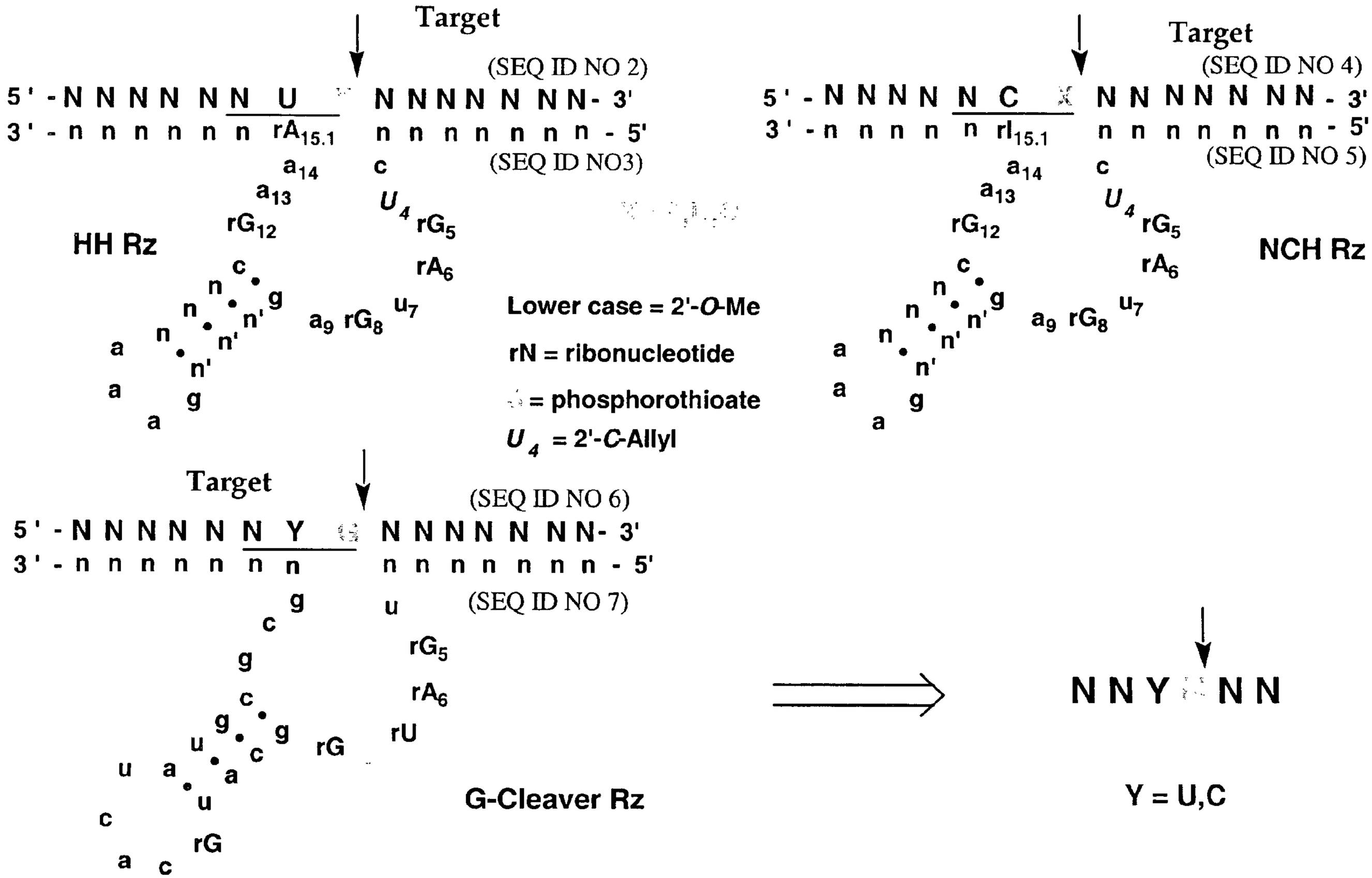


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

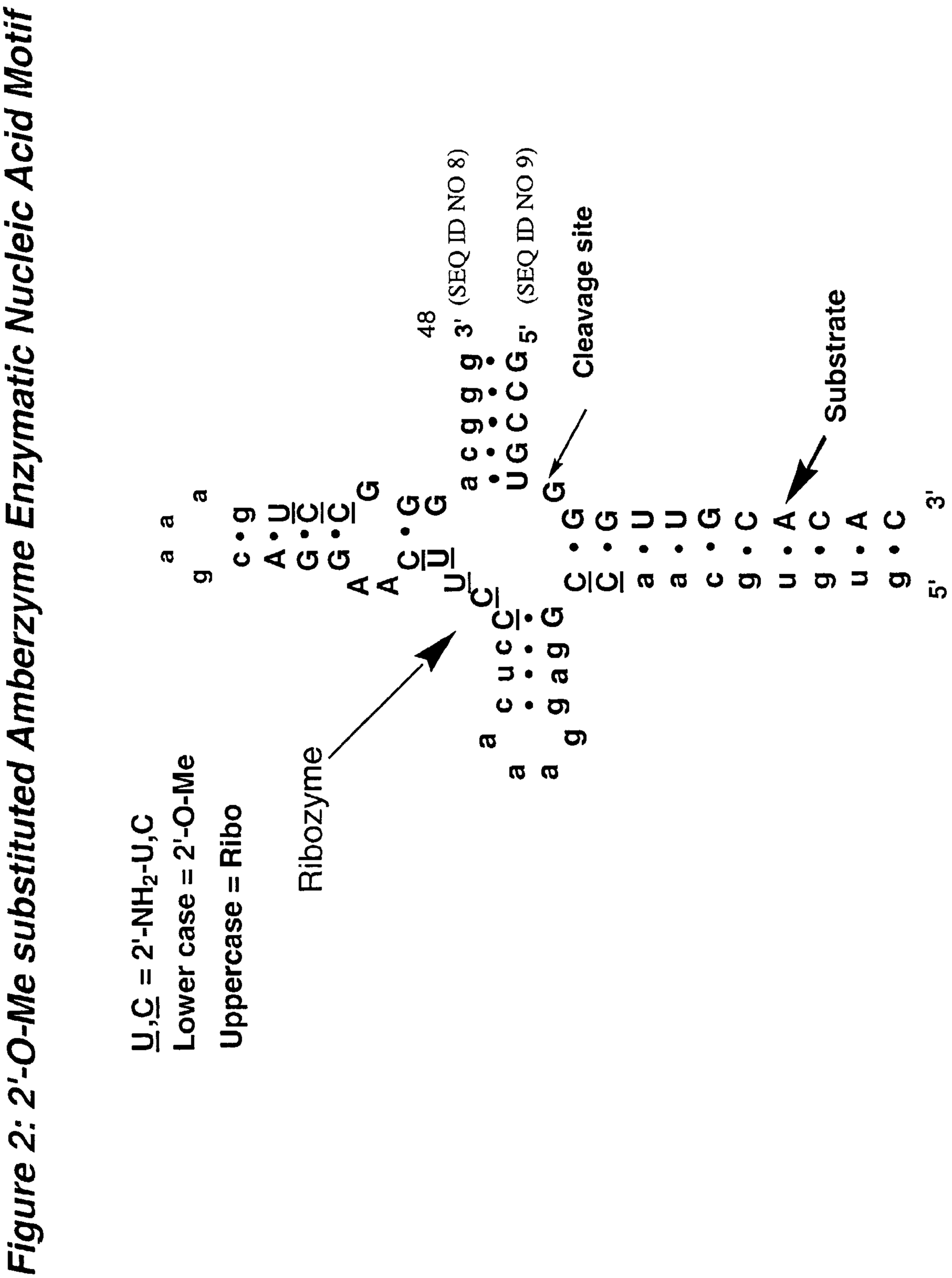
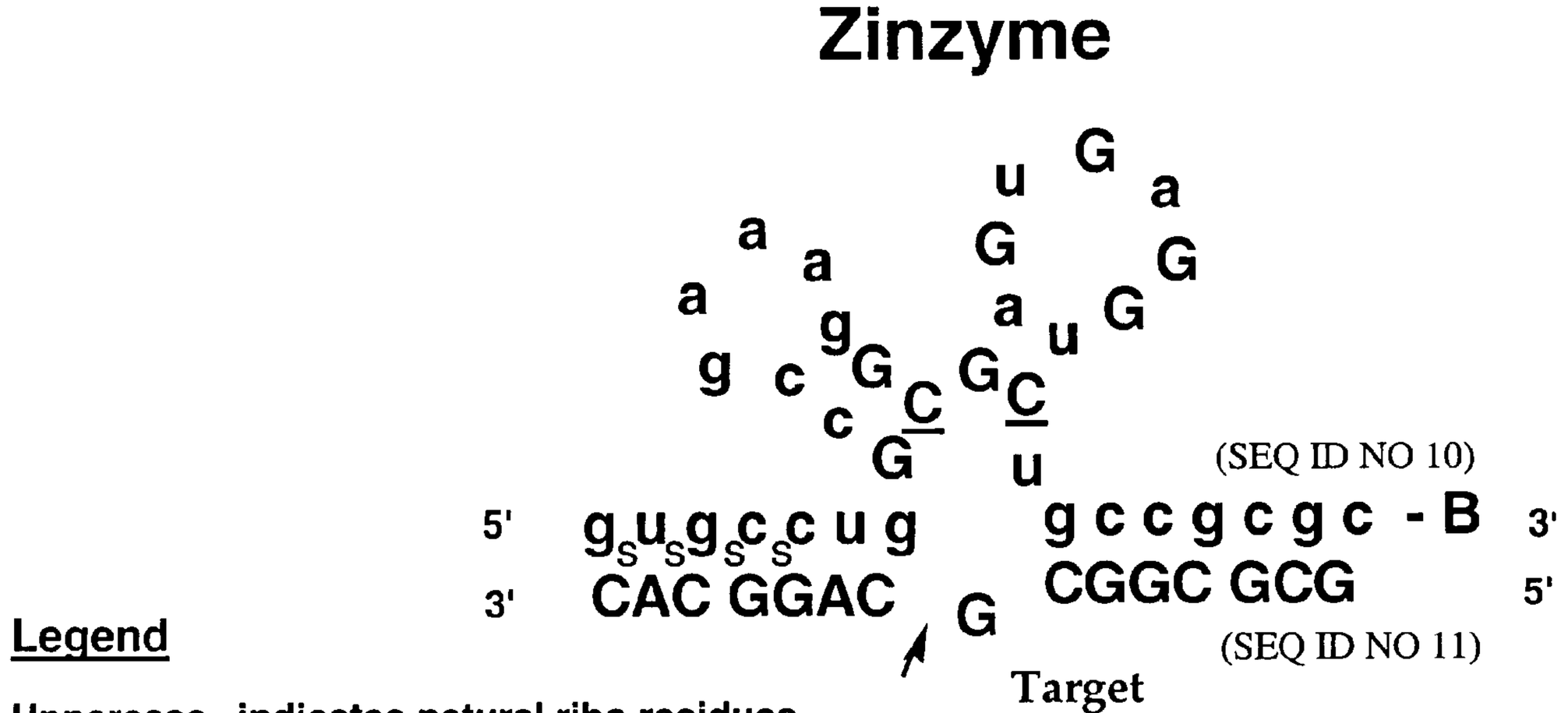


Figure 3: Stabilized Zinzyme Ribozyme Motif



Legend

Uppercase indicates natural ribo residues

C indicates 2' - deoxy-2'-amino Cytidine

Lowercase indicates 2'-O-methyl

S indicates phosphorothioate/phosphorodithioate

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif

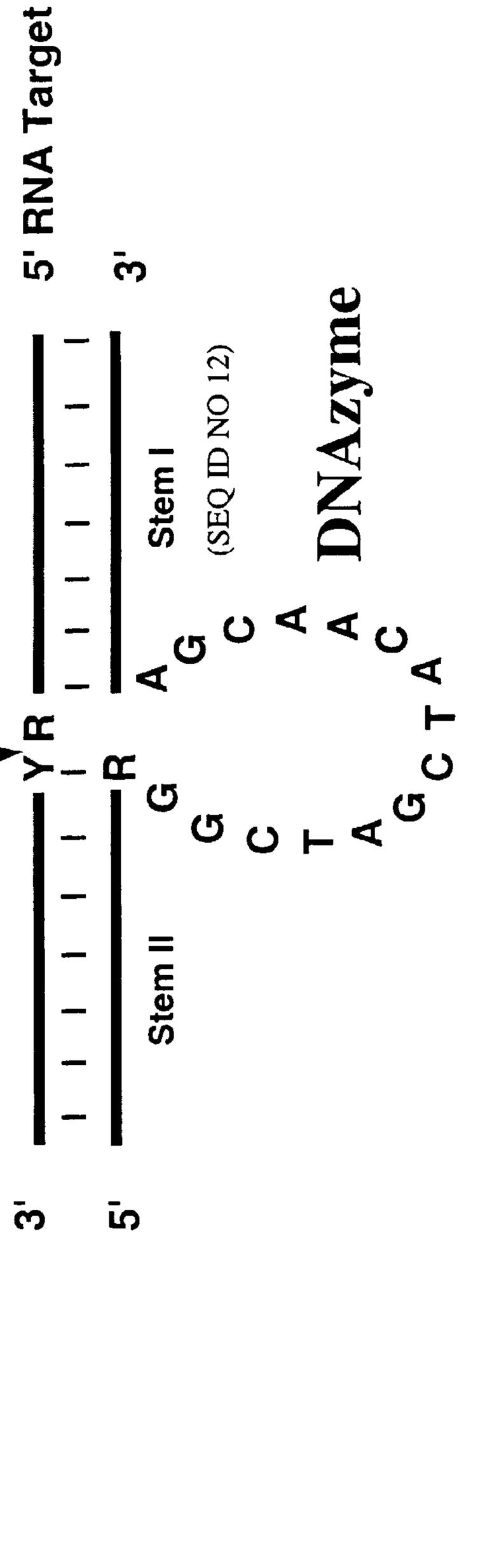


Figure 5: Synthesis of Folate Linked phosphoramidite

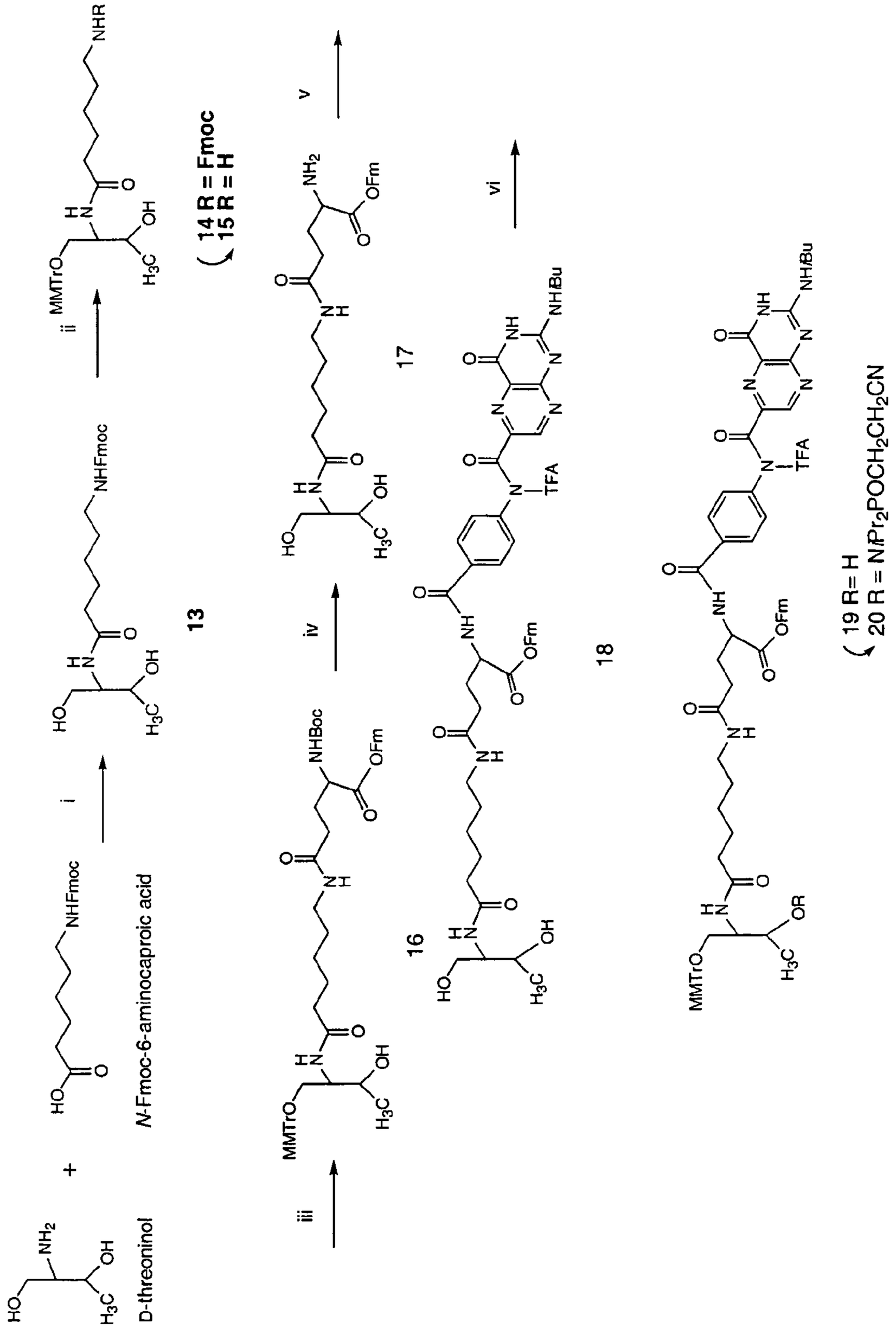
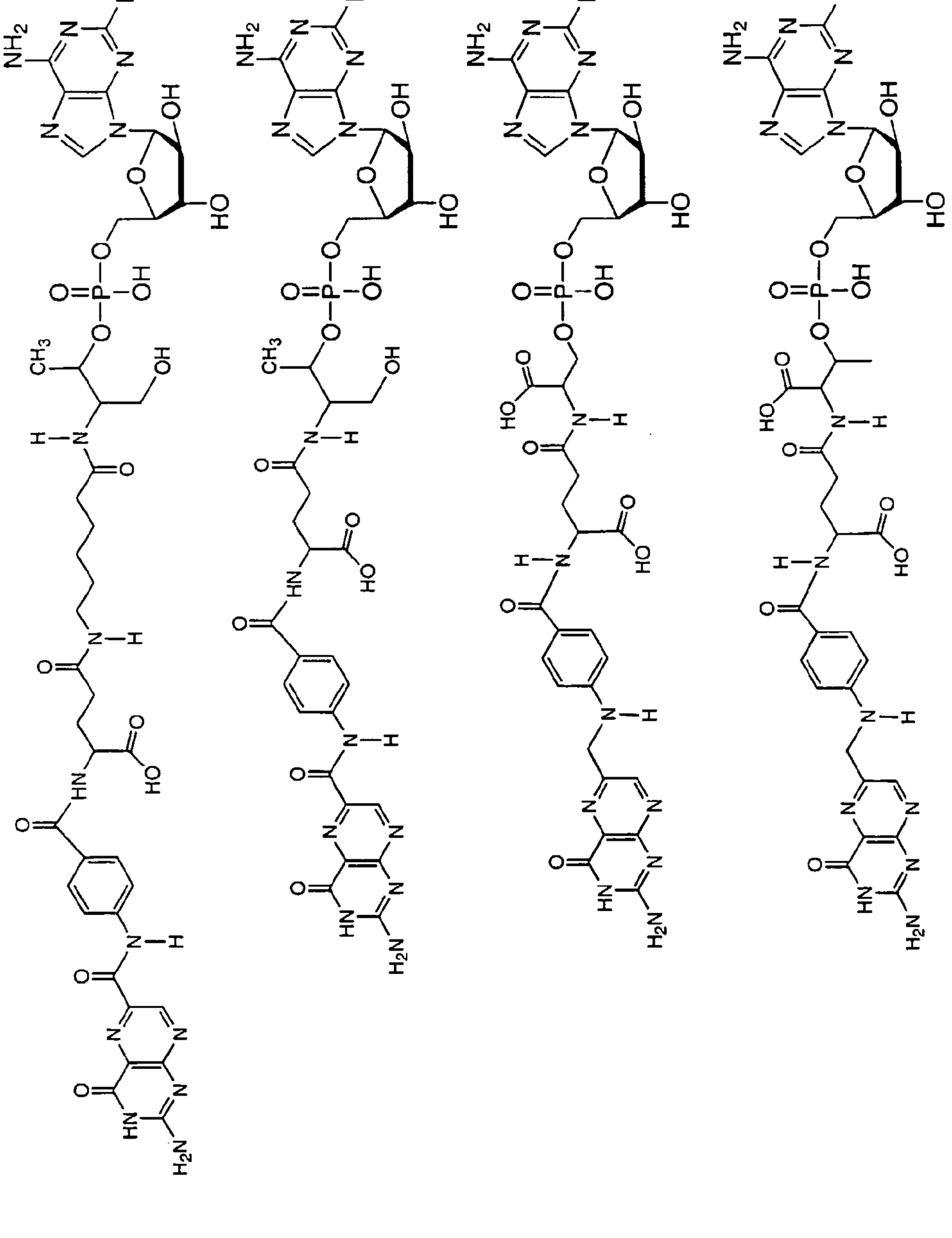


Figure 6: Fludarabine-Folate conjugates



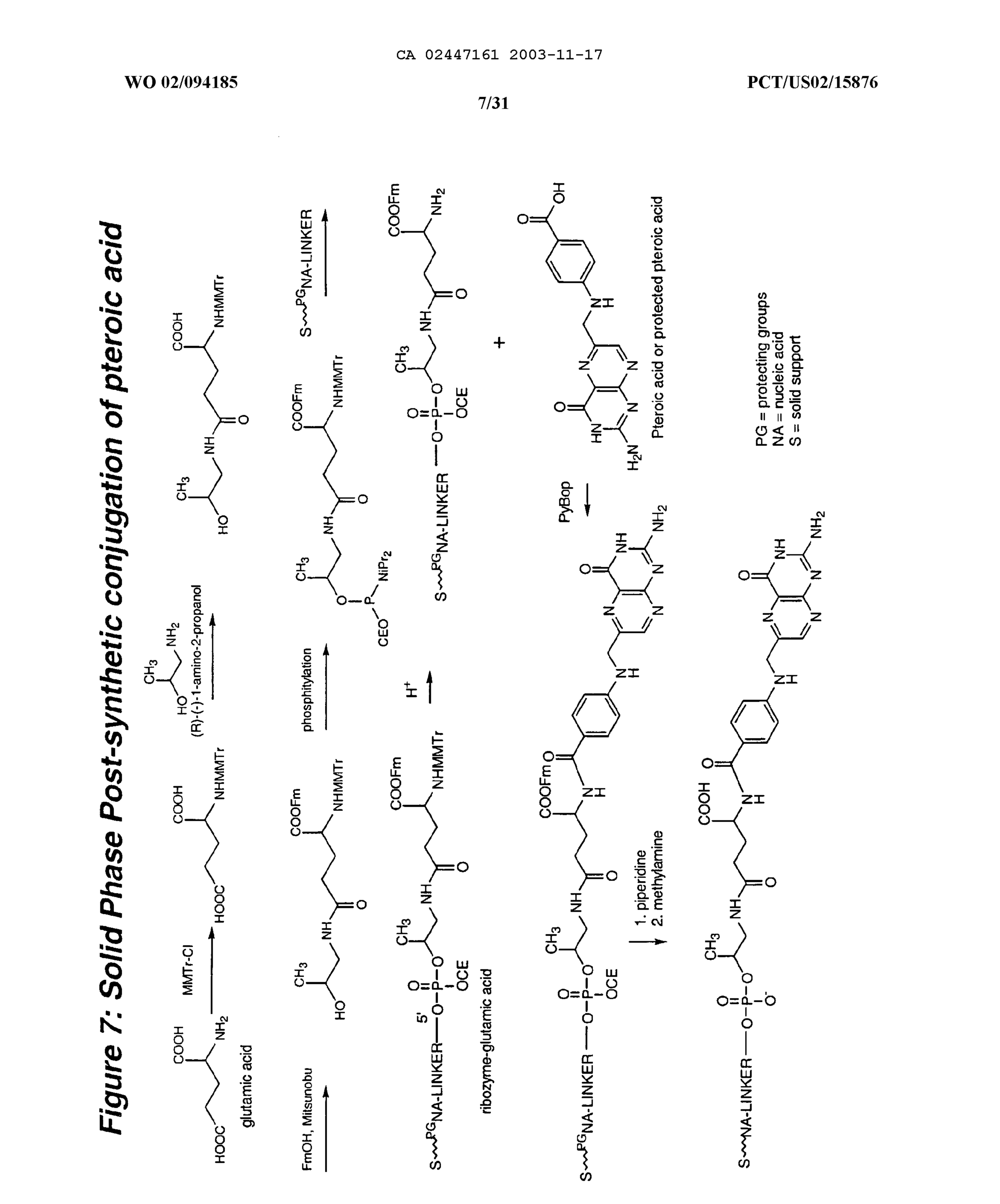


Figure 7: Solid Phase Post-synthetic conjugation of pteroic acid
glutamic acid
MMTr-Cl
(R)-(-)-1-amino-2-propanol
FmOH, Mitsunobu
phosphitylation
H+
ribozyme-glutamic acid
PyBop
Pteroic acid or protected pteroic acid
1. piperidine
2. methylamine
PG = protecting groups
NA = nucleic acid
S = solid support

Figure 8: Chemo-enzymatic synthesis of pteroic acid synthon

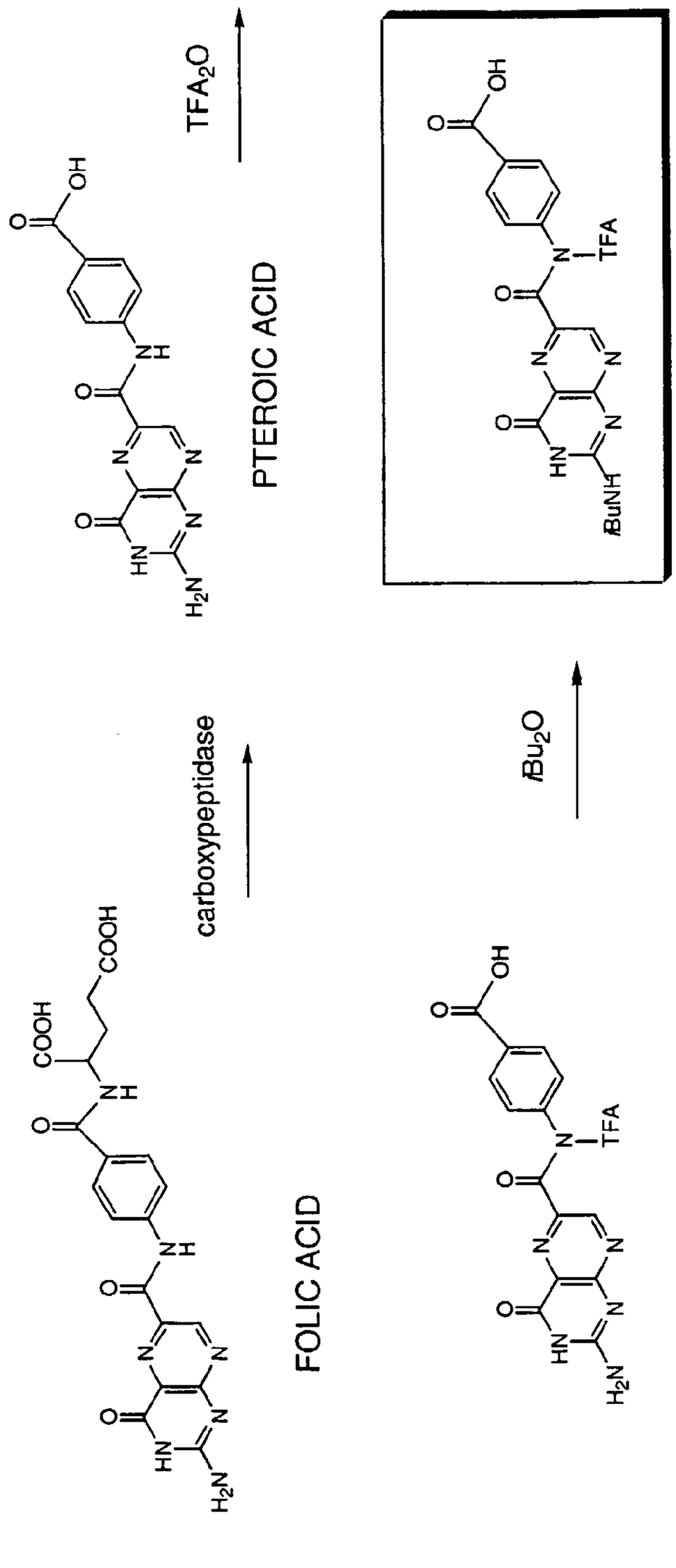


Figure 9

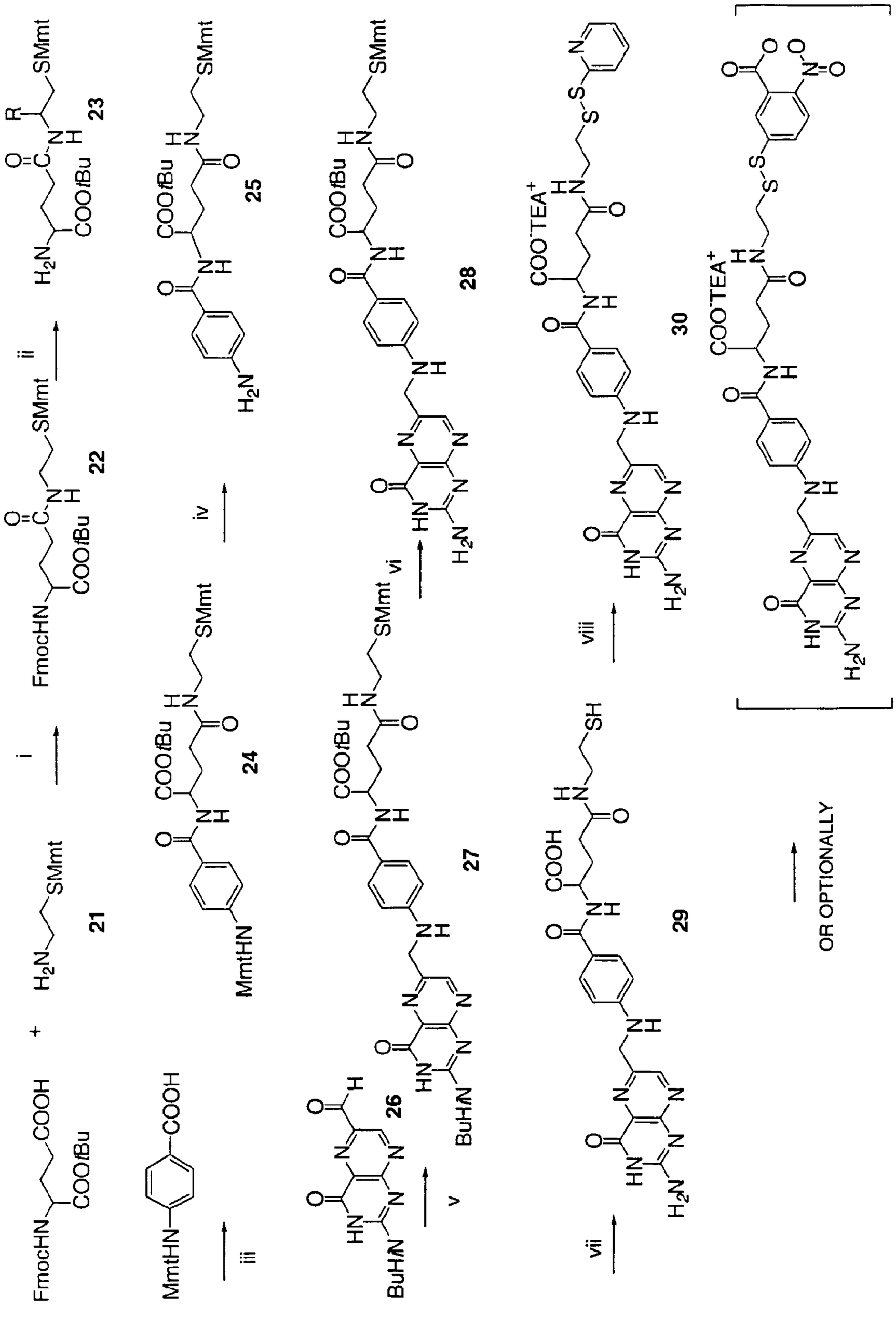

FmocHN
COOtBu
COOH
i
+
H2N
SMmt
21
MmtHN
24
ii
iii
iv
v
vi
vii
viii
22
23
25
26
27
28
29
30
BuHN
OR OPTIONALLY

Figure 10

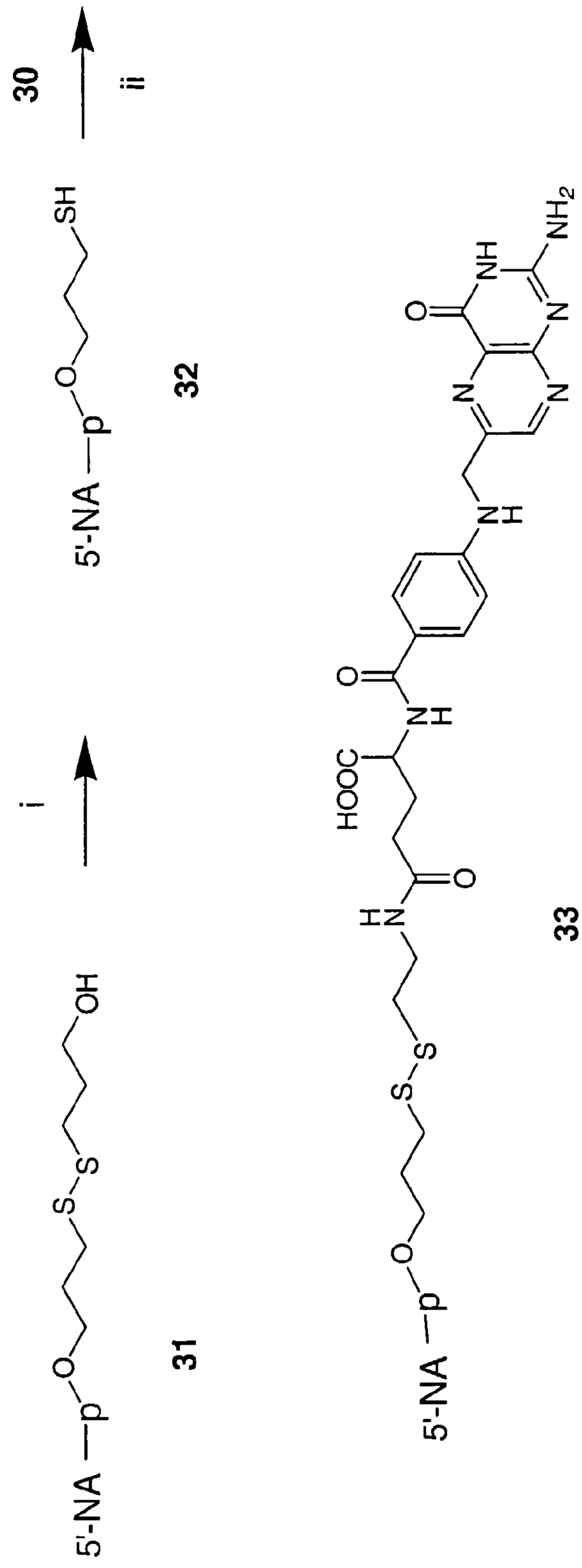

5'-NA—p—O—(CH2)3—SH
30
ii
5'-NA—p—O—(CH2)3—S—S—(CH2)3—OH
31
i
5'-NA—p—O—(CH2)3—S—S—(CH2)3—NH—C(=O)—CH(COOH)—(CH2)2—C(=O)—NH—C6H4—CH2—NH—C7H4N4O—NH2
32
33
NA = Nucleic Acid, such as enzymatic nucleic acid
p = phosphorous moiety

Figure 11

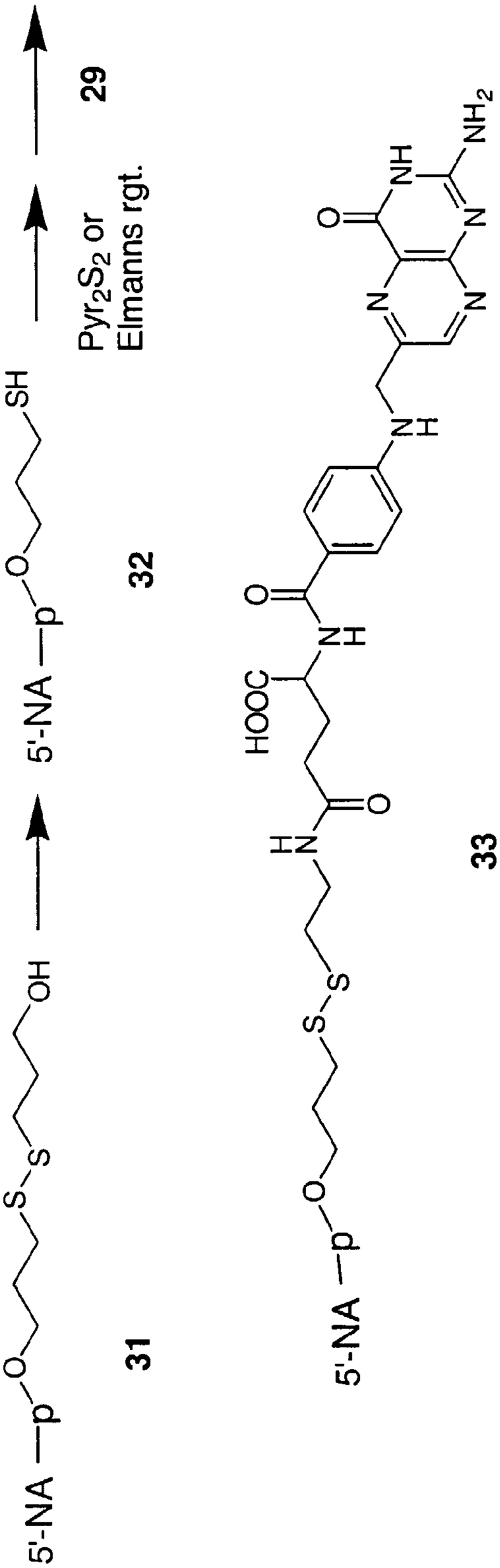

5'-NA—p
O
SH
Pyr2S2 or
Elmanns rgt.
29
31
5'-NA—p
O
S
S
OH
32
5'-NA—p
O
S
S
HOOC
NH
O
NH
NH2
O
N
N
N
33
NA = Nucleic Acid, such as enzymatic nucleic acid
p = phosphorous moiety

Figure 12: Solid Phase Post-synthetic conjugation of pteroic acid

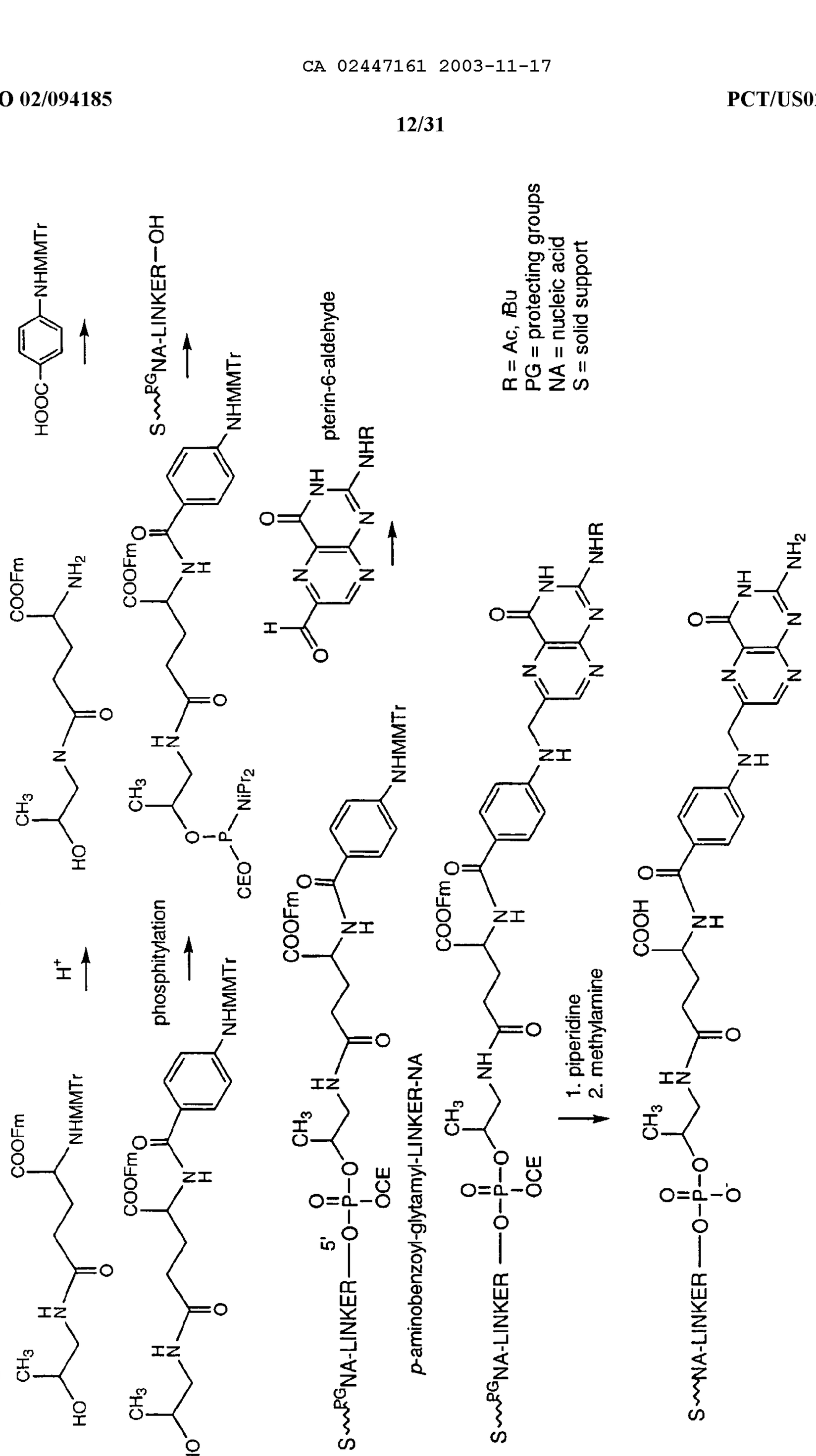
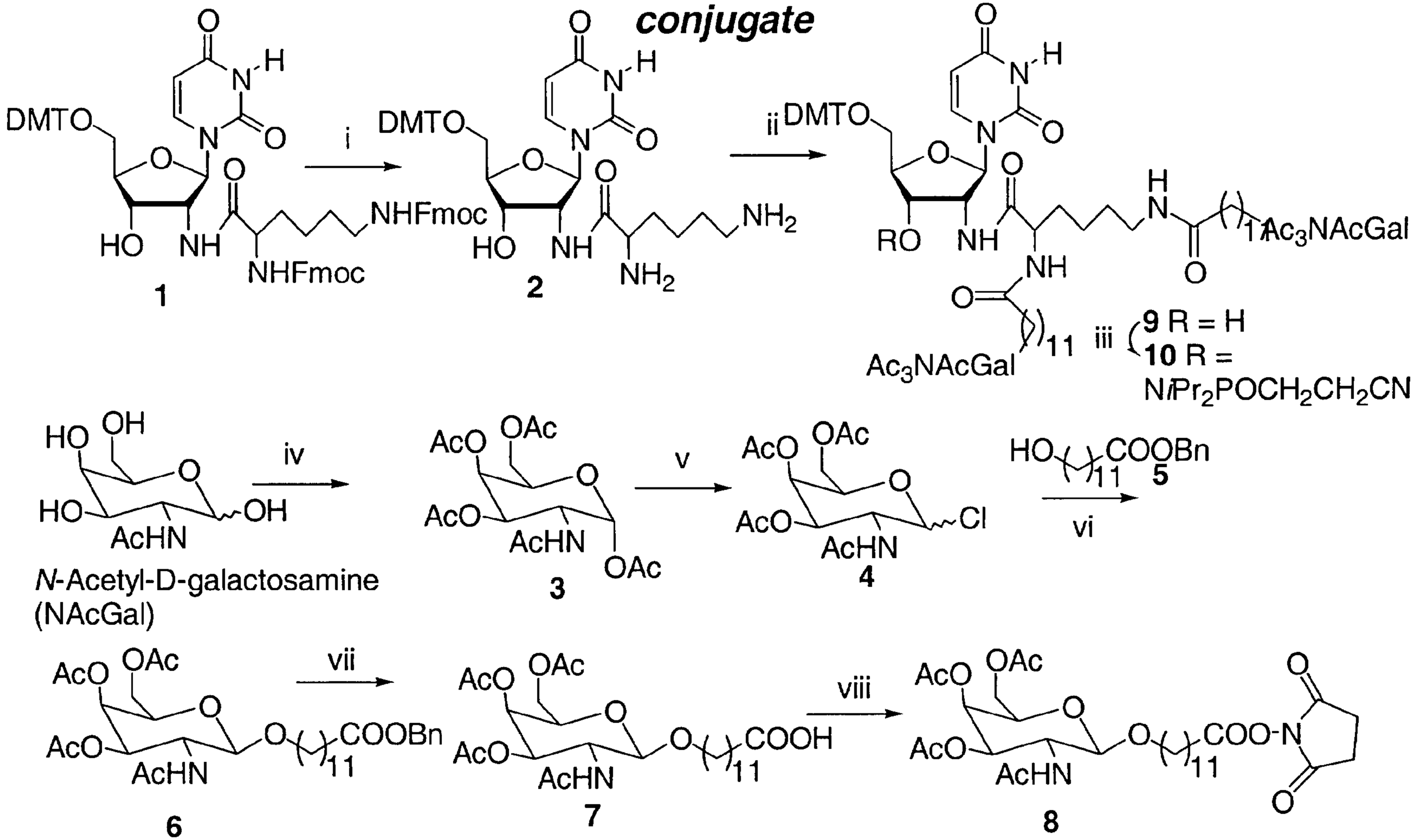
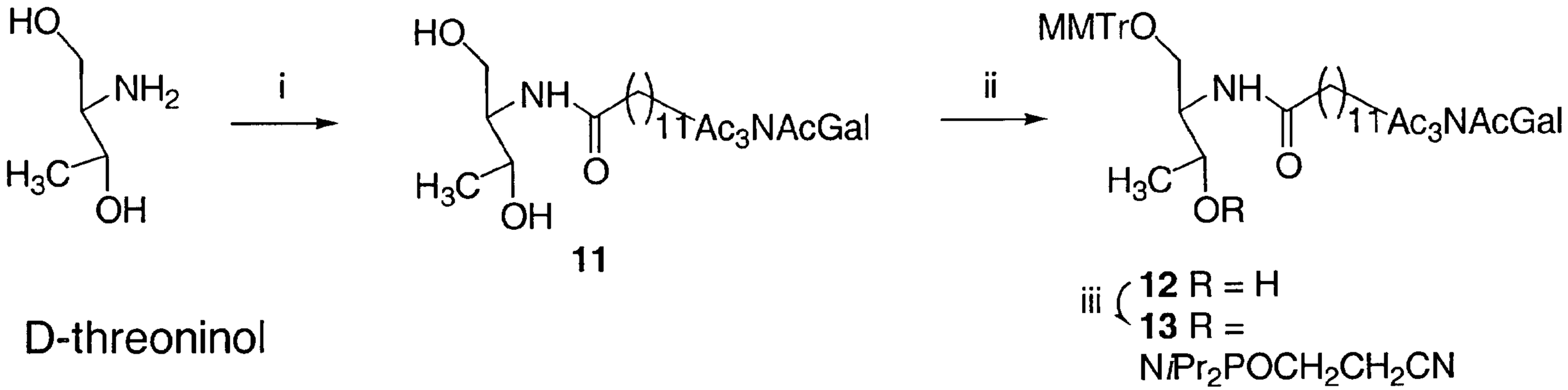


Figure 13: Synthesis of N-acetyl-D-galactosamine-2'-aminouridine conjugate



Reagents and Conditions: (i) diethylamine, DMF, (ii) **8**, diisopropylethylamine, DMF, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2, (iv) Ac_2O, TEA, CH_3CN, (v) HCl, Ac_2O, (vi) $Hg(CN)_2$, MS 4A, CH_3NO_2-toluene 1:1, (vii) H_2, 5% Pd-C, ethanol, (viii) *N*-hydroxysuccinimide, DCC, THF.

Figure 14: Synthesis of N-acetyl-D-galactosamine-D-threoninol conjugate



D-threoninol

Reagents and Conditions: (i) **7**, DCC, *N*-hydroxysuccinimide, (ii) MMTr-Cl, pyridine, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2.

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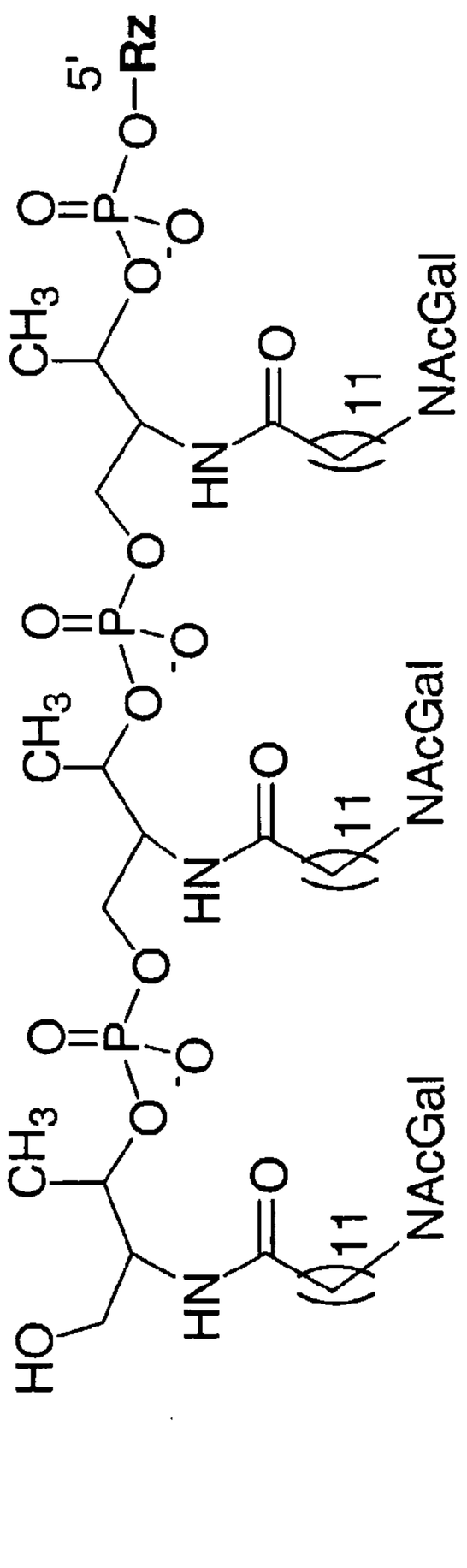
Figure 15: Conjugation of targeting ligands to the 5'-end of a Ribozyme



N-acetyl-D-galactosamine conjugate

Figure 16: Synthesis of dodecanoic acid linker

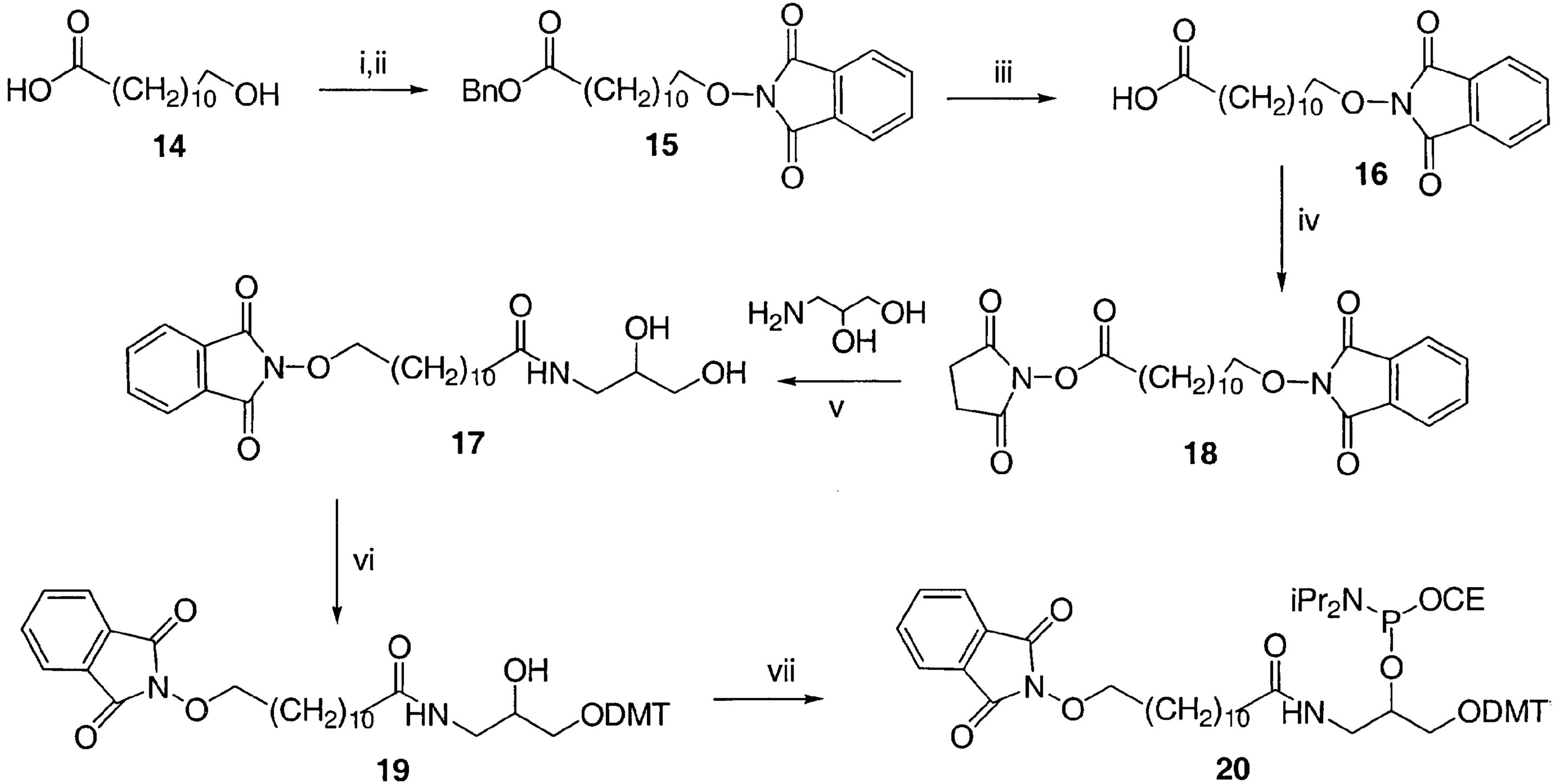


Figure 17: Oxime linked Nucleic Acid/Peptide Conjugate

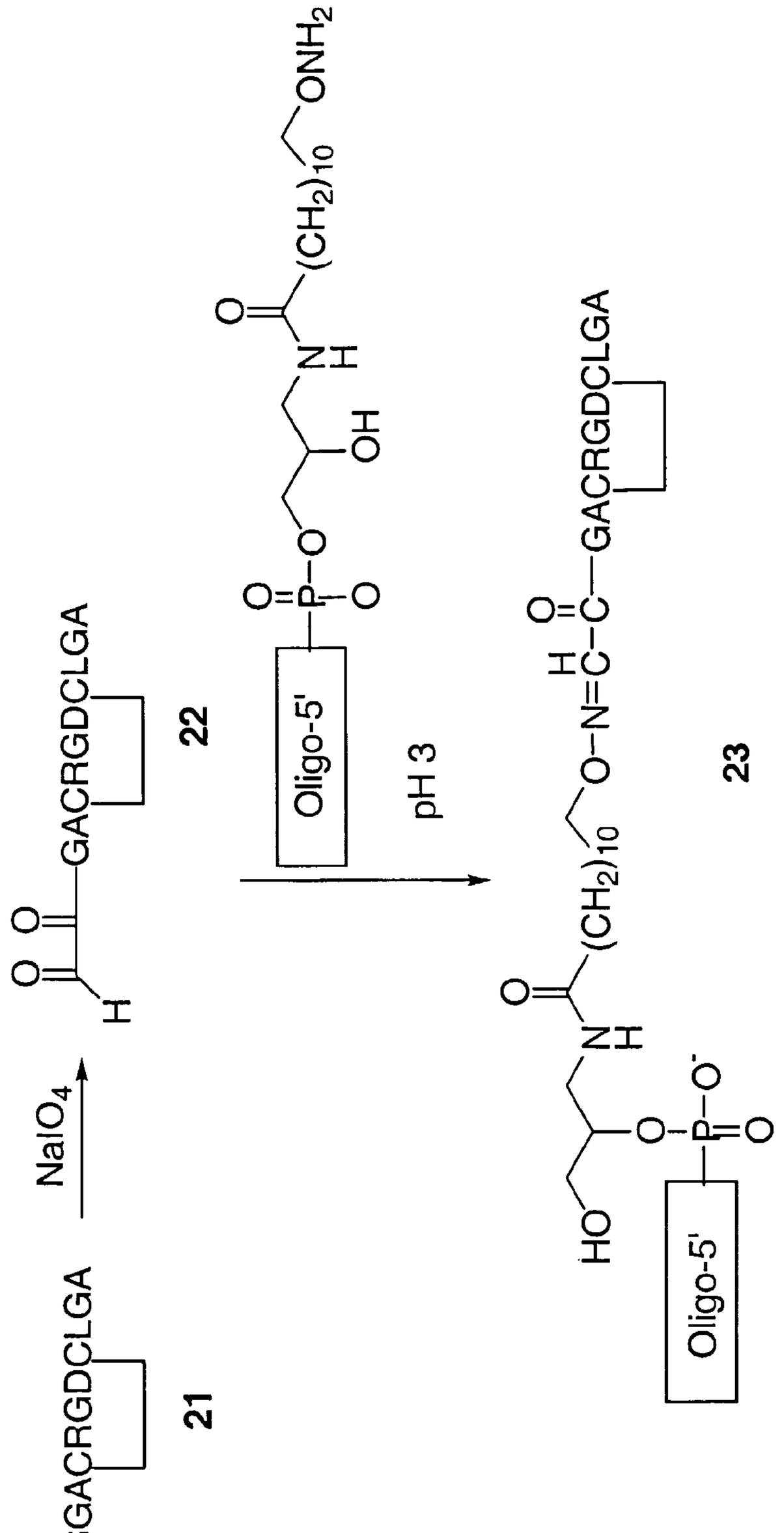
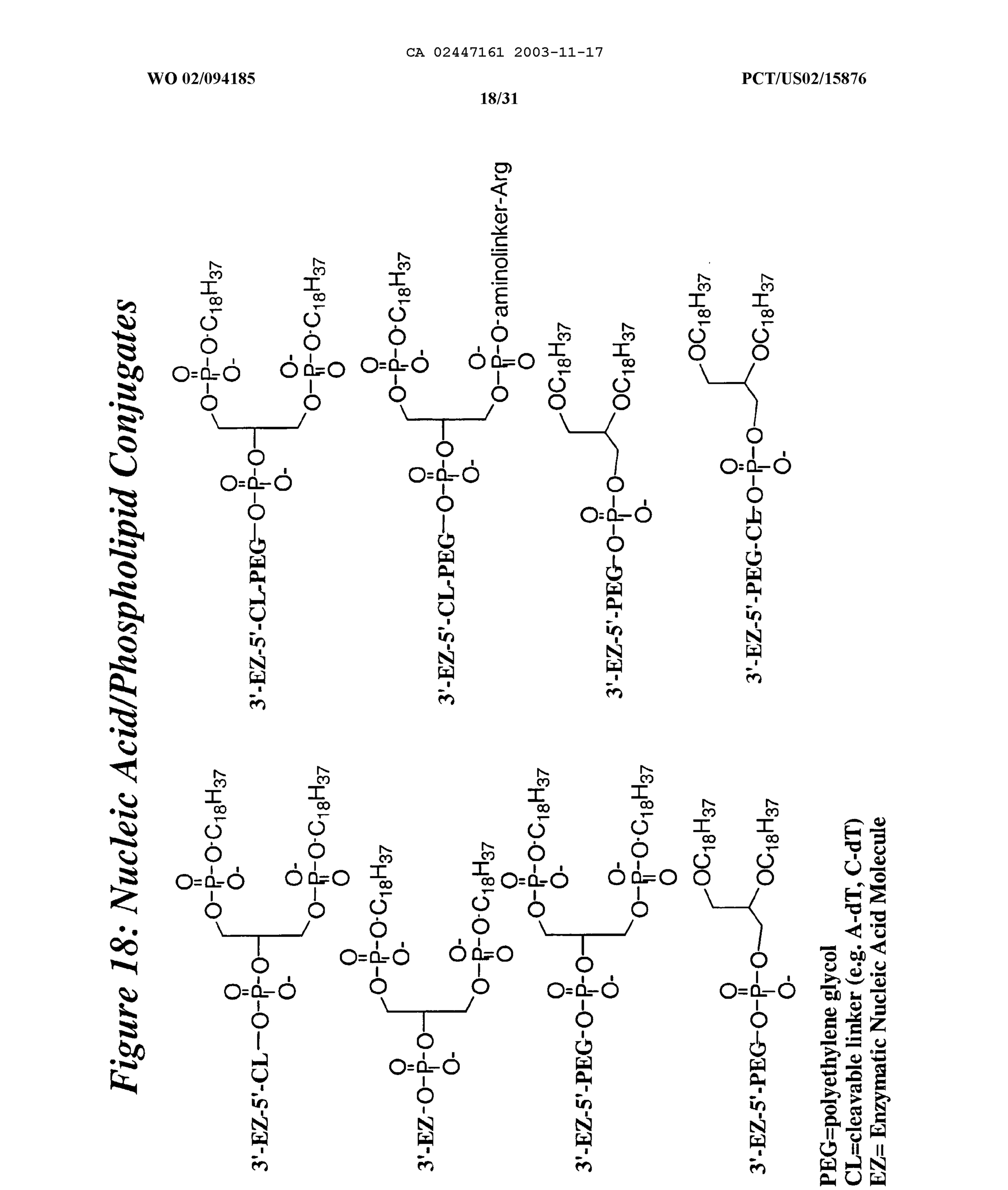


Figure 18: Nucleic Acid/Phospholipid Conjugates



PEG=polyethylene glycol
CL=cleavable linker (e.g. A-dT, C-dT)
EZ= Enzymatic Nucleic Acid Molecule

Figure 19: Angiozyme Phospholipid Conjugate

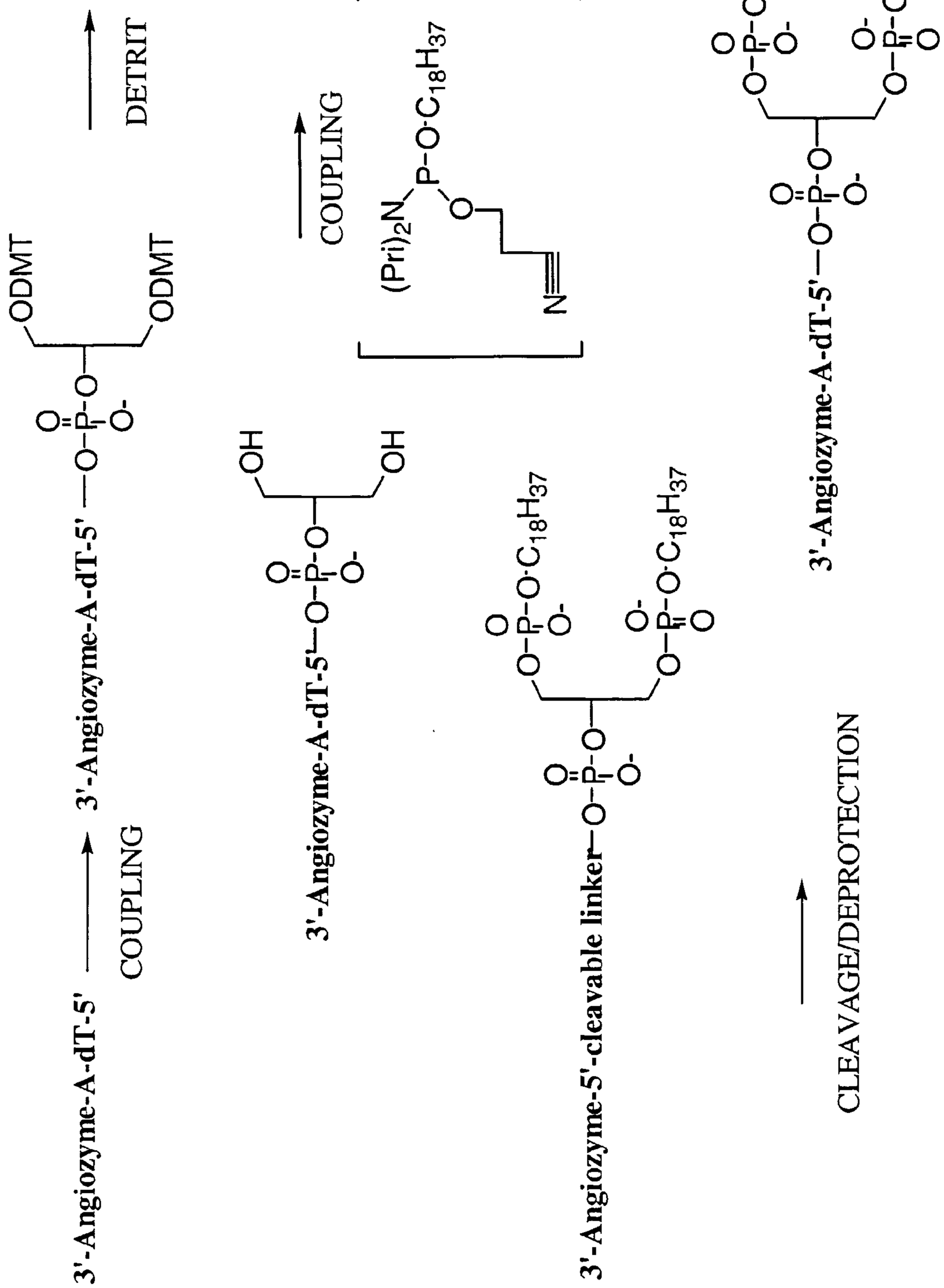
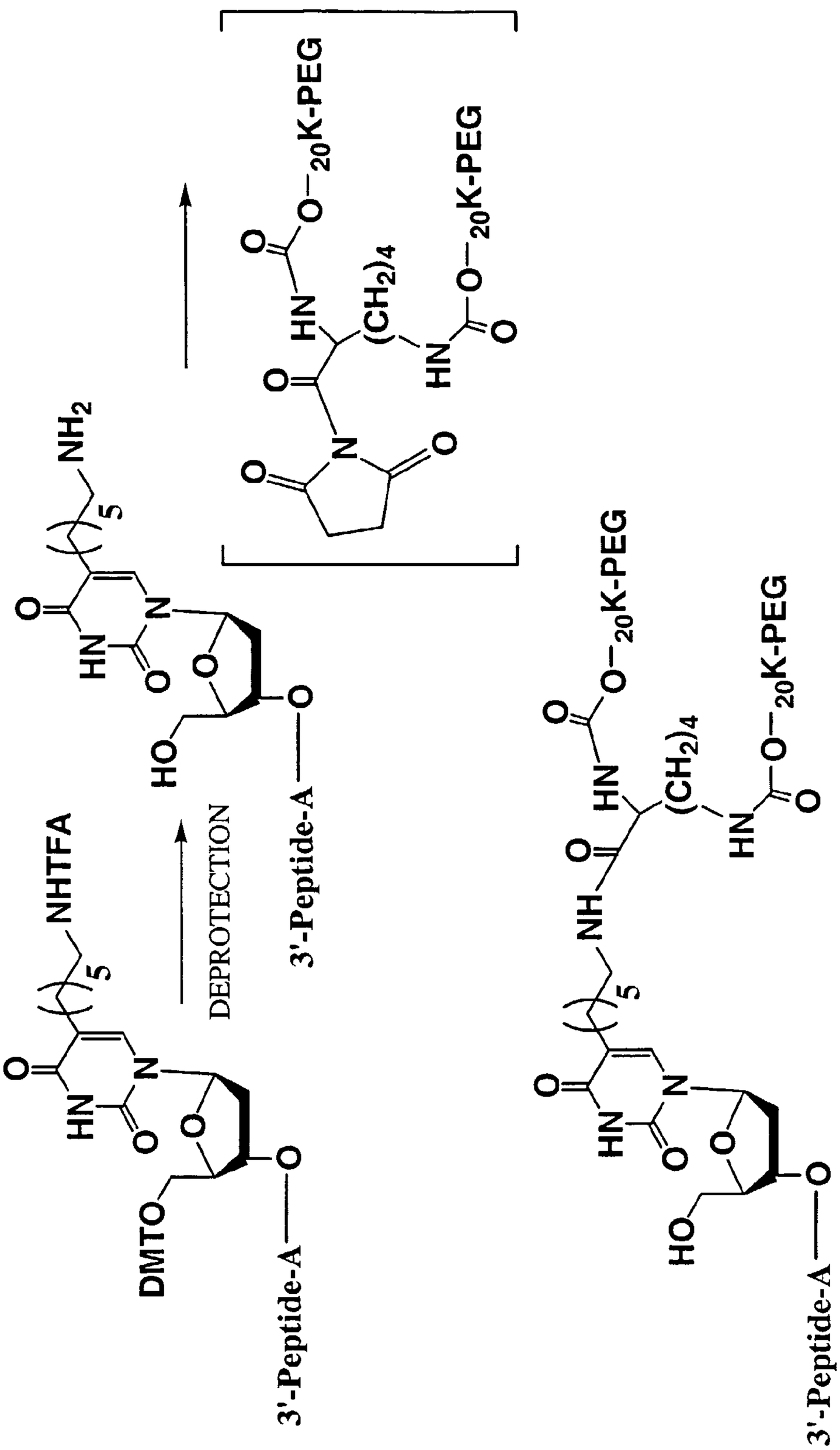


Figure 20: Peptide PEG Conjugate



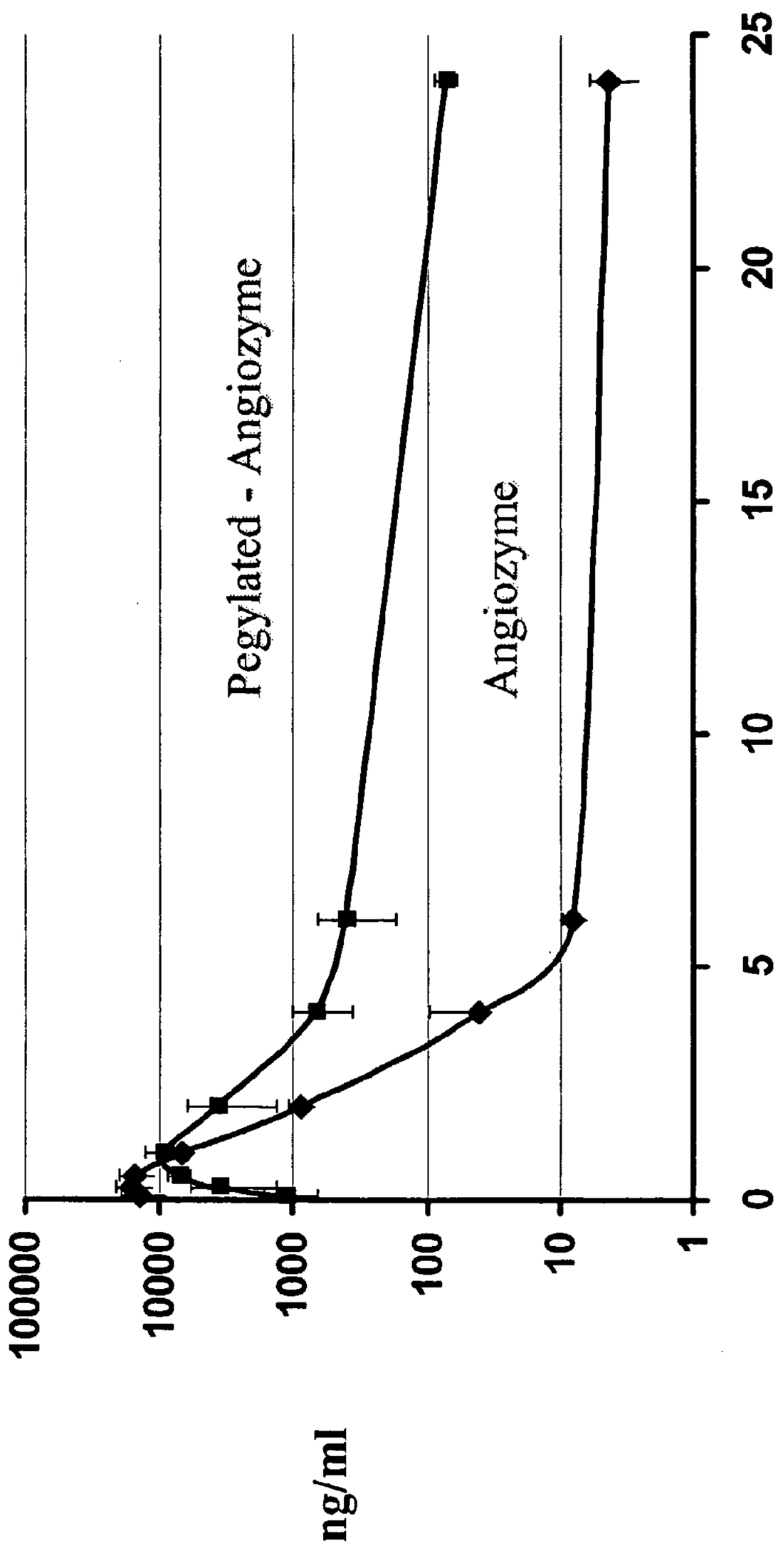


Figure 21: 40-KDa PEG-Angiozyme vs Angiozyme

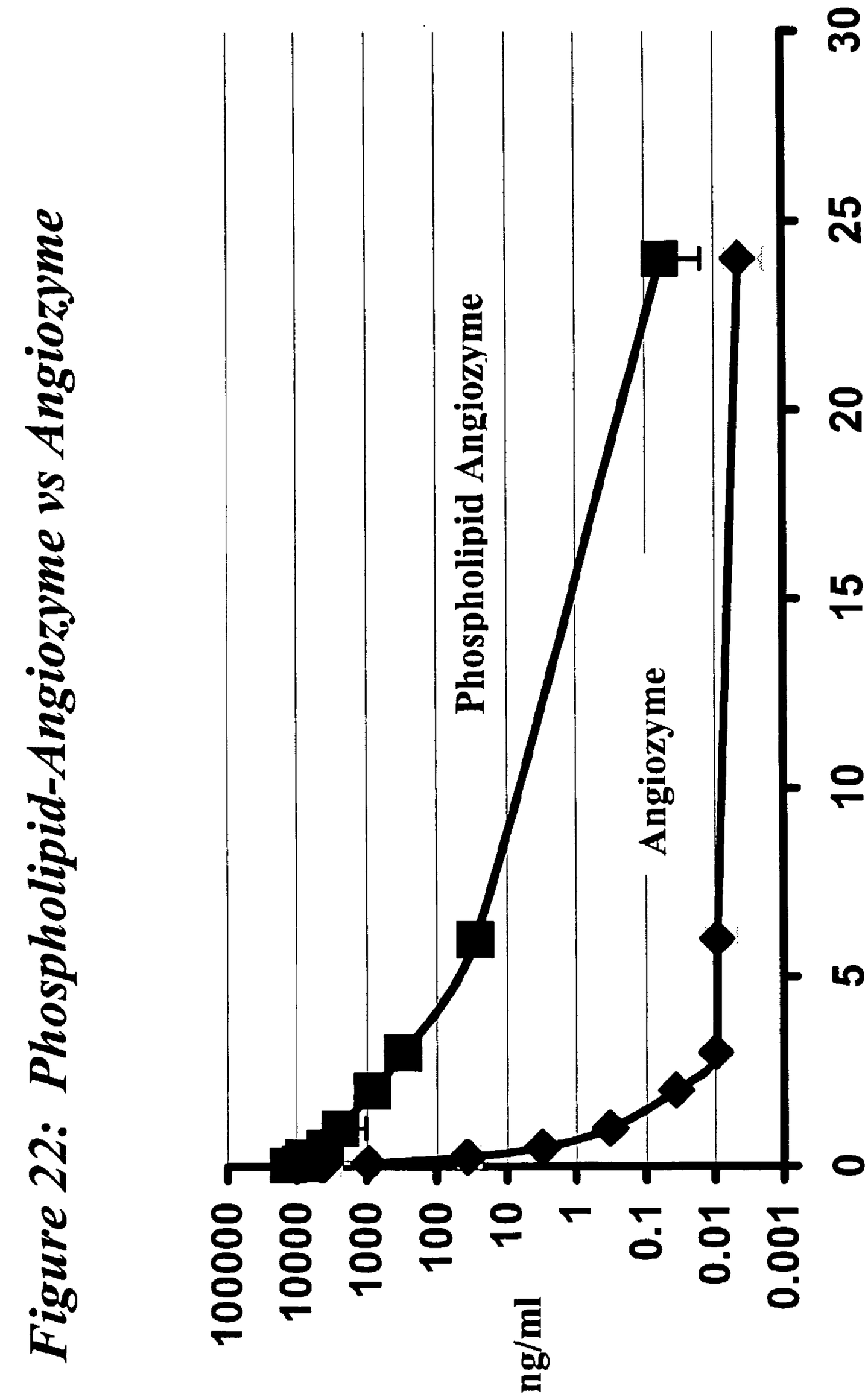


Figure 22: Phospholipid-Angiozyme vs Angiozyme
Phospholipid Angiozyme
Angiozyme
Time hours
ng/ml
100000
10000
1000
100
10
1
0.1
0.01
0.001
0
5
10
15
20
25
30

Figure 23: Oligonucleotide-NAcGalactosamine post-synthetic coupling

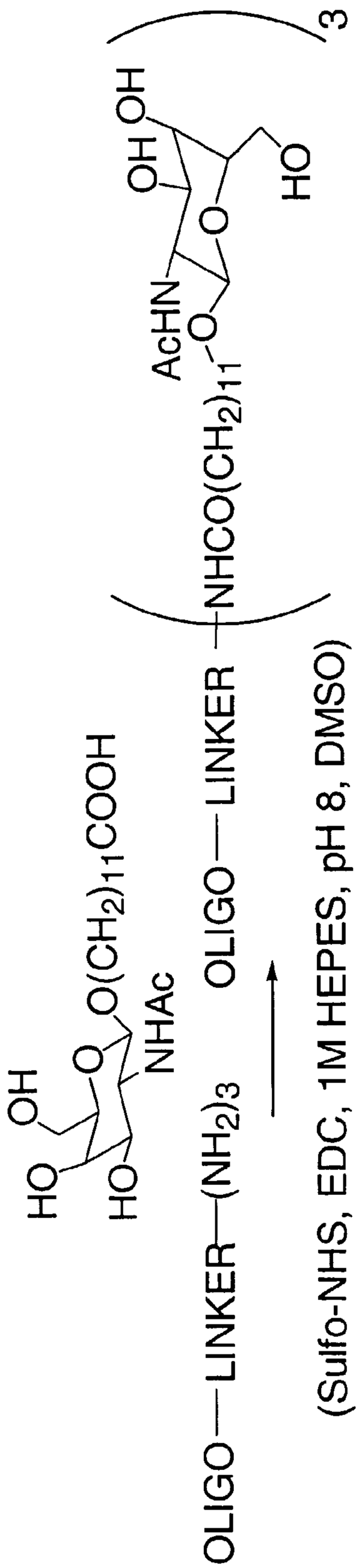


Figure 24a: Protein with cleavable linker

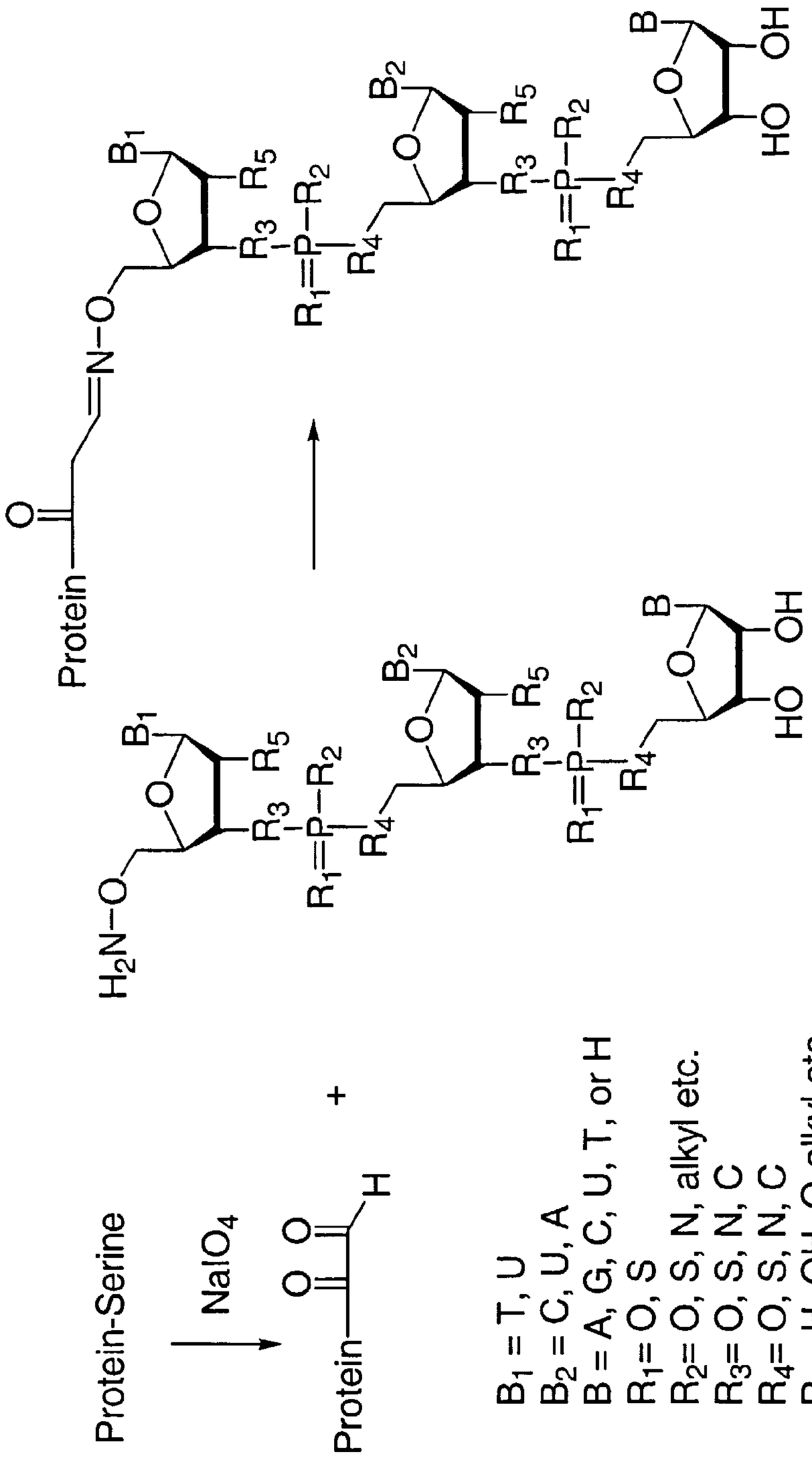


Figure 24b: Protein cleavable linker PEG Conjugate

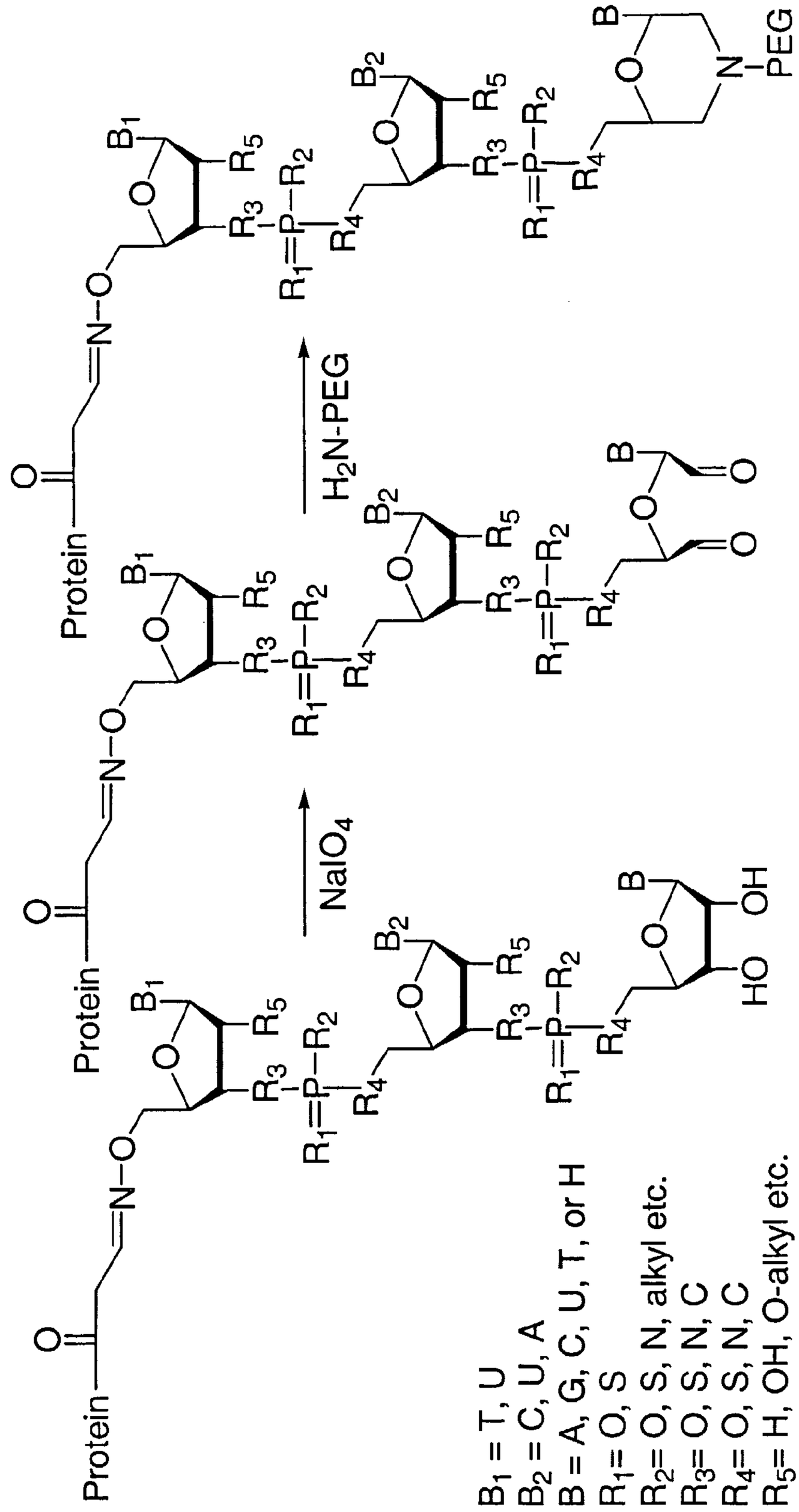


Figure 25: Protein PEG conjugate with cleavable linker

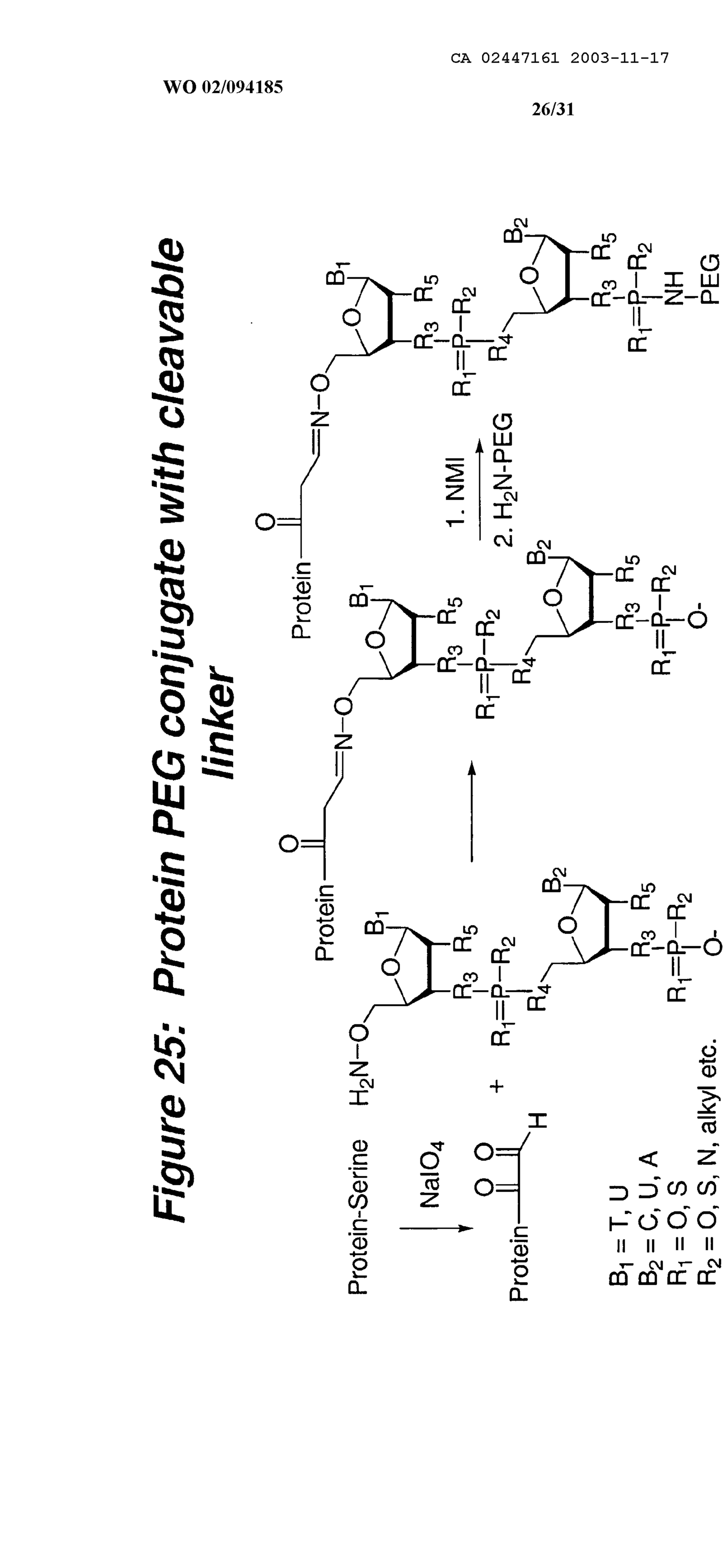


Figure 26a: PEG with cleavable linker

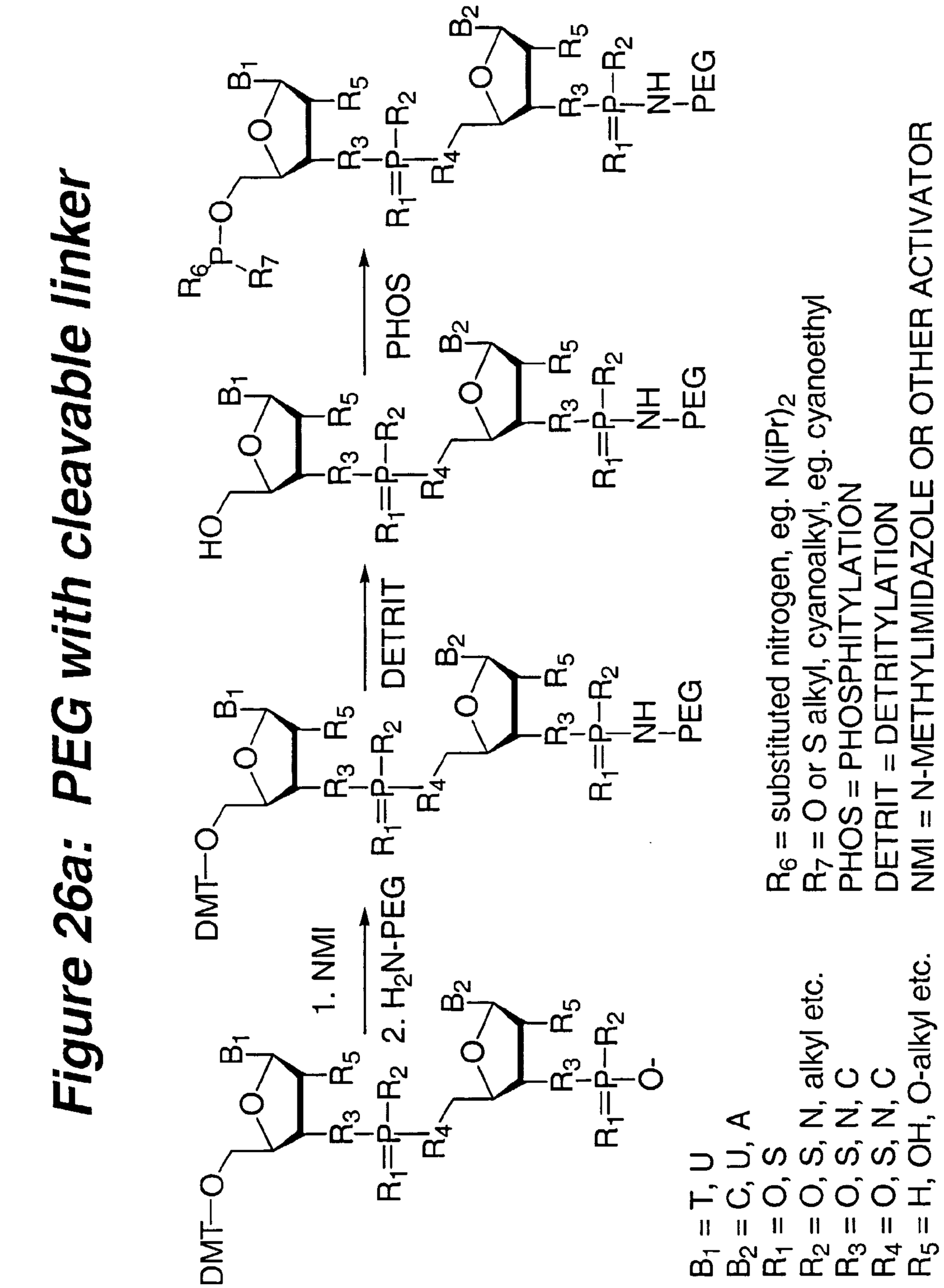


Figure 26b: Protein PEG conjugate with cleavable linker

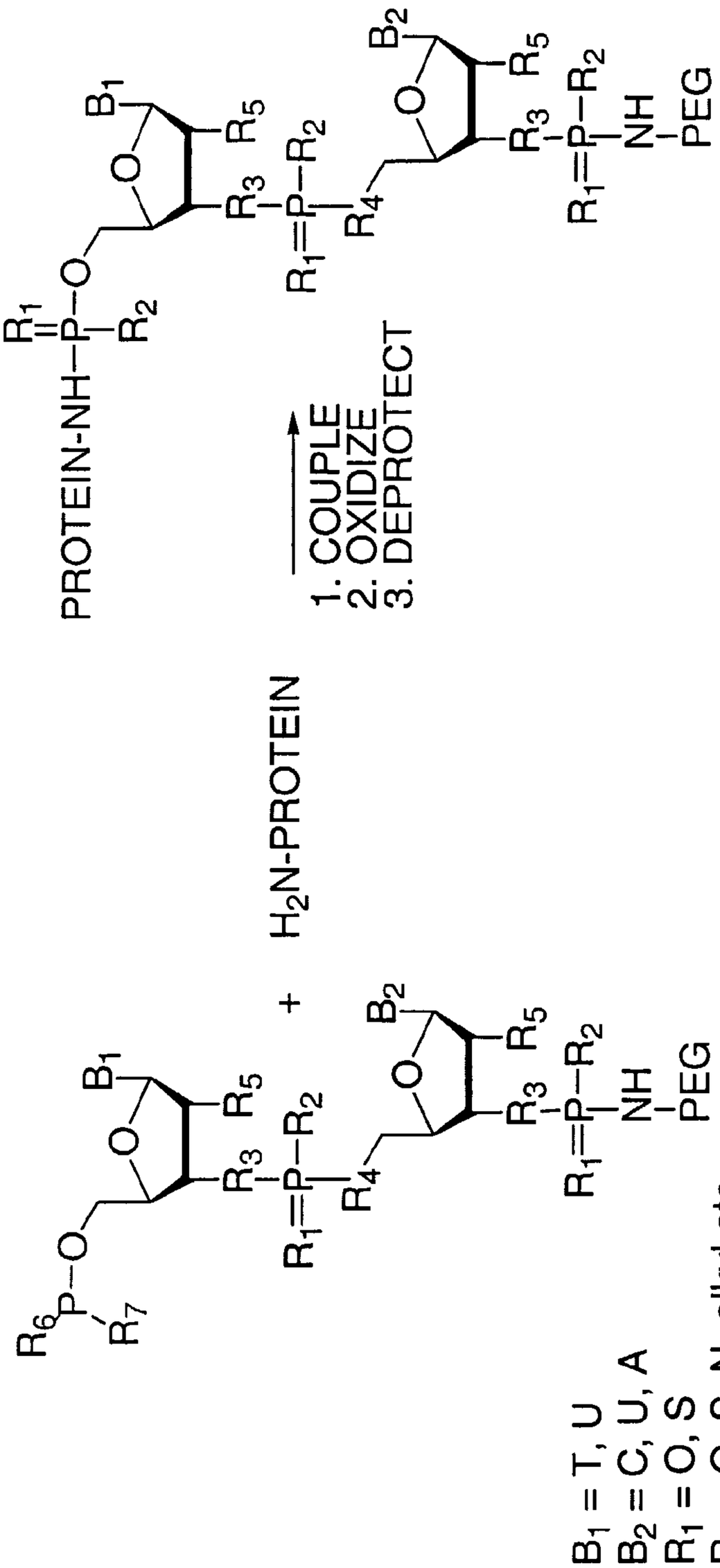
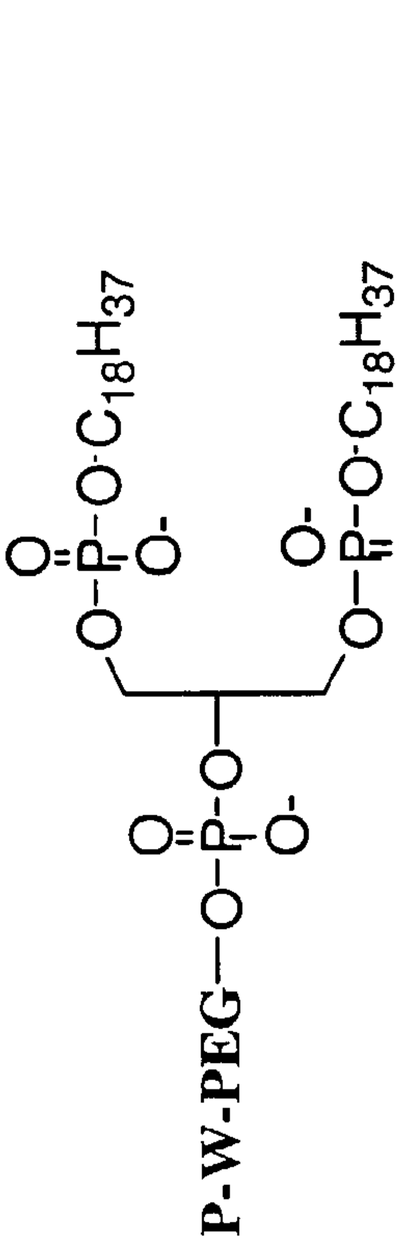
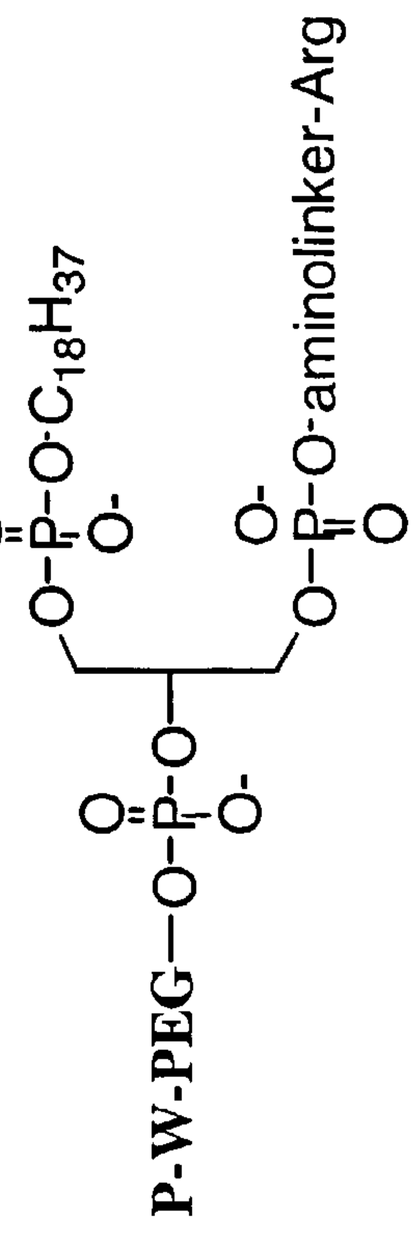
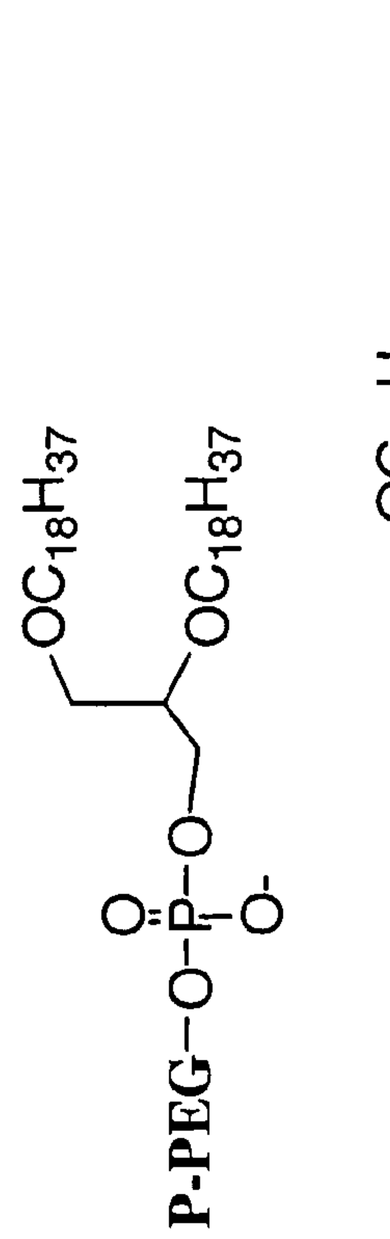
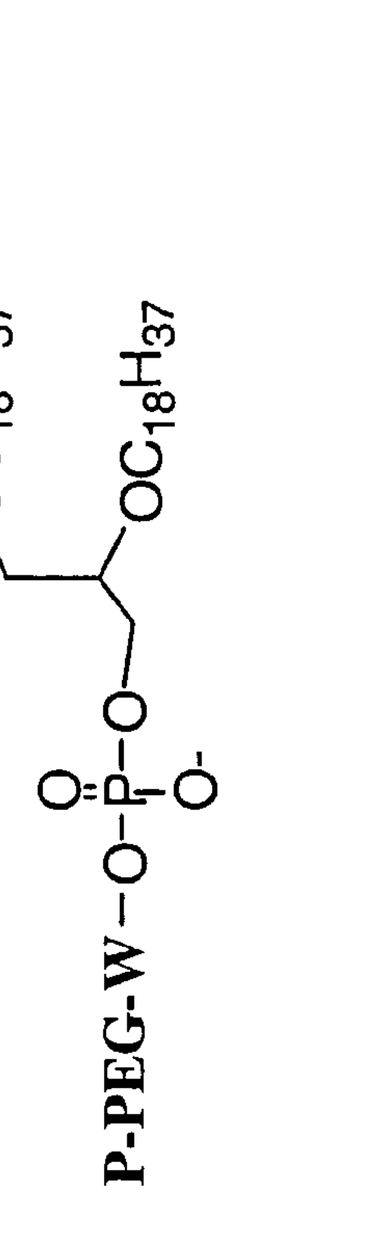


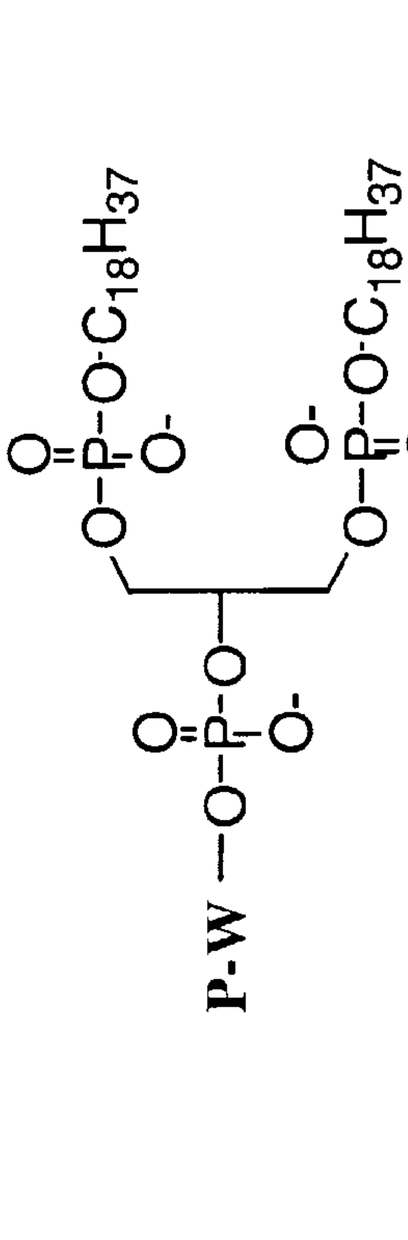
Figure 27: Peptide or Protein/Phospholipid Conjugates

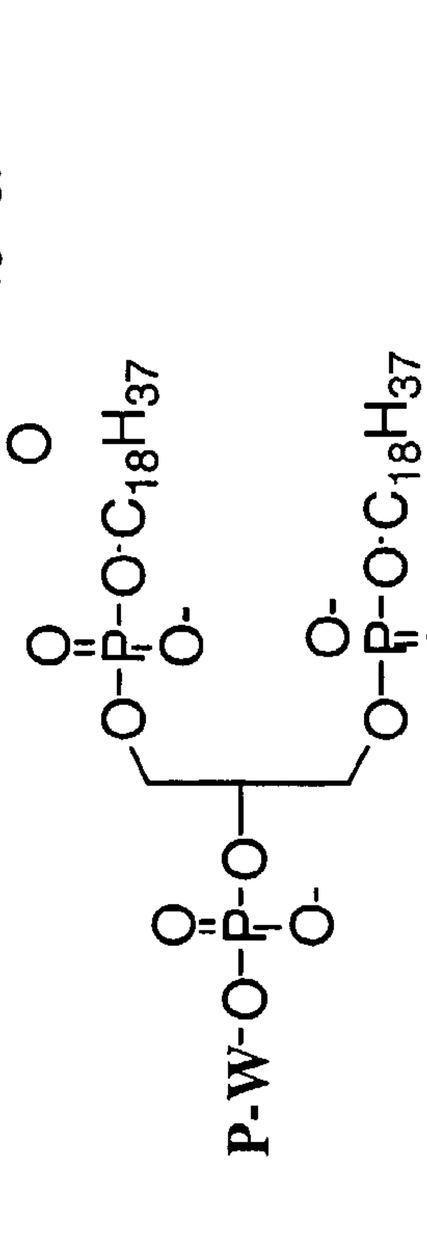


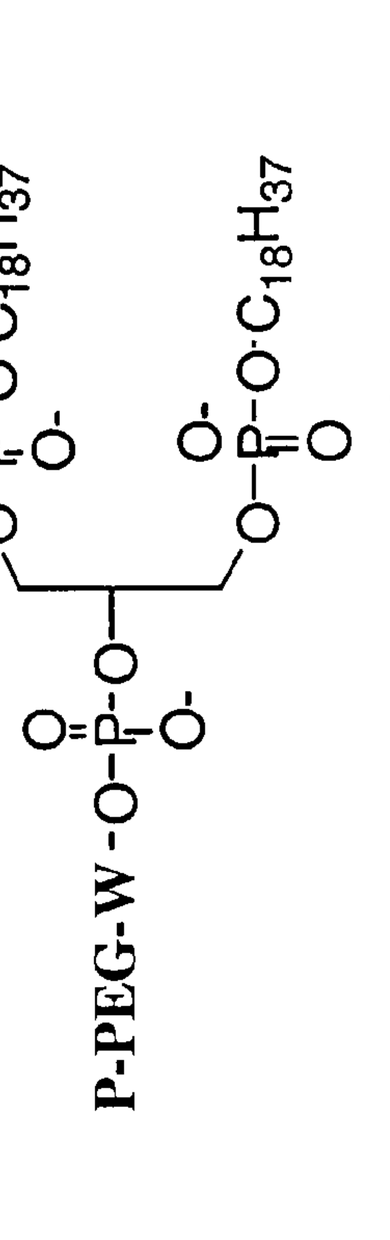


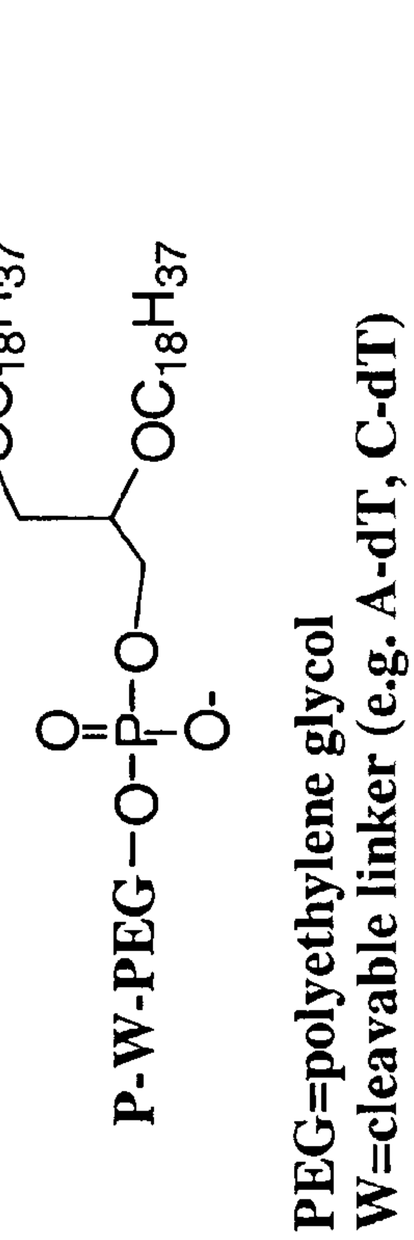






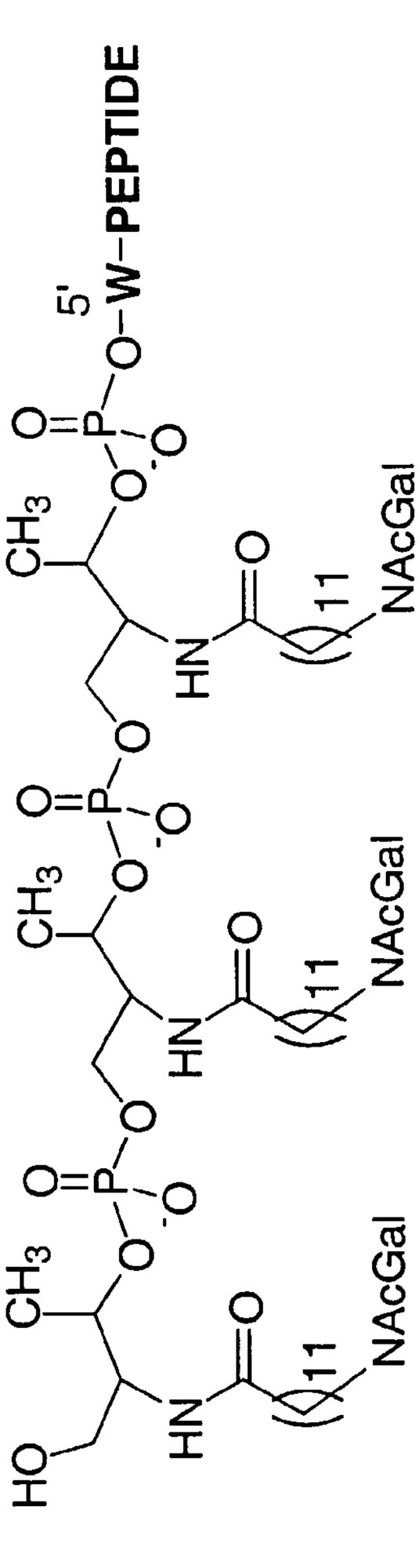






PEG=polyethylene glycol
W=cleavable linker (e.g. A-dT, C-dT)
P= Peptide/Protein

Figure 28: Conjugation of targeting ligands to a peptide protein



N-acetyl-D-galactosamine conjugate

W = cleavable linker (eg. A-dT, C-dT dimer)

Figure 29: Protein/PEG conjugate with cleavable linker

