FORM 1

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

610687

APPLICATION FOR A STANDARD PATENT

Xoma Corporation, incorporated in Delaware, of 2910 Seventh Street, Berkeley, California, 94710, UNITED STATES OF AMERICA, hereby apply for the grant of a standard patent for an invention entitled:

Disulphide Derivatives as Linking Agents

~~Linking Agents and Methods~~

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
151700	US	3 February 1988

The address for service is:-

Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia

DATED this THIRTIETH day of JANUARY 1989

Xoma Corporation

AUSTRALIAN PATENT OFFICE

By: M.J. Anderson

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 83290
S&F CODE: 63070

REPRINT OF RECEIPT
3005091 30/01/89

APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 5.3.91

5845/2

PATENT DECLARATION FORM (CONVENTION)

COMMONWEALTH OF AUSTRALIA

Patents Act 1952

Regulation 12 (2)

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

To be signed by the applicant(s) or in the case of a body corporate to be signed by a person authorised by the body corporate.

In support of the Convention application made for a patent for an invention entitled

(a) Insert title of invention. (a) LINKING AGENTS AND METHODS

I Martin H. Goldstein, Vice President, General Counsel

(b) Insert full name(s) of declarant(s). of ~~I/We~~ (b) Xoma Corporation

(c) Insert address(es) of declarant(s). ~~of~~ (c) 2910 Seventh Street, Berkeley, California 94710 United States of America

do solemnly and sincerely declare as follows:–

~~1. I am/We are the applicant(s) for the patent~~

(OR, IN THE CASE OF AN APPLICATION BY A BODY CORPORATE.)

1. I am/~~We are~~ authorised by Xoma Corporation the applicant for the patent to make this declaration on its behalf.

2. The basic application(~~s~~) as defined by Section 141 of the Act was/were made in the following country ~~or countries~~ on the following date(~~s~~) namely:–

(d) Insert country in which basic application(s) was/were filed. (e) Insert date of basic application(s). (f) Insert full names of basic applicant(s).

in (d) the United States of America on (e) 03 February 1988
by (f) Dayton Thomas Reardan & Dane Allen Goff
in (d) ..... on (e) .....
by (f) .....
in (d) ..... on (e) .....
by (f) .....

~~3. I am/We are the actual inventor(s) of the invention referred to in the basic application.~~

(OR, WHERE A PERSON OTHER THAN THE INVENTOR IS THE APPLICANT)

(g) Insert full name(s) of actual inventor(s) (h) Insert address(es) of actual inventor(s).

3. (g) Dayton Thomas Reardan and Dane Allen Goff
of (h) 151 Falcon Way, Hercules, California 94547; and 1177 Noel Drive, Menlo Park, California 94025 respectively, both in the United States of America

is/are the actual inventor(s) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows:

(i) Set out how applicant(s) derive(s) title from actual inventor(s) i.e., assignee of the invention from the actual inventor(s).

(i) assignment dated 8 March 1988 from the actual inventors to the said applicant.

4. The basic application(~~s~~) referred to in paragraph 2 of this Declaration was/~~were~~ the first application(~~s~~) made in a Convention country in respect of the invention the subject of the application.

Attestation or legalization not required.

Declared at Berkeley this 16th day of January 198 9

To:

The Commissioner of Patents

Xoma Corporation

By: Martin H. Goldstein (Signature of Declarant(s))

Name: Martin H. Goldstein

Title: Vice President, General Counsel

(12) PATENT ABRIDGMENT (11) Document No. AU-B-28902/89
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 610687

(54) Title
DISULPHIDE DERIVATIVES AS LINKING AGENT

International Patent Classification(s)
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(71) Applicant(s)
XOMA CORPORATION

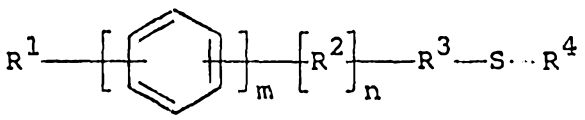
(72) Inventor(s)
DANE ALLEN GOFF; DAYTON THOMAS REARDAN

(74) Attorney or Agent
SPRUSON & FERGUSON, GPO Box 3898, SYDNEY NSW 2001

(56) Prior Art Documents
AU 556328 16937/83 C07D 213/71

(57) Claim

1. A compound having the formula



in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of $-NH-C(O)-$, $-C(O)-NH-$, and $-C(O)-$;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,

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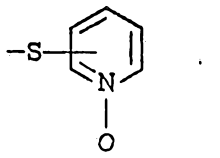
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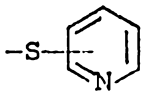
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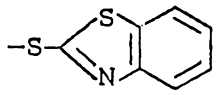
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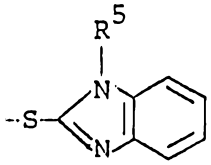
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where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

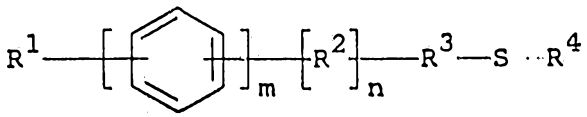
n is zero or 1;

and analogs comprising water-soluble salts and water-soluble derivatives thereof.

11. A method for linking a first compound containing a carbohydrate moiety to a second compound containing a thiol group, said method comprising:

(a) oxidizing said first compound to convert said carbohydrate moiety to an aldehyde group; and

(b) reacting said first and second compounds with a compound having the formula



in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of -NH-C(O)-, -C(O)-NH-, and -C(O)-;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,

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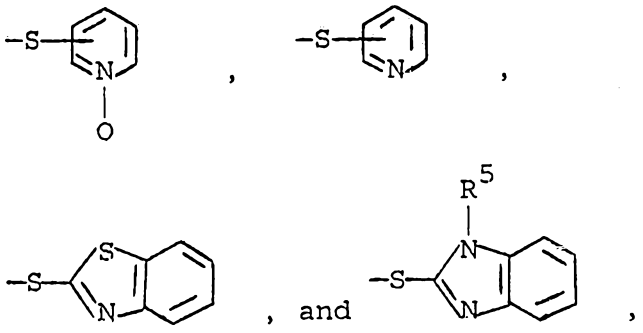


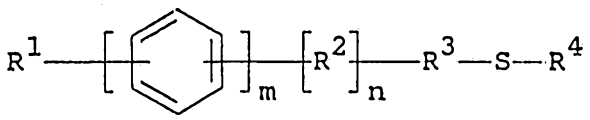
where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

n is zero or 1;

and analogs comprising water-soluble salts and water-soluble derivatives thereof.

21. A method for linking a first compound containing a carboxyl group to a second compound containing a thiol group, said method comprising reacting said first and second compounds with a compound having the formula



in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of -NH-C(O)-, -C(O)-NH-, and -C(O)-;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,

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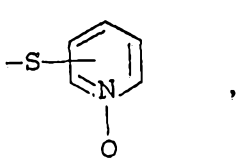
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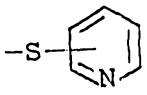
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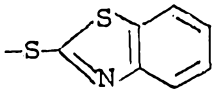
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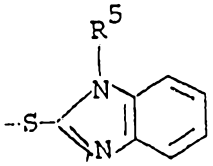

,

where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

S & F Ref: 83290

FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION 610687

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:
Accepted:
Published:

This document contains the amendments made under Section 49 and is correct for printing.

Priority:

Related Art:

Name and Address of Applicant: Xoma Corporation
2910 Seventh Street
Berkeley California 94710
UNITED STATES OF AMERICA

Address for Service: Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower, 31 Market Street
Sydney, New South Wales, 2000, Australia

Complete Specification for the invention entitled:

Disulphide Derivatives as Linking Agent

~~Linking Agents and Methods~~

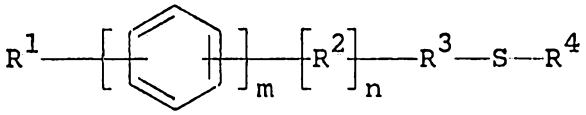
The following statement is a full description of this invention, including the best method of performing it known to me/us

AUSTRALIAN PATENT OFFICE 58/5/3

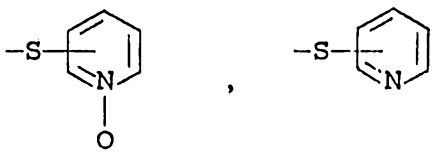
~~LINKING AGENTS AND METHODS~~
DISULPHIDE DERIVATIVES AS LINKING AGENT

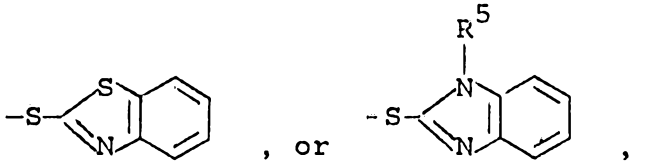
ABSTRACT OF THE DISCLOSURE

Compounds containing carbohydrate moieties or carboxyl groups are linked to either compounds containing thiol moieties or electron-deficient moieties by the use of linking agents of the formula



in which R^1 is NH_2– or NH_2–NH–; R^2 is –NH–C(O)–, –C(O)–NH–, or –C(O)–; R^3 is C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene, arylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, or amino-substituted C_1-C_{10} alkylene; R^4 is H, acetyl,





where R^5 is C_1-C_5 alkyl; m is zero or 1; and n is zero or 1.

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~~LINKING AGENTS AND METHODS~~
DISULPHIDE DERIVATIVES AS LINKING AGENT

BACKGROUND OF THE INVENTION

This invention relates to the formation of conjugates, and in particular to the joining of one species to another at a carbohydrate or carboxyl moiety on one of the species, utilizing a thioether or disulfide bond as part of the linkage.

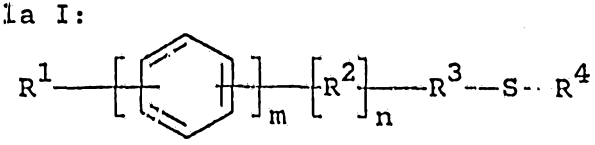
The linkage of compounds of various types to carbohydrate or carboxyl moieties is desirable for a variety of reasons. In the formation of conjugates involving immunoglobulins, for instance, linkage at specific regions on the immunoglobulin is often desirable for purposes of maintaining the accessibility of antigen-binding sites or of sites on the Fc chains for complement binding. As a further example, certain solid supports used in affinity chromatography have carbohydrate or carboxyl groups available for binding, and the same is true for other solid phase materials as well such as those used in two-phase immunoassays.

Many of the species which are sought to be linked to these carbohydrate or carboxyl moieties are species which lack the ability to react directly.

SUMMARY OF THE INVENTION

Novel compositions and methods are provided herein for the formation of linkages of the type described above at carbohydrate or carboxyl moieties. The novel compositions are linking agents falling within the following generic formula:

Formula I:



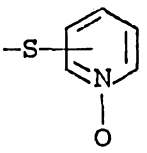
in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of $-NH-C(O)-$, $-C(O)-NH-$, and $-C(O)-$;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene; and

R^4 is a member selected from the group consisting of H, acetyl,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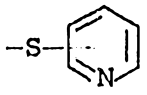
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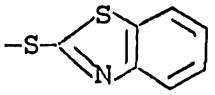
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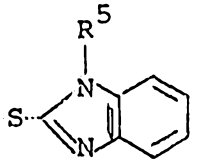

,

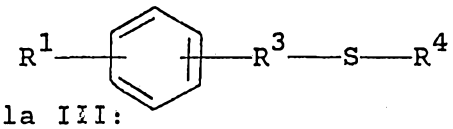
where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

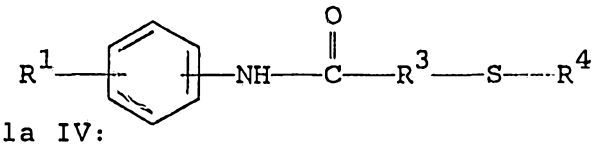
n is zero or 1.

Within the scope of this formula, certain embodiments are preferred, notably those of the formulas given below. (In each of these formulas, R^1, R^3, and R^4 are as defined above.)

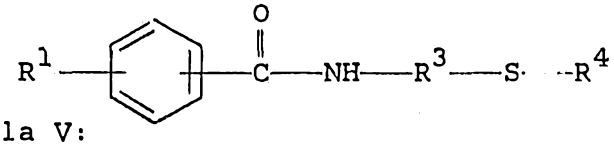
Formula II:



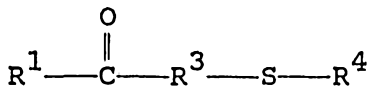
Formula III:

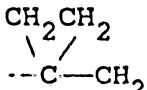


Formula IV:



Formula V:



In each of these formulas, the terms "alkylene" and "alkyl" refer to saturated divalent and monovalent hydrocarbon radicals, respectively, and are intended to include straight-chain, branched-chain and cyclic structures. Examples of alkylene groups are $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$ and longer chains; and $-CH(CH_3)-$, $-C(CH_3)_2-$, $-CH(CH_3)\cdot CH_2-$, $-CH(CH_3)-CH(CH_3)-$, $-CH(CH_3)_2-CH_2-$,  and the reverses thereof (i.e., left-to-right). Preferred alkylene groups are those having 1 to 6 carbon atoms, the most preferred being those having 1 to 4 carbon atoms.

The term "cyclic alkylene" refers to a saturated divalent cyclic hydrocarbon radical, with the two points of attachment being at any two locations on the ring. Examples are cyclopentylene, cyclohexylene, and cycloheptylene. A preferred group is 1,2-cyclohexylene

(in which the two points of attachment are on adjacent carbon atoms on the ring).

The term "arylene" refers to a divalent radical containing at least one aromatic ring, with the two points of attachment being at any two locations on the ring (or rings, in the case of multi-ring groups). Examples are phenylene, particularly 1,2-phenylene, and naphthylene.

In Formulas I, II, III and IV, the two points of attachment on the phenyl ring may be ortho-, meta- or para- with respect to each other. Compounds in which the points of attachment are meta- or para- are preferred, with para- the most preferred. In the R^4 definition, the sulfur substituent on the pyridine and pyridine N-oxide may be in the 2-, 3-, or 4-position. The 2- and 4-positions are preferred, with the 2-position being the most preferred.

The following are examples of compounds within these formulas:

S-acetyl-4-(4-aminophenyl)-1-butanethiol
S-acetylthioacetic acid, 4-aminoanilide
2-pyridyl-3'-propanoyl disulfide, 4-aminoanilide
S-acetylthioacetic acid hydrazide
2-pyridyl-3'-propanoyl disulfide hydrazide
2-pyridyl-1'-methyl-3'-propanoyl disulfide hydrazide
2-pyridyl-1',1'-dimethyl-3'-propanoyl disulfide hydrazide
2-pyridyl-1',2'-dimethyl-3'-propanoyl disulfide hydrazide
2-pyridyl-2',2'-dimethyl-3'-propanoyl disulfide hydrazide
2-pyridyl 1'-(2'-ethanoyl)-cyclopropane disulfide hydrazide
2-pyridyl-1',1'-dimethyl-2'-amino-3'-propanoyl disulfide hydrazide

2-pyridyl-1′-isopropyl-2′-ethanoyl disulfide hydrazide

2-pyridyl-1′-phenyl-2′-ethanoyl disulfide hydrazide

2-pyridyl-1′-methyl-2′-ethanoyl disulfide hydrazide

2-pyridyl-1′,1′-dimethyl-2′-ethanoyl disulfide hydrazide

2-pyridyl 1′-(2′-ethanoyl)-cyclohexane disulfide hydrazide

2-pyridyl 1′-(2′-ethanoyl)-cycloheptane disulfide hydrazide

The invention further extends to water-soluble salts and derivatives of these compounds. The salts may be formed by anionic moieties to complement the amine (or hydrazine) terminus of the compounds in cationic form. Examples of such salts are acetate salts, trifluoroacetate salts, hydrohalide salts, particularly hydrochloride and hydrobromide salts, and toluenesulfonic acid salts. The derivatives may be compounds having the same formula but with the addition of a charge group at a point on the molecule where it does not interfere with the coupling ability of either of the end groups on the molecule. Examples of such derivatives are those bearing sulfonic acid ($-SO_3-$) groups and those bearing nonreactive amino groups (such as diethylamino, for example). The most preferred among these are the trifluoroacetate salts.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The compounds of the present invention are prepared by conventional techniques well known among those skilled in the art, selected in accordance with the desired substituent groups of the formulas as listed above. Compounds with aminophenyl terminal groups

(as in Formulas II, III and IV with R^1 = NH_2–), for instance, may be prepared from the corresponding nitrophenyl analogs by reduction. An example is reduction with stannous chloride. Compounds where R^1 is a hydrazino group may be prepared from an N-protected hydrazino starting material or an appropriately substituted carbazate.

Compounds of Formula II may be prepared from the corresponding nitrophenyl alcohols. Compounds of Formula III with an amino group as R^1 may be prepared by reacting an appropriately substituted N-hydroxysuccinimide ester with an appropriately substituted 4-aminoaniline. Other standard coupling methods for this formula may also be used.

Compounds with a carbonylaminomethylene group may be prepared by coupling an N-protected amino benzoic acid with the appropriate amino disulfide or S-protected amino thiol. Compounds of Formula IV with a hydrazino group as R^1 may be prepared by coupling an N-protected hydrazino benzoic acid with the appropriate thiol-containing amine. Compounds of Formula V with a hydrazino group as R^1 may be prepared by coupling a thiol-containing carboxylic acid with an appropriately substituted carbazate.

The preparation of other compounds within these formulas, as well as the derivatives described above, is done with the appropriate variations on the above, as will be readily apparent to those skilled in the art.

Salts are readily prepared by conversion of the compounds in carbamate form with the appropriate acids, and the free base may be formed by treatment with base.

These compounds are useful in placing a sulfhydryl or disulfide functional group at the site of a carbohydrate group or a carboxyl moiety on another compound, and likewise for linking two compounds at

specified functional groups, i.e., a carbohydrate or carboxyl moiety on one and a thiol or electron-deficient moiety on the other. In the case of the thiol moiety, the reaction at the corresponding end of the linking agent will produce a disulfide group, preferably by disulfide exchange. In the case of an electron-deficient moiety (such as maleimide or an α-halo carbonyl group), the reaction will be a nucleophilic displacement or addition reaction.

The utility of these linking agents is in providing linkages between species in a site-specific manner with respect to at least one of the species, and in some cases with site specificity on both species. These agents may thus be used for example in linking proteins or other molecules to carbohydrates or carboxyl groups on other proteins or substances such as column supports and glass, or any substance containing the particular types of functional groups with which phenylamines, hydrazines or hydrazides will react. They can also be used to introduce a sulfhydryl moiety for purposes of site specificity for reaction with an electron-deficient moiety of another species, such as for example an ethylene group between two highly electronegative groups (i.e., maleimide).

The linking reactions used to form these linkages may be done according to conventional techniques well known among those skilled in the art. In the case of a species containing a carbohydrate moiety, the carbohydrate is first converted to an aldehyde. It is then reacted with the amino terminus of the linking agent in accordance with known conventional reaction conditions to form an imine or hydrazone linkage which can then be reduced if necessary. When more than one carbohydrate moiety is present on the species, certain specific carbohydrates may be selectively oxidized in some cases, depending on the type of molecule bearing the carbohydrate groups. In the case of

immunoglobulins, for instance, carbohydrate side chains may be selectively oxidized by the use of galactose oxidase or periodate under mild conditions. The reaction at the other end of the linking agent, as mentioned above, will depend on whether the reaction is one of disulfide exchange or nucleophilic displacement. In either case, conventional procedures known to those skilled in the art may be used.

In the case of a species containing the carboxyl moiety the amino terminus of the linking agent is coupled to form an amide bond.

A wide variety of pairs of species may be linked by the linking agents of the present invention. Examples are proteins and macromolecules in general, linked to other proteins or macromolecules or to smaller molecular species, such as bifunctional chelators, luminescent agents and NMR shift reagents. A particularly useful example is the coupling of immunoglobulins at the Fc region to toxins or labels to impart the site specificity characteristic of the immunoglobulin to the toxin or label. Examples of such labels are enzymes, radioisotopes (through bifunctional chelators), and fluorescent agents (also possibly through bifunctional chelators).

The following examples are offered for purposes of illustration, and are intended neither to limit nor define the invention in any manner. In these examples, the following abbreviations are used:

NMR: nuclear magnetic resonance at 60 MHz; all chemical shifts given in δ values relative to tetramethylsilane; "s" = singlet, "br s" = broad singlet, "d" = doublet, "t" = triplet, "m" = multiplet, "ar." = aromatic

IR: infrared spectra; values given in cm^{-1}; "sh" = shoulder, "br" = broad

LRMS: low resolution mass spectroscopy, intensity given relative to the base peak

TLC: thin-layer chromatography; values given in R_f (ratio to the front)

UV/VIS: ultraviolet/visible spectra; values given in relative absorption

TFA: trifluoroacetic acid

EtOAc: ethyl acetate

Ac: acetyl

s.m.: starting material

EXAMPLE 1

Preparation of S-Acetyl-4-(4-aminophenyl)-1-butanethiol.

This example illustrates the preparation of one of the compounds within the scope of the present invention, S-acetyl-4-(4-aminophenyl)-1-butanethiol, whose structural formula is that of Formula I above, in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$ and R^4 is acetyl.

A solution of S-acetyl-4-(4-nitrophenyl)-1-butanethiol (1.0g, 3.95 mMol) in 25 mL of methanol was heated in a bath at 60°C under a nitrogen atmosphere with $SnCl_2 \cdot 2H_2O$ (4.45g, 19.8 mMol) for 6 hours. The composition was not changing after 4 hours (using thin-layer chromatography, hereinafter "TLC"), although some starting material still remained. (The product was observed to have a R_f of 0.0 in a 90:10 mixture of hexanes and ethyl acetate, as opposed to a R_f of 0.45 for the starting material.) An additional 0.60 g of $SnCl_2 \cdot 2H_2O$ was then added. After 6 hours, the reaction mixture was cooled on ice and then brought to pH 7 with 100 mL of cold 50% saturated aqueous $NaHCO_3$.

A voluminous white precipitate formed. This was extracted with four 100 mL portions of ethyl acetate. The organic layers were rinsed with water, dried over Na_2SO_4, and concentrated in vacuo. NMR indicated the presence of the thioacetyl group (a positive indication of the existence of the desired product). The crude yield was 0.60 g (68%).

The crude product was subjected to flash chromatography on SiO_2, eluting with 90/10 hexanes/ethyl acetate to 80/20 hexanes/ethyl acetate. The major product eluted with 20% ethyl acetate and had a R_f of 0.08 in 90/10 hexanes/ethyl acetate, yielding 300 mg of an oil (34% yield). Identity of the product as S-acetyl-4-(4-aminophenyl)-1-butanethiol was confirmed by NMR, TLC, IR, LRMS and UV/VIS as follows:

NMR ($CDCl_3$): 6.88 (d, 2H, ar.); 6.50 (d, 2H, ar.); 3.48 (s, 2H, NH_2); 2.85 (m, 2H, CH_2); 2.47 (m, 2H, CH_2); 2.32 (s, 3H, SAc); 1.57 (m, 4H, $2CH_2$)

IR (NaCl, neat): 3440, 3390 (NH_2); 2930, 1680 (s, SAc); 1618, 1528 (NH_2); 1278, 1135, 960, 830 (strong NO_2 absorbance at 1350 cm^{-1} in s. m. gone)

LRMS: 223 (M^+, 14.0%); 181 (M^+, $-COCH_3$, 22.0%); 106 (100%)

TLC (90/10 hexanes/EtOAc): R_f 0.08 (ninhydrin positive)

UV/VIS: ($CHCl_2$) 296 (0.33), 250 (1.41); (CH_3OH) 288 (0.05), 234 (0.69), 214 (0.85)

EXAMPLE 2

Preparation of 4-(4-Aminophenyl)-1-butanethiol.

The structural formula of this compound corresponds to that of Formula I above, in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$ and R^4 is H.

A solution of S-acetyl-4-(4-aminophenyl)-1-butanethiol (1.3 g, 5.8 mMol) in ethanol (25 mL) was stirred with 1M aqueous ammonia (25 mL) at room temperature in the dark under nitrogen overnight. The pH was then adjusted to 6.0 with 2M HCl and the solution extracted with three 75-mL portions of EtOAc. The organic layers were rinsed with water, dried over Na_2SO_4 and concentrated in vacuo to give a pale yellow liquid weighing 0.94 g (90% yield). The identity of the product as 4-(4-aminophenyl)-1-butanethiol was confirmed by NMR, TLC, IR and UV/VIS as follows:

NMR ($CDCl_3$): 6.92 (d, 2H, ar.); 6.53 (d, 2H, ar.); 3.53 (s, 2H, NH_2); 2.28-2.75 (m, 4H); 1.08-1.92 (m, 5H)

IR (NaCl, neat): 3440, 3360, 3020, 2930, 2860, 1620 (s), 1515 (s), 1435, 1280 (s), 1185, 1130, 830

TLC (50/50 hexanes/EtOAc): R_f 0.65

UV/VIS: (CH_2Cl_2) 296 (0.261), 246 (1.05); (CH_3OH) 290 (0.147), 238 (1.02), 210 (0.702)

EXAMPLE 3

Preparation of 2-Pyridyl-4′-[1-(4-aminophenyl)]butyl Disulfide.

The structural formula corresponds to that of Formula I above, in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$ and R^4 is 2-pyridylthio. A solution of 4-(4-aminophenyl)-1-butanethiol (0.94 g, 5.2 mMol) in EtOAc (20 mL) was treated with 2,2′-dipyridyldisulfide (1.15 g, 5.2 mMol) as a solid. After all the solid had dissolved, 4 drops of $BF_3 \cdot (C_2H_5)_2O$ was added and the solution was teated at a bath temperature of 55°C. The disulfide exchange was sluggish, so after 2 hours, 17 mL of benzene was added and the temperature was increased to 65°C. After a further 20 hours, the solvent was removed in vacuo and the crude product was purified on a chromatotron (SiO_2, Harrison Research), eluting with a step gradient of 5-20% EtOAc in hexanes. The mixed disulfide was obtained as a pale yellow oil weighing 0.41 g (27% yield). The identity of the product as 2-pyridyl-4′-[1-(4-aminophenyl)]butyl disulfide was confirmed by NMR, TLC and IR as follows:

NMR ($CDCl_3$): 8.45 (m, 1H, pyridyl); 7.42-7.82 (m, 2H, pyridyl); 6.75-7.18 (m, 1H, pyridyl); 6.92 (d, 2H, phenyl); 6.55 (d, 2H, phenyl); 3.58 (s, 2H, NH_2); 2.25-3.00 (m, 4H, $2CH_2$); 1.38-2.00 (m, 4H, $2CH_2$)

IR (NaCl, neat) 3430, 3340, 2930 (s), 1620, 1575, 1520, 1450, 1420, 1280, 1120, 830, 765

TLC (50/50 EtOAc/hexanes): R_f 0.74

EXAMPLE 4

1. Preparation of S-Acetylthioacetic Acid, 4-(t-Butoxycarbonylamino)anilide.

This compound was prepared as a precursor to the trifluoroacetate salt (described below).

A solution was prepared consisting of S-acetylthioacetic acid, N-hydroxysuccinimide ester (1.29 g, 5.6 mMol) and 4-(t-butoxycarbonylamino)aniline (1.5 g, 5.6 mMol) in ethyl acetate (30 mL). The solution was stirred at room temperature for 3.5 hours, then at 45°C overnight. The reaction mixture remained colorless throughout.

The reaction mixture was then rinsed with two 50 mL portions of 10% saturated aqueous $NaHCO_3$, then with 50 mL of H_2O. The organic layers were dried over Na_2SO_4 and concentrated in vacuo to give a yellow oil. This was flash chromatographed twice on a SiO_2 column slurried in 50/50 ethyl acetate/petroleum ether, eluting with the latter as well. The fastest running fractions from each were pooled and concentrated. The resulting solid was recrystallized from methylene chloride/petroleum ether to give 1.22 g (67% yield) of a white solid with melting point 161-162°C. The latter was identified as S-acetylthioacetic acid, 4-(t-butoxycarbonylamino)anilide by IR, NMR, UV/visible, and TLC, as follows:

NMR ($CDCl_3$): 8.00 (br s, 1H, NH); 7.33 (s, 5H, ar.); 6.55 (br s, 1H, NH); 3.65 (s, 2H, CH_2); 2.43 (s, 3H, SAc); 1,52 (s, 9H, t-butyl)

IR (KBr): 3350 (s), 1695 (s, SAc), 1660 (s, amide C=O), 1550 (s), 1410, 1315, 1235, 1170, 1065, 825, 705, 630

UV/VIS: (CH_3OH) 262 (0.597), 210 (0.518); (CH_2Cl_2) 270 (sh, 0.750), 258 (0.873)

TLC (50/50 EtOAc/hexanes): R_f 0.75

2. Preparation of S-Acetylthioacetic Acid, 4-Aminoanilide, Trifluoroacetate Salt.

This compound has the structural formula of Formula III above, in which R^1 is 4-amino, R^3 is methylene, and R^4 is acetyl, in the form of the trifluoroacetate salt of the primary amine at the left end of the structure as shown.

The product of Section 1 of this example in the amount of 200 mg was dissolved in 5 mL of freshly distilled trifluoroacetic acid (hereinafter "TFA"), and stirred in the dark at room temperature under a nitrogen atmosphere for 1.5 hours. The solvent was then removed in vacuo at a temperature less than 30°C to give a pale yellow oil in approximately quantitative yield. Its identity was confirmed as that of S-acetylthioacetic acid, 4-aminoanilide, trifluoroacetate salt by NMR as follows:

NMR (D_2O): 7.57 (d, 2H, ar.); 7.33 (d, 2H, ar.); 3.82 (s, 2H, $\underline{CH_2}$SAc); 2.42 (s, 3H, SAc)

EXAMPLE 5

1. Preparation of 2-Pyridyl-3'-propanoyl Disulfide, 4-(t-Butoxycarbonylamino)anilide.

This compound was prepared as the precursor of the trifluoroacetate salt (described below).

A solution of 2,2'-dipyridyl disulfide (Aldrich, 1.5 g, 6.8 mMol) in 10 mL of dry ethyl acetate was treated with 3-mercaptopropionic acid (0.73 g, 6.89 mMol) in 10 mL of dry ethyl acetate. Then four drops of $BF_3 \cdot (C_2H_5)_2O$ were added and the reaction stirred overnight at room temperature under a nitrogen atmosphere. The reaction was concentrated to dryness in vacuo, then slurried with 10 mL of cold ethyl acetate and filtered of all solid to give a pale yellow solution. To the latter were added 4-t-butoxycarbonylamino aniline (1.82 g, 6.8 mMol) in 10 mL of dry ethyl acetate and dicyclohexylcarbodiimide (1.40 g, 6.8 mMol),

also in 10 mL ethyl acetate. The reaction was again stirred overnight at room temperature, then filtered to remove dicyclohexylurea. The filtrate was concentrated in vacuo, then subjected to flash chromatography on SiO_2, eluting with 30/70 ethyl acetate/hexanes.

A first fraction at R_f 0.8 (with 50/50 ethyl acetate/hexanes) was an oil not further characterized. A second fraction at R_f 0.62 was a pale yellow solid, 0.48g, identified by NMR as 4-t-butoxycarbonylamino aniline. A closely following third fraction at R_f 0.50 was a solid, 0.66g (representing 23% yield) with melting point 139-140°C. This was recrystallized from ethanol to yield a solid with a melting point of 154.5-155.5°C. Its identity was confirmed as that of 2-pyridyl-3'-propanoyl disulfide, 4-(t-butoxycarbonylamino)anilide by NMR, IR, and elemental analysis, as follows:

NMR ($CDCl_3/CD_3OD$): 8.30 (m, 1H, pyridyl); 7.50-7.80 (m, 2H, pyridyl); 7.37 (s, 4H, phenyl ring); 6.87-7.27 (m, 1H, pyridyl); 3.00 (m, 2H); 2.75 (m, 2H); 1.50 (s, 9H, t-butyl)

IR (KBr): 3340 (Br); 1690,1655 (both C=O); 1520 (s), 1390, 1165, 1075, 842, 770

Elemental Analysis, found(required): C: 56.11(56.27), H: 5.83(5.72), N: 10.27(10.36), S: 15.75(15.81)

2. Preparation of 2-Pyridyl-3'-propanoyl Disulfide, 4-Aminoanilide, Trifluoroacetate Salt.

This compound has the structural formula of Formula III above, in which R^1 is 4-amino, R^3 is $-(CH_2)_2-$, and R^4 is 2-pyridylthio, in the form of the trifluoroacetate salt of the primary amine at the left end of the structure as shown.

A solution was prepared consisting of 150 mg of the product of Section 1 of this Example in 5 mL of freshly distilled TFA. The solution was stirred at room temperature under a nitrogen atmosphere for 1.5

hours in the dark. No yellow color (which would indicate liberated 2-pyridylthiol) was observed. The solvent was removed in vacuo at a temperature less than 30°C to give a solid in quantitative yield. The solid was soluble in water, methanol and ethyl acetate, and its identity was confirmed as that of 2-pyridyl-3′-propanoyl disulfide, 4-aminoanilide, trifluoroacetate salt by proton NMR and UV/visible, as follows:

^{1}H NMR (D_2O): 8.62 (m, 1H, pyridyl); 8.18 (m, 2H, pyridyl); 7.56-7.90 (m, partially obscured, 1H, pyridyl); 7.50 (pseudo-d, 4H, phenyl ring); 3.33 (t, 2H); 2.95 (t, 2H)

UV/VIS (H_2O): 242 (with poorly defined broad absorbance to about 310nm)

EXAMPLE 6

Preparation of S-Acetylthioacetic Acid, N-t-Butoxycarbonyl Hydrazide.

This compound has the structural formula of Formula V above in which R^1 is hydrazino, R^3 is methylene, and R^4 is acetyl, in the form of the t-butyl carbamate.

A mixture of S-acetylthioacetic acid, N-hydroxysuccinimide ester (1.5 g,6.49 mMol) and t-butylcarbazate (0.86 g, 6.49 mMol) was stirred in dry ethyl acetate under a nitrogen atmosphere at room temperature for 24 hours, then at 60°C for an additional 6.5 hours. The reaction mixture was then cooled on ice, rinsed with two 50 mL portions of saturated aqueous $NaHCO_3$ followed by water, then dried over Na_2SO_4 and concentrated in vacuo. A major product spot was observed by TLC (using 50/50 ethyl acetate hexanes) at R_f 0.42.

The crude product was subjected to flash chromatography on SiO_2 eluting with 50/50 ethyl acetate/hexanes, which yielded a very pale yellow oil,

1.22 g (76% yield). Residual starting material was crystallized out, and the remaining mixture was concentrated to dryness, dissolved in 20 mL ethyl acetate, and rinsed with four 25 mL portions of 20% saturated aqueous $NaHCO_3$ followed by 25 mL of water, then dried over Na_2SO_4 and concentrated in vacuo to yield the final product, an oil whose identity was confirmed as that of S-acetylthioacetic acid, N-t-butoxycarbonyl hydrazide by NMR, IR, and UV/visible, as follows:

NMR ($CDCl_3$): 8.50 (br s, 1H, NH); 7.00 (br s, 1H, NH); 3.63 (s, 2H, CH_2SAc); 2.38 (s, 3H, SAc); 1.46 (s, 9H, t-butyl)

IR (NaCl, neat): 3290 (br), 2990, 1690 (br), 1490, 1375, 1255, 1165

UV/VIS: (CH_2Cl_2) 248; (CH_3OH) 218

This compound can be converted to the trifluoroacetate salt by procedures analogous to those described in the other examples in this specification. The compound may also be converted to other salts within the scope of the present invention by other conventional procedures.

EXAMPLE 7

1. Preparation of 2-Pyridyl-3'-propanoyl Disulfide, N-t-Butoxycarbonyl Hydrazide.

This compound was prepared as a precursor of the trifluoroacetate salt (described below).

A solution of 2,2'-dipyridyl disulfide (3.8 g, 17 mMol) in 20 mL of ethyl acetate was treated with 1.8 g (17.2 mMol) of 3-mercaptopropionic acid in 10 mL of ethyl acetate and 5 drops of $BF_3 \cdot (C_2H_5)_2O$. The reaction mixture was stirred for 5 hours under nitrogen in the dark, then filtered and concentrated in vacuo. The resulting solid residue was slurried in 20 mL of cold ethyl acetate and refiltered. Then 1.98 g (15 mMol) of t-butylcarbazate was added, followed by 3.09 g (15 mMol) of dicyclohexylcarbodiimide in 10 mL of dry

ethyl acetate. The reaction was stirred at room temperature for 18 hours in the dark, then filtered and concentrated in vacuo to yield a yellow oil. The oil was subjected to flash chromatography on SiO_2, eluting with 50/50 ethyl acetate/petroleum ether (35-60°C).

Three fractions were collected, the second containing the desired product at R_f 0.40 (50/50 ethyl acetate/hexanes), 1.56 g (28% yield) of an oil, whose identity was confirmed as that of 2-pyridyl-3'-propanoyl disulfide, N-t-butoxycarbonyl hydrazide by NMR, IR, and UV/visible, as follows:

NMR ($CDCl_3$): 9.46 (br s, 1H, NH); 8.43 (m, 1H, pyridyl); 7.63 (m, 2H, pyridyl); 7.50 (br s, 1H, NH); 7.10 (q, 1H, pyridyl); 3.07 (m, 2H, CH_2); 2.77 (m, 2H, CH_2); 1.47 (s, 9H, t-butyl)

IR (NaCl, neat): 3280 (br), 2990, 1730 (sh), 1685 (br), 1420, 1372, 1250, 1165, 770

UV/VIS: CH_2Cl_2) 286 (1.05), 250 (1.30); (CH_3OH) 284 (0.74), 238 (1.49), 214 (sh, 1.11)

2. Preparation of 2-Pyridyl-3'-propanoyl Disulfide Hydrazide, Trifluoroacetate Salt.

This compound has the structural formula of Formula V above in which R^1 is hydrazino, R^3 is $-(CH_2)_2-$ and R^4 is 2-pyridylthio, in the form of the trifluoroacetate salt of the primary amine at the left end of the structure as shown.

A portion of the product of Section 1 of this Example (200 mg) was dissolved in freshly distilled TFA (5 mL) and stirred in the dark at room temperature under a nitrogen atmosphere for 1 hour, during which time a very faint pink color developed. The solvent was then removed in vacuo at a temperature less than 30°C to give a pale yellow oil in approximately quantitative yield. The identity of the oil was confirmed as that of 2-pyridyl-3'-propanoyl disulfide hydrazide, trifluoroacetate salt by NMR, as follows:

NMR (D_2O): 8.73 (m, 1H, pyridyl); 8.33 (m, 2H, pyridyl), 7.88 (m, 1H, pyridyl); 3.23 (m, 2H, CH_2); 2.93 (m, 2H, CH_2)

EXAMPLE 8

Conjugate Preparation.

This example illustrates the preparation of a conjugate of ricin A-chain with IND1 antibody through a carbohydrate moiety on the latter, using a linking agent in accordance with the present invention. The linking agent used is that prepared in Example 5, part 2. The following abbreviations are used in this example:

RTA:	Ricin toxin A-chain
NaOAc:	sodium acetate
DMSO:	dimethyl sulfoxide
DTT:	dithiothreitol
SDS PAGE:	sodium dodecyl sulfide polyacrylamide gel electrophoresis
PBS:	phosphate-buffered saline
SPDP:	N-succinimidyl-3-(2-pyridyldithio)-propionate
EIA:	enzyme immunoassay

1. Periodate Oxidation of Carbohydrate Moiety on Antibody and Reaction With Linking Agent.

1 mL of 5 mg/mL IND1 antibody in PBS was spun through a G-50 column equilibrated with 0.1M NaOAc + 0.15M NaCl, pH 5, and diluted 1:1 with 1 mL of 0.1M NaOAc buffer. The antibody was oxidized with 10mM sodium periodate for 20 minutes at 0°C in the dark, then quenched with 10mM glycerol for 20 minutes at 0°C in the dark. 250 µL of the quenched reaction was spun through G-50 into fresh acetate buffer, pH 5, then diluted 1:1 with acetate buffer to a final volume of 500 µL (3.3µM).

A solution of the trifluoroacetate salt of 2-pyridyl-3'-propanoyl disulfide, 4-aminoanilide was

prepared by combining 5-8 mg of the salt (which was a yellow oil) with 10 µL DMSO to give a 1.5-2M solution. This was then diluted about 50× with ethanol to produce a 30mM 10× concentrated working stock solution. 55 µL of this solution was added to the reaction mixture described above, resulting in a final linking agent concentration of 300µM (a linker:antibody ratio of about 100). The reaction was allowed to proceed for 15 h at 4°C in the dark with gentle agitation. The solution was then treated with 10mM $NaCNBH_3$ (aqueous) for 4 h at 4°C with gentle agitation. The antibody was then spun through G-50 into SPDP buffer and concentrated to 1.3 mg/mL for the coupling reaction with RTA.

2. Reaction With RTA and Characterization of the Immunotoxin.

RTA was concentrated to 6.4 mg/mL, and treated with 50mM DTT for 1 hour at room temperature to reduce sulfhydryls, then spun through G-50 into SPDP buffer, pH 7.5. The concentration of the reduced RTA was 4.75 mg/mL. A tenfold molar excess of RTA was added to IND1 (272 µL of 4.75 mg/mL RTA added to 0.5 mL 1.3 mg/mL IND1), inverted to mix and allowed to stand overnight at 4°C without stirring. The presence of immunotoxin in the crude reaction was confirmed by SDS PAGE (Coomassie) and quantitated by densitometer scanning. The mono-conjugate appeared to be 36% of the crude reaction mixture. The di-conjugate was present in about 10% yield. About 40% of the IND1 antibody was unreacted.

3. Purification of Immunotoxin.

The impure reaction product was loaded on AcA-44 in SPDP, pH 7.5. Two peaks of protein were eluted from the column; the seond peak containing antibody and immunotoxin was concentrated (100 µL, 1.77 mg/mL) and loaded onto a 0.8 mL column of Affigel Blue. After loading by gravity, the column was washed with 10

volumes of PBS, pH 7 at a flow rate of anout 15 mL/h. A high salt, high pH step was then applied (0.1M phosphate, 0.5M NaCl, pH 8) to elute the immunotoxin. The immunotoxin was concentrated to about 60 µL (0.9 mg/mL) for SDS PAGE and activity assays (EIA and whole cell kill).

4. Activity Assay Results.

Densitometry of the Coomassie stained SDS PAGE gel showed that the Affi-gel Blue purified material contained 10% free antibody, 20% monoconjugate and 9% di-RTA conjugate. As much as 55% of the stain was present as a diffuse high molecular weight band that resulted from periodate oxidation of the antibody.

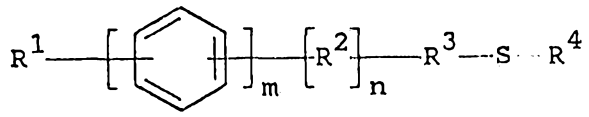
EIA binding was determined for both the starting material and the final product. EIA for the immunotoxin was 23.3% (relative to 100% for unmodified IND1) and the whole cell kill activity was >200 ng/mL for the Affi-gel Blue purified material.

The foregoing is offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that variations and modifications in terms of the molecular structures, preparation procedures and reaction conditions may be made without departing from the spirit and scope of the invention.

~~WHAT IS CLAIMED IS:~~

The claims defining the invention are as follows:

1. A compound having the formula



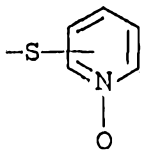
in which

R^1 is a member selected from the group consisting of NH_2– and NH_2–NH–;

R^2 is a member selected from the group consisting of –NH–C(O)–, –C(O)–NH–, and –C(O)–;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,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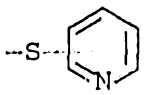
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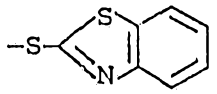
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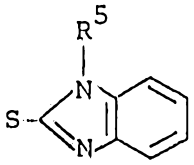

,

where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

n is zero or 1;

and analogs comprising water-soluble salts and water-soluble derivatives thereof.

2. A compound in accordance with claim 1 in which R^1 is 4-amino, R^3 is $-(CH_2)_3-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

3. A compound in accordance with claim 1 in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

4. A compound in accordance with claim 1 in which R^1 is 4-amino, R^2 is $-NH-C(O)-$, R^3 is $-CH_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

5. A compound in accordance with claim 1 in which R^1 is 4-amino, R^2 is $-NH-C(O)-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

6. A compound in accordance with claim 1 in which R^1 is 4-amino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

7. A compound in accordance with claim 1 in which R^1 is 4-amino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

8. A compound in accordance with claim 1 in which R^1 is 4-hydrazino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

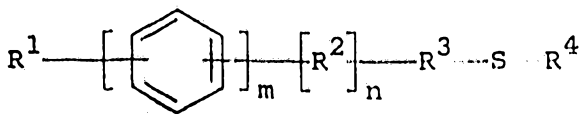
9. A compound in accordance with claim 1 in which R^1 is hydrazino, R^2 is $-C(O)-$, R^3 is $-CH_2-$, R^4 is acetyl, m is zero and n is 1; or the trifluoroacetate salt thereof.

10. A compound in accordance with claim 1 in which R^1 is hydrazino, R^2 is $-C(O)-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is zero and n is 1; or the trifluoroacetate salt thereof.

11. A method for linking a first compound containing a carbohydrate moiety to a second compound containing a thiol group, said method comprising:

(a) oxidizing said first compound to convert said carbohydrate moiety to an aldehyde group; and

(b) reacting said first and second compounds with a compound having the formula



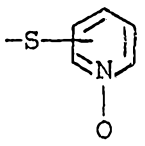
in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of $-NH-C(O)-$, $-C(O)-NH-$, and $-C(O)-$;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,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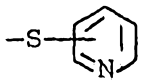
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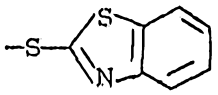
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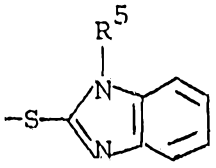
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where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

n is zero or 1;

and analogs comprising water-soluble salts and water-soluble derivatives thereof.

12. A method in accordance with claim 11 in which R^1 is 4-amino, R^3 is $-(CH_2)_3-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

13. A method in accordance with claim 11 in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

15. A method in accordance with claim 11 in which R^1 is 4-amino, R^2 is $-NH-C(O)-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

16. A method in accordance with claim 11 in which R^1 is 4-amino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

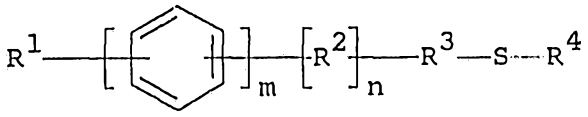
17. A method in accordance with claim 11 in which R^1 is 4-amino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

18. A method in accordance with claim 11 in which R^1 is 4-hydrazino, R^2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

19. A method in accordance with claim 11 in which R^1 is hydrazino, R^2 is $-C(O)-$, R^3 is $-CH_2-$, R^4 is acetyl, m is zero and n is 1; or the trifluoroacetate salt thereof.

20. A method in accordance with claim 11 in which R^1 is hydrazino, R^2 is $-C(O)-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is zero and n is 1; or the trifluoroacetate salt thereof.

21. A method for linking a first compound containing a carboxyl group to a second compound containing a thiol group, said method comprising reacting said first and second compounds with a compound having the formula



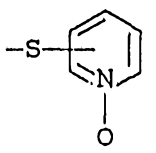
in which

R^1 is a member selected from the group consisting of NH_2- and NH_2-NH-;

R^2 is a member selected from the group consisting of $-NH-C(O)-$, $-C(O)-NH-$, and $-C(O)-$;

R^3 is a member selected from the group consisting of C_1-C_{10} alkylene, phenyl-substituted C_1-C_{10} alkylene, benzyl-substituted C_1-C_{10} alkylene, amino-substituted C_1-C_{10} alkylene, C_5-C_7 cyclic alkylene and arylene;

R^4 is a member selected from the group consisting of H, acetyl,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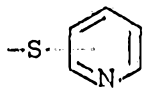
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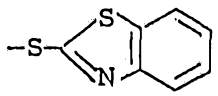
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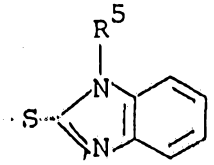
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where R^5 is C_1-C_5 alkyl;

m is zero or 1; and

22. A method in accordance with claim 21 in which R^1 is 4-amino, R^3 is $-(CH_2)_3-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

23. A method in accordance with claim 21 in which R^1 is 4-amino, R^3 is $-(CH_2)_4-$, R^4 is acetyl, m is 1 and n is zero; or the trifluoroacetate salt thereof.

24. A method in accordance with claim 21 in which R^1 is 4-amino, R_2 is $-NH-C(O)-$, R^3 is $-CH_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

25. A method in accordance with claim 21 in which R^1 is 4-amino, R_2 is $-NH-C(O)-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

26. A method in accordance with claim 21 in which R^1 is 4-amino, R_2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

27. A method in accordance with claim 21 in which R^1 is 4-hydrazino, R_2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is acetyl, m is 1 and n is 1; or the trifluoroacetate salt thereof.

28. A method in accordance with claim 21 in which R^1 is 4-hydrazino, R_2 is $-C(O)-NH-$, R^3 is $-(CH_2)_2-$, R^4 is 2-pyridylthio, m is 1 and n is 1; or the trifluoroacetate salt thereof.

29. A method in accordance with claim 21 in which R^1 is hydrazino, R_2 is $-C(O)-$, R^3 is $-CH_2-$, R^4 is acetyl, m is zero and n is 1; or the trifluoroacetate salt thereof.

30. A compound as defined in claim 1 substantially as hereinbefore described with reference to any one of the Examples.

31. A method for linking a first compound containing a carbohydrate moiety to a second compound containing a thiol group said method as defined in claim 11, substantially as hereinbefore described with reference to any one of the Examples.

32. A method for linking a first compound containing a carboxyl group to a second compound containing a thiol group said method as defined in claim 21, substantially as hereinbefore described with reference to any one of the Examples.

33. The product of the method of any one of claims 11 to 29.

DATED this TWENTY-SECOND day of FEBRUARY 1991
Xoma Corporation

Patent Attorneys for the Applicant
SPRUSON & FERGUSON