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SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

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

PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELICIT CATALYTIC ANTIBODIES,

I, **Richard J. Massey, Senior Vice President** of **Igen, Inc., 1530 East Jefferson Road, Rockville, Maryland 20852, United States of America**, do solemnly and sincerely declare as follows:-

1. I am authorized by Igen, Inc., the applicant for the patent, to make this declaration on its behalf.

2. The basic application, as defined by Section 141 of the Act, was made in United States of America on **4 MAY 1988** by **Richard C. Titmas; David E. Hansen; Wonpyo Hong; Paul M. Booth; Michael J. Powell; Anthony R. Rees;** and **Richard J. Massey.**

3. Richard C. Titmas; David E. Hansen; Wonpyo Hong; Paul M. Booth; Michael J. Powell; Anthony R. Rees; and Richard J. Massey, of 12905 Crookston Lane # 103, Rockville, Maryland 20851; 23 Orchard Street, Amherst, Massachusetts 01002; 10921 Candlelight Lane, Potomac, Maryland 20854; 12116 Island View Circle, Germantown, Maryland 20874; 5 War Admiral Court, Gaithersburg, Maryland 20852; 1530 East Jefferson Street, Rockville, Maryland 20852; and 5 Valerian Court, Rockville, Maryland 20852, all in United States of America [respectively], are the actual inventors of the invention and the facts upon which the applicant is entitled to make the application are as follows:

   The said applicant is the assignee of the actual inventors.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Rockville, Maryland, USA, this 17 day of October, 1990.

By Richard J. Massey
Richard J. Massey
Senior Vice President

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA

AU8937393

(12) PATENT ABRIDGMENT (11) Document No. AU-B-37393/89
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 643186

(54) Title
PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELICIT CATALYTIC ANTIBODIES

International Patent Classification(s)
(51)[5] C12P 021/08 C07C 271/24 C07K 015/12 A61K 039/395
(51)[4] C07C 079/46 C12N 009/14 C12N 009/16
C12N 009/48

(21) Application No. : 37393/89 (22) Application Date : 04.05.89

(87) PCT Publication Number : WO89/10961

(30) Priority Data

(31) Number (32) Date (33) Country
190271 04.05.88 US UNITED STATES OF AMERICA

(43) Publication Date : 29.11.89

(44) Publication Date of Accepted Application : 11.11.93

(71) Applicant(s)
IGEN, INC.

(72) Inventor(s)
RICHARD C. TITMAS; DAVID E. HANSEN; WONPYO HONG; PAUL M. BOOTH; MICHAEL J. POWELL; ANTHONY R. REES; RICHARD J. MASSEY

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

(57) Claim

1. A method for catalyzing the cleavage or formation of a specific amide, ester or glycosidic bond within a molecule which comprises contacting said molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of said amide, ester or glycosidic bond under conditions suitable for said cleavage or formation to take place, said monoclonal antibody having been prepared by a process comprising the steps of:

(a) selecting the specific amide, ester or glycosidic bond to be cleaved or formed;

(b) selecting an antigen comprising an analog of said amide, ester or glycosidic bond to be cleaved or formed, and also comprising moieties surrounding said analog of said amide, ester or glycosidic bond, said moieties substantially corresponding to some or all of the moieties surrounding the amide, ester or glycosidic bond to be cleaved or formed;

(c) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

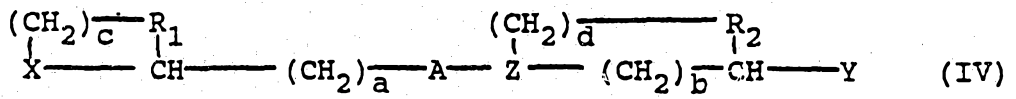
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(d) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(e) screening said plurality of monoclonal antibodies to identify a monoclonal anitbody which catalyzes the cleavage or formation of said amide, ester or glycosidic bond.

4. An immunogen comprising:

(a) a hapten of formula IV



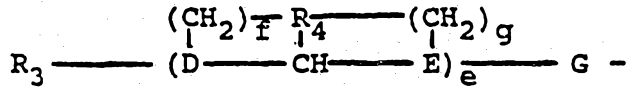
(IV)

or a physiologically acceptable salt thereof, wherein:

a and b are the same or different and each is an integer from 0 to 10;

c and d are the same or different and each is 0 or 2;

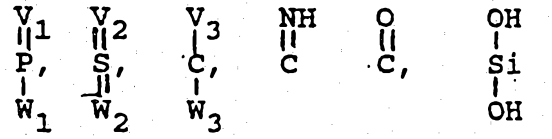
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy,
(C_1-C_4)alkoxycarbonyl, or

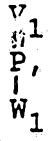


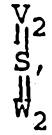
provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

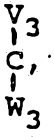
e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

A is  ;

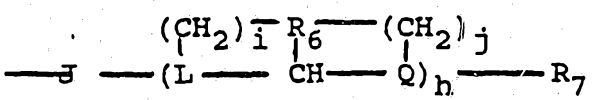
Z is O, NH, CH_2, or S when A is , provided that

Z is N or CH when d is 2; Z is O, NH or CH_2 when A is , ;

provided that Z is N or CH when d is 2; Z is CH_2 or CF_2 when A is , provided that Z is CH or CF when d is 2; and

Z is NH when A is , provided that Z is N when d is 2; Z is CF_2 when A is C=O, provided that Z is CF when d is 2; and Z is O or CH_2 when A is Si or B, provided that Z is CH when d is 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or
(C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1

is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy,
(C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S provided that when c is 2, G is N, CH or CF;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is

N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

V_3 is OH, NH_2, or $NHNH_2$;

W_1 is OH, NH_2, NH(C_1-C_{10})alkyl, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C1-C10) alkyl or $NHNH_2$;

W_3 is H or CH_2NH_2;

and wherein

one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$, provided that if Z is O or NH when V_1 is O and W_1 is OH, then at least one of R_1, R_2, R_4 and R_6 is bound to one of said remaining substitutents R_1, R_2, R_4 and R_6.

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

OPI DATE 29/11/89 APPLN. ID 37393 / 89
AOJP DATE 04/01/90 PCT NUMBER PCT/US89/01951

PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
C12N 9/14, 9/16, 9/48
C12N 9/88, C07K 15/00
C07C 79/46

A1

(11) International Publication Number: WO 89/10961

(43) International Publication Date: 16 November 1989 (16.11.89)

(21) International Application Number: PCT/US89/01951

(22) International Filing Date: 4 May 1989 (04.05.89)

(30) Priority data:
190,271 4 May 1988 (04.05.88) US

(60) Parent Application or Grant
(63) Related by Continuation
US 190,271 (CIP)
Filed on 4 May 1988 (04.05.88)

(71) Applicant *(for all designated States except US)*: IGEN, INC. [US/US]; 1530 East Jefferson Street, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : TITMAS, Richard, C. [GB/US]; 12905 Crookston Lane ,103, Rockville, MD 20851 (US). HANSEN, David, E. [US/US]; 23 Orchard Street, Amherst, MA 01002 (US). HONG, Wonpyo [KR/US]; 10921 Candlelight Lane, Potomac, MD 20854 (US). BOOTH, Paul, M. [GB/US]; 12116 Island View Circle, Germantown, MD 20874 (US). POWELL, Michael, J. [GB/US]; 5 War Admiral Court, Gaithersburg, MD 20852 (US). REES, Anthony, R. [GB/US]; 1530 East Jefferson Street, Rockville, MD 20852 (US). MASSEY, Richard, J. [US/US]; 5 Valerian Court, Rockville, MD 20852 (US).

(74) Agent: EVANS, Barry; Curtis, Morris & Safford, 530 Fifth Avenue, New York, NY 10036 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US.

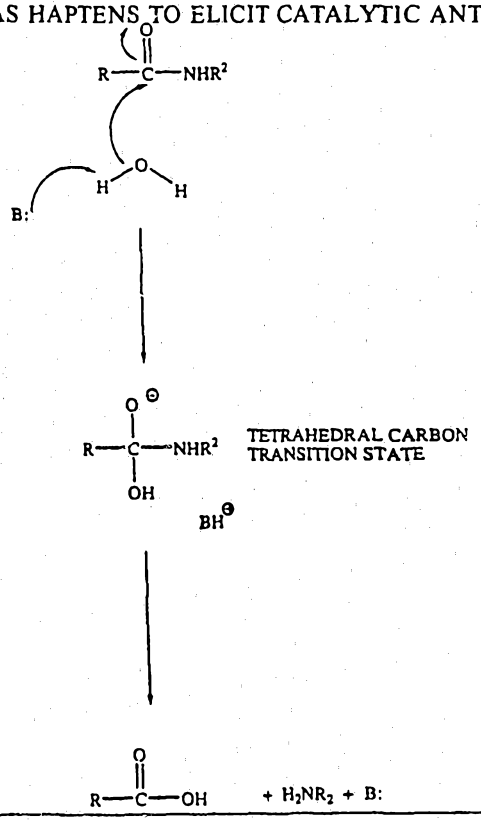
Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

643186

(54) Title: PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELICIT CATALYTIC ANTIBODIES

(57) Abstract

Antigens capable of eliciting antibodies which can catalyze chemical reactions, in particular, the cleavage or formation of a peptide linkage, comprising a hapten or a hapten and a suitable carrier molecule are disclosed. Antibodies which are catalytically active for chemical reactions, in particular, the cleavage or formation of a selected amide, ester or glycosidic bond, and which are elicited by such antigenes are disclosed as well as methods for producing the antibodies and methods for catalyzing the cleavage or formation of an amide, ester or glycosidic bond in a molecule.



PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELI ANTIBODIES

FIELD OF TEE INVENTION

The invention pertains generally to antibodies, antigens, haptens and immunogens capable of eliciting antibodies which include a paratope that binds to and thereby stabilizes a transition state in the cleavage or formation of an amide bond, a peptide bond linkage or an ester bond so that the cleavage or formation is catalyzed by the antibodies.

This application is a continuation-in-part U.S. Application Serial No. 190,271, filed May 4, 1988, which in turn is a continuation-in-part of U.S. Application Ser. No. 674,253, filed November 27, 1984, which is a continuation-in-part of U.S. Application Ser. No. 556,016, filed November 29, 1983, the contents of which applications are hereby incorporated by reference into this application.

Several publications are referenced in this application by Arabic numerals within parentheses. Full citation for these references are found at the end of the specification immediately preceding the claims. The references more fully describe the state-of-the-art to which this invention pertains as well as certain aspects of the invention itself.

BACKGROUND OF THE INVENTION

There are numerous enzymes which have been identified as capable of catalyzing various chemical reactions. Similarly, it has been discovered that antibodies can be elicited to catalyze a variety of chemical reactions (U.S. Appln. Ser. No. 674,253). It is well known that antibodies and enzymes share a fundamental similarity in that both are specialized proteins that bind to other molecules. However, there

are important physiological differences between antibodies and enzymes.

Antibodies typically bind to a molecule or antigen so that the antigen is marked as foreign to the organism that produced the antibody. The binding of the antibody to the antigen enables the antigen to be removed from the organism. Enzymes are biological catalysts which bind a molecule in such a way that the activation energy of a reaction involving a molecule or substrate is lowered, thereby increasing the rate of the reaction.

Linus Pauling hypothesized that there are two types of interactions between proteins and the molecules that bind them. Antibodies bind molecules in their ground states most strongly while enzymes bind molecules in higher energy states most strongly.

Pauling attempted to explain the mechanism of enzyme catalysis based upon such binding. During the course of the chemical reaction, the reactants undergo one or more transitions through intermediate structures or transition states which are energically less favorable than either the reactant or the product. The hydrolysis reaction of a peptide linkage or an ester bond in an aqueous medium passes through a tetrahedral carbon transition state, as depicted in Figs. 1 (peptide) and 2 (ester). In the transition state, a tetrahedral carbon atom is bonded to: a carbon atom of the acid portion of the peptide linkage or ester bond; two oxygen atoms, one corresponding to the carbonyl group and the other corresponding to a hydroxyl ion or water molecule of the medium; and either the oxygen atom of the alcohol portion of an ester or the nitrogen atom of the amine portion of the peptide linkage. The transition state can be neither isolated nor detected since it exists for only about 10^{-13} sec.

In molecular terms, these transition states reflect changes in bond lengths and bond angles as well as the formation and breakage of bonds. The energy required to achieve a transition state is denoted as the activation energy which may also be considered as the difference in energy between the energy of the transition state and the energy of the reactants. According to Pauling's hypothesis, an enzyme preferentially binds the transition state of a reaction, thereby stabilizing it relative to the substrate and products and reducing the activation energy of the reaction, thus increasing the reaction rate. For example, aspartic proteinases are enzymes which are known to catalyze the hyrolysis of peptide linkages within a protein molecule.

By extending this explanation, Pauling also predicted that stable analogs of a transition state would bind tightly to an enzyme. It has been suggested that the term "transition state analog" might be used to describe an inhibitor of this kind (1).

Pauling's prediction has become the basis for the now well established approach to enzyme inhibitor design. The strategy for designing enzyme inhibitors has suggested a strategy for preparing catalytic antibodies whereby antigens are designed based upon mechanistic principles so that antibodies raised in response to such antigens will catalyze a chemical reaction by carrying out the reaction mechanism implicit in the design of the antigen. This strategy has been attempted a number of times.

For example, a transition-state analog mimicking an intramolecular 6-member ring cyclization transition state was used to elicit a monoclonal antibody which acted as a stereospecific, enzyme-like catalyst (2). Specifically, the monoclonal antibody so elicited

accelerated, by about a factor of 170, the formation of a single enantiomer of a δ-lactone from the corresponding racemic δ-hydroxyester.

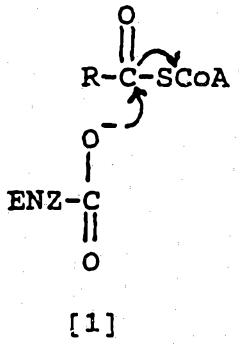
Similarily, monoaryl phosphonate esters, designated as analogs of the transition state in the hydrolysis of carboxylic esters, were synthesized and used as haptens to elicit specific monoclonal antibodies capable of catalyzing the hydrolysis of carboxylic esters (3). Certain of the antibodies elicited were reportedly found to be catalytic and selective for the hydrolysis of particular aryl esters.

Phosphonamidates or phosphonate analog-ligands having conformations that substantially correspond to the conformation of a hydrolytic transition state of an amide or ester ligand and which have been used to produce antibodies are described in U.S. Patent 4,659,567 to Tramontano et al. (Tramontano). Antibodies so produced purportedly include a paratope that binds to and stabilizes the tetrahedral carbon atom of the amide or ester hydrolysis transition state of the ligand to hydrolyze the ligand at a predetermined site.

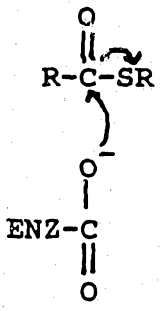
Analog-ligands which can be used to produce antibody catalysts for the hydrolysis of esters and amides are also described in European Patent Application 0,251,093 of Kollmorgen Corp. (Kollmorgen).

In enzyme catalysis, groups on both the substrate and the enzyme which are not involved in the chemical mechanism of bond making and breaking make an important contribution to catalysis. This is illustrated by examining the action of the enzyme succinyl - CoA acetoacetate transferase, shown below, which involves nucleophilic attack of the enzyme's glutamate carboxyl on the thioester succinyl - CoA [1] to give an anhydride

intermediate. The enzyme forms anhydride intermediates from "non-specific" substrates [2], as well.



[1]



[2]

Even though the chemical reactivities of the two substrates, [1] and [2], are similar, e.g. towards alkaline hydrolysis, the enzyme reaction proceeds up to 3×10^{12} fold faster with the so called specific substrate [1](4). The non-reacting part of the substrate, i.e., the CoA residue, lowers the activation energy by 72 $KJmol^{-1}$ compared with [2].

It has also been explicitly noted that to obtain catalysis, the Gibbs free energy of the enzyme-substrate and enzyme-product complex must be increased so that the transition state can be reached easily (5). This represents destabilization of the enzyme-substrate complex which can occur by physical strain, desolvation and other mechanisms.

Thus, the haptens disclosed in Tramontano do not provide the correct architecture to elicit antibodies that are capable of catalyzing the cleavage of a predetermined peptide sequence in a native protein. These haptens do not provide the correct side-chain

groups for production of antibodies that can react with predetermined sites on a protein and cause selective proteolysis in a sequence specific manner. Furthermore, these haptens do not incorporate amino acid side-chain sub-sites on either side of the transition state analog. Without these sub-sites, the haptens cannot provide for the elicitation of catalytic antibodies capable of recognizing a specific amino acid sequence and selectively proteolyzing a peptide linkage within that sequence.

OBJECTS, FEATURES AND ADVANTAGES OF THE INVENTION

It is an object of the invention to provide a rational design approach for designing haptens which are capable of molecular recognition and hydrolytic rate enhancement.

It is also an object of the invention to provide haptens which include an array of atoms which mimics the transition state in the cleavage or formation of an amide, ester or glycosidic bond in a molecule and/or which mimics one or more high energy conformations of an amide, ester or glycosidic bond.

It is a further object of the invention to provide haptens which mimic the native conformation of biomolecules and which have complimentarity with biomolecules.

It is another object of the invention to provide catalytic antibodies which are capable of catalyzing the cleavage or formation of an amide, ester or glycosidic bond in a molecule.

It is yet another object of the invention to provide catalytic antibodies capable of recognizing a

specific amino acid sequence in a molecule containing numerous amino acids.

It is a further object of the invention to provide catalytic antibodies which are capable of catalyzing the cleavage or formation of a specific amide or ester bond within a specific amino acid sequence of a molecule.

It is yet another object of the invention to provide a method for catalyzing the cleavage or formation of an amide, ester or glycosidic bond in a molecule.

It is a further object of the invention to provide a method for catalyzing the cleavage or formation of a specific amide or ester bond within a specific amino acid sequence of a molecule containing numerous amino acids joined by amide bonds.

These and other features and advantages of the invention will become readily apparent from the ensuing detailed description, and the novel features will be particularly pointed out in the appended claims.

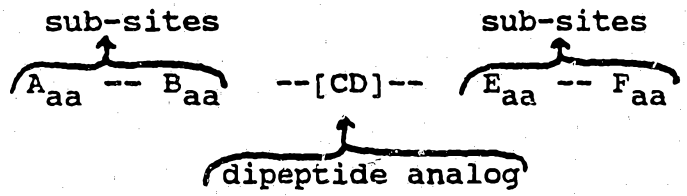
SUMMARY OF THE INVENTION

The invention is broadly directed to antigens capable of eliciting through immunogenic methods catalytic antibodies which can catalyze the cleavage or formation of an amide, peptide, ester or glycosidic bond in a molecule. The invention is directed to antigens capable of eliciting through immunogenic methods catalytic antibodies which can catalyze the selective cleavage or formation of a predetermined amide bond in a native polypeptide sequence. In general, the antigens can be a hapten or an immunogen comprising a hapten coupled (linked, conjugated) to a carrier molecule via a suitable coupling moiety. The haptens include structural elements which are designed to (i) mimic one or more high energy intermediates or transition states in

the cleavage or formation of the amide, ester or glycosidic bond (ii) mimic one or more high energy conformations of the amide, ester or glycosidic bond to be cleaved or (iii) mimic both one or more high energy intermediates or transition states and which mimic one or more high energy conformations in the cleavage or formation of the amide, ester or glycosidic bond.

The haptens according to the invention provide the correct side chain groups for production of antibodies that can react with predetermined sites on a protein and can catalyze selective proteolysis in a sequence specific manner. The haptens further incorporate amino acid side-chain sub-sites surrounding the amide bond analog. These sub-sites provide for the elicitation of catalytic antibodies capable of recognizing a specific amino acid sequence and selectively proteolyzing a peptide linkage within that sequence.

Such catalytic antibodies are elicited with the haptens of the present invention. For example, a hapten according to the invention, shown below,



incorporates not only the dipeptide analog [CD] but also sub-site amino acid residues A, B, E, F. These subsite amino acid residues can be part of a cyclic structure as well as a linear structure. The optimum number of sub-site residues is determined by the size of the antibody combining site. It is likely that the only essential criterion for effective binding of antibody to a peptide is that complementarity between the antigen combining site of the antibody and the molecular surface of the

binding peptide is maintained with regard to both shape and charge.

The haptens according to the invention are designed in such a way such that antibodies raised against these haptens can selectively stabilize one or any of the high energy intermediates or transition states in the cleavage or formation of an amide, peptide, ester or glycosidic bond. These haptens fall into three general classes: one, those in which the hybridization of the atom corresponding to the carbonyl carbon of the scissile bond of the amide or ester bond is converted from sp^2 to sp^3 hybridization; two, those in which any of the atoms corresponding to the amide, ester or glycosidic bond is replaced by a different atom; and three, those in which the atoms corresponding to the amide, ester or glycosidic bond are part of a monocyclic or bicyclic system.

Peptide sequences containing dipeptide analogs, according to the invention, at the bond that is required to be hydrolyzed by the catalytic antibodies of the present invention define a sequence that the catalytic antibody will hydrolyze in a native protein. The binding energy of the antibody is distributed in such a way as to allow both sequence specific recognition and chemical reactivity with the native protein or peptide of interest.

It has been reported that it is not necessary to prepare peptides longer than eight amino-acid residues (octapeptides) to demonstrate all continuous epitopes (6). It has also been demonstrated that antibodies bind to peptides in a reproducible manner (7). It has also been established that optical isomerism of the amino acids used has a powerful influence on the strength and specificity of antibody binding by dipeptides. Consequently, the importance of L and D amino acid

residues in the immunizing antigen will have a profound effect on the chirality of the antibody combining site generated. In generating catalytic antibodies according to the invention with predetermined specificity for particular sequential (continuous) or assembled epitopes in a native protein, the relationship between measurable properties of a protein and its immunogenic sites are important (8). With the ready availability of protein sequences, the most widely used algorithm is based on the likelihood of finding a sequential epitope at the site of a local maximum in the hydrophilicity profile (9). Surface accessibility profiles (10) and protein flexibility (11) also provide information on the antigen sites in a native protein sequence. With knowledge of these sites and the importance of these epitopes in receptor mediated interactions or other disease associated mechanisms, peptide haptens having dipeptide analogs within these important "bioactive" epitopes can be designed in accordance with the invention. The catalytic antibodies elicited with these haptens can then be utilized, for example, to digest epitopes on viral proteins or tumor derived growth factors or other peptides involved in life-threatening situations (e.g., tumor necrosis factor in bacterial sepsis, etc.).

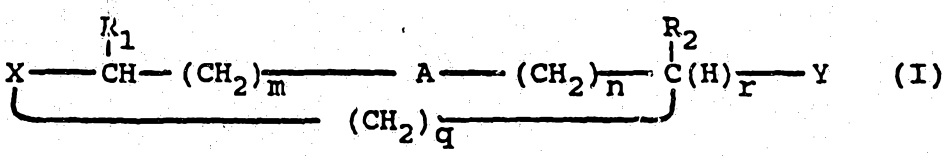
Thus, the haptens of the invention are distinguished from prior analog-ligands in that they have been rationally designed from knowledge of mechanistic features of enzyme catalysis and provide suitable templates for generating antibody combining sites endowed with catalytic properties. Consequently, they incorporate all the necessary features to provide for antibodies capable of molecular recognition and catalytic function.

Accordingly, the invention is a method for catalyzing the cleavage or formation of a specific amide, ester or glycosidic bond within a molecule. The method comprises contacting the molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of the amide, ester or glycosidic bond under conditions suitable for the cleavage or formation to take place; the monoclonal antibody having been prepared by a process comprising the steps of: selecting the specific amide, ester or glycosidic bond to be cleaved or formed; selecting an antigen comprising an analog of the amide, ester or glycosidic bond to be cleaved or formed, and also comprising moieties surrounding the analog of the amide, ester or glycosidic bond, which moieties substantially correspond to the moieties surrounding the amide, ester or glycosidic bond to be cleaved or formed; exposing cells capable of producing antibodies to the antigen and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the cleavage or formation of the amide, ester or glycosidic bond.

In another aspect, the invention is a method for catalyzing the cleavage or formation of a specific amide or ester bond in a molecule which comprises contacting the molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of the amide or ester bond under conditions suitable for the cleavage or formation to take place; the monoclonal antibody having been prepared by a process comprising the steps of: selecting the specific amide or ester bond to be cleaved or formed; selecting an antigen comprising an

analog of the amide or ester bond to be cleaved or formed, and also comprising moieties surrounding the analog of said amide or ester bond, the moieties substantially corresponding to some or all of the moieties surrounding the amide or ester bond to be cleaved or formed; exposing cells capable of producing antibodies to the antigen and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the cleavage or formation of the amide or ester bond.

In one aspect, the invention is directed to haptens of formula I

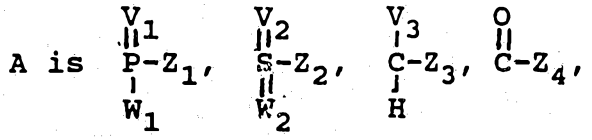


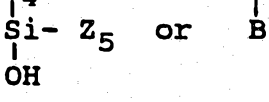
(I)

wherein

R_1 and R_2 may be the same or different and each is a side chain of a naturally occurring amino acid, a hydroxy containing side chain of a naturally occurring amino acid wherein said hydroxy group may be glycosylated, phosphorylated, sulphonylated or protected by a hydroxy protecting group, a primary amido containing side chain of a naturally occurring amino acid wherein said amido group may be glycosylated, or (C_1-C_4)alkyl, $-CH_2CH(CO_2H)_2$, $-(CH_2)_2S(O)CH_3$, $-(CH_2)_2S(O)_2CH_3$, $-(CH_2)_3NH_2$ or $-(CH_2)_3ONHC(=NH)NH_2$;

m, n and q may be the same or different and each is 0 or an integer from 1 to 10 and r is 0 or 1 provided that if r is 1, then there is no bond between X and the carbon bonded to R_2;

A is 



V_1 is O or S;

V_2 is O or a lone pair of electrons;

V_3 and V_4 are OH or NH_2;

W_1 is OH, NH_2, SH or H;

W_2 is O or a lone pair of electrons;

X is hydrogen, oxygen, amino, amino protected by a protecting group selected from the group consisting of terminal amino protecting groups, amino bonded to the C terminus of a naturally occurring amino acid to form a peptide bond, amino bonded to the C terminus of a peptide to form a peptide bond, said amino acid and peptide being unprotected or protected by said protecting group, or X is alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups may be unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl; Y is hydrogen, carboxyl, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, a carbonyl bonded to the N terminus of a naturally occurring amino acid to form a peptide bond, carbonyl bonded to the N terminus of a peptide to form a peptide bond, said amino acid and peptide being protected or unprotected by said protecting group, or Y is (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl

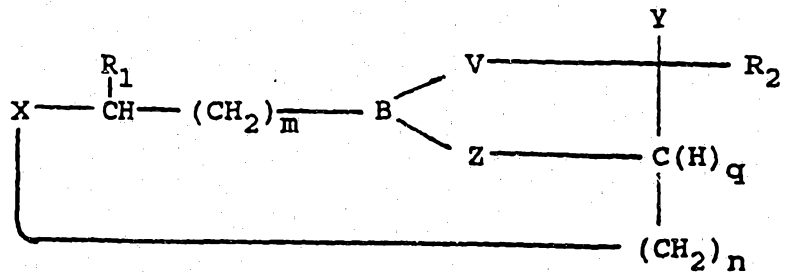
wherein the aforementioned phenyl groups may be unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl;

and wherein

each of said substituents R_1, R_2, X and Y may be unbound or bound to one or more of said remaining substituents R_1, R_2, X and Y and if bound, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_s-S-S-(CH_2)_t-$, $-(CH_2)_t-$, $-S-(CH_2)_t-S-$, $-(CH_2)_s-S-(CH_2)_t-$, $-(CH_2)_s-CH=CH-(CH_2)_t-$, $-(CH_2)_s-NH-CO-(CH_2)_t-$, $-(CH_2)_s-NH-(CH_2)_t-$ and $-(CH_2)_s-$phenyl$-(CH_2)_t-$;

Z_1, Z_2, Z_3, Z_4, Z_5 and Z_6 may be unbound or bound to said linker moiety; and if unbound Z_1 is O, NH, CH_2 or S, Z_2 is O, NH or CH_2, Z_3 is CH_2 or CF_2, Z_4 is CF_2 or CF_2CO and Z_5 and Z_6 are O or CH_2 provided that if Z_1 is O or NH and if V_1 is O and if W_1 is OH, then r is either 0 or r is 1 and at least one of said substituents R_1, R_2, X or Y is bound to one or more of said remaining substituents R_1, R_2, X and Y, and further provided that if Z_3 is CH_2 and V_3 is OH, then r is either 0 or r is 1 and at least one of said substituents R_1, R_2, X or Y is bound to one or more of said remaining substituents R_1, R_2, X and Y; and if bound Z_1 and Z_2 are N or CH, Z_4 is CF or CFCO and Z_3, Z_5 and Z_6 are CH and further provided that if Z_1, Z_2, Z_3, Z_4, Z_5 or Z_6 is bound to said linker moiety, it is covalently bound to said linker moiety by substitution at an appropriate atom of said linker moiety; and s and t may be the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_t-$ in which case t is an integer from 1 to 10.

The invention is also directed to boron-containing haptens having formula II



(II)

wherein

R_1, R_2, X, Y and m are defined as in formula I above;

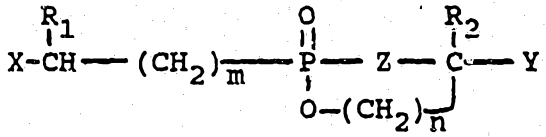
V is O, CH_2 or NH;

Z is O, CH_2 or NH;

n is 0 or 1; and

q is 1 or 2 provided that if q is 2, then there is no bond between X and the carbon bonded to Z.

The invention is further directed to phosphorus containing haptens of formula III



(III)

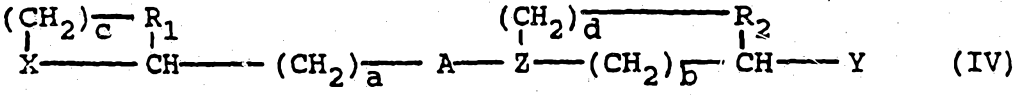
wherein

R_1, R_2, X, Y and m are defined as before;

Z is O, CH_2 or NH; and

n is 0 or an integer from 1 to 10.

In another aspect, the invention relates to haptens of formula IV



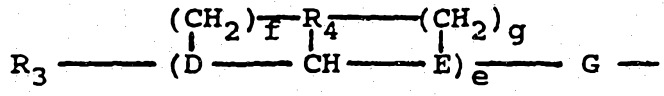
(IV)

or a physiologically acceptable salt thereof, wherein:

a and b are the same or different and each is an integer from 0 to 10;

c and d are the same or different and each is 0 or 2;

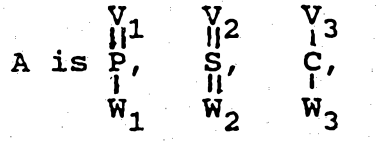
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or

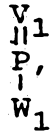


provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

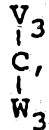
e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

A is  or ;

Z is O, NH, CH_2, or S when A is , provided that

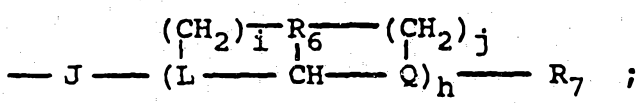
Z is N or CH when d is 2; Z is O, NH or CH_2 when A is ,

provided that Z is N or CH when d is 2; Z is CH_2 or CF_2 when A is , provided that Z is CH or CF when d is 2;

Z is NH when A is , provided that Z is N when d is 2; Z is CF_2 when A is C=O, provided that Z is CF when d is 2;

and Z is O or CH_2 when A is Si or B, provided that Z is CH when d is 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1 is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen, $CONH_2$ or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal

carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S provided that when c is 2, G is N, CH or CF;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

V_3 is OH, NH_2, or $NHNH_2$;

W_1 is OH, NH_2, NH(C_1-C_{10})alkyl, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

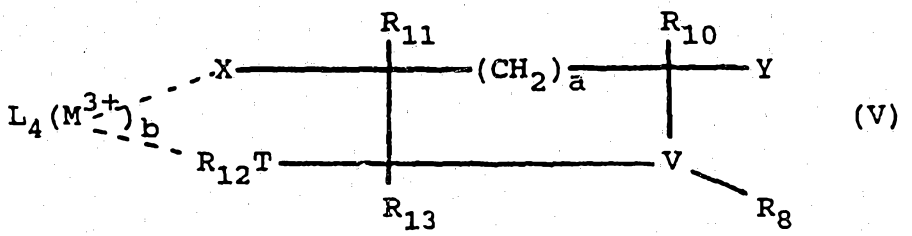
W_3 is H or CH_2NH_2;

and wherein

one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-phenyl-(CH_2)_v-$, provided that if Z is O or NH when V_1 is O and W_1 is OH, then at least one of R_1, R_2, R_4 and R_6 is bound to one of said remaining substituents R_1, R_2, R_4 and R_6.

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

The invention also relates to haptens of formula V



(V)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

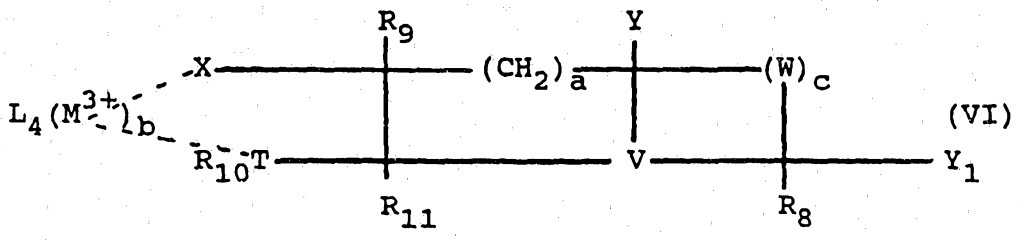
V is N, CH or CF;

X is defined as X above in formula IV;

Y and Y_1 are the same or different and each is defined as Y above in formula IV; and wherein

one or more of R_4, R_6, R_9, R_{10} and R_{11} are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9, R_{10} and R_{11} provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety as defined above in formula IV.

In another aspect the invention is directed to haptens of formula VI



or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 and R_9 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

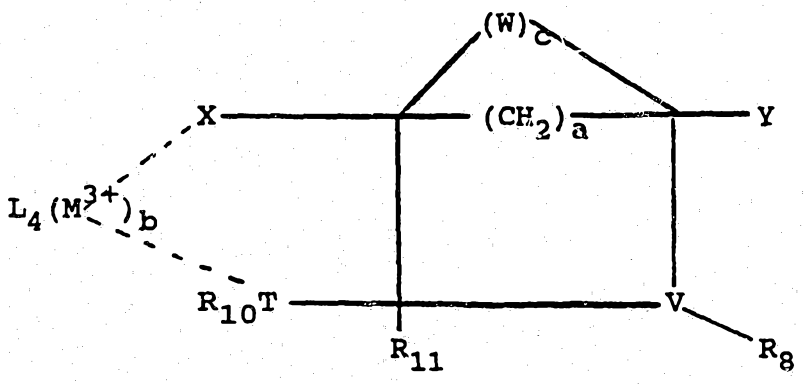
X is defined as X above in formula IV;

Y and Y_1 are the same or different and each is defined as Y above in formula IV;

and wherein

one or more of R_4, R_6, R_8 and R_9 are unbound or bound to one or more of said remaining substituents R_4, R_6, R_8 and R_9 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety as defined above in formula IV.

The invention is also directed to haptens of formula VII



(VII)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9 is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

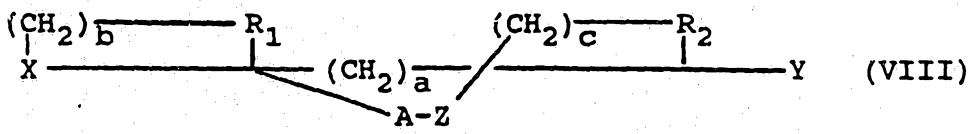
X is defined as X above in formula IV;

Y and Y_1 are the same or different and each is defined as Y above in formula IV and Y is additionally a side chain of a naturally occurring amino acid or an analog of the side chain;

and wherein

one or more of R_4, R_6, R_9 and Y, when Y is a side chain of a naturally occurring amino acid or analog of said side chain, are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9 and Y provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety as defined above in formula IV.

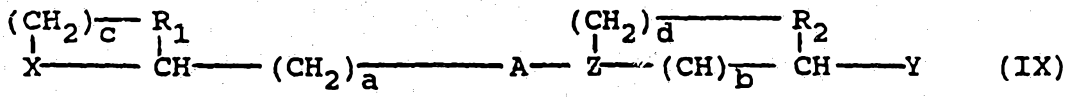
In still another aspect, the invention is a hapten of formula VIII



(VIII)

or a physiologically acceptable salt thereof, wherein b is 0 or 2 and a, c, X, Y, A, Z, R_1, and R_2 are as defined above in formula IV.

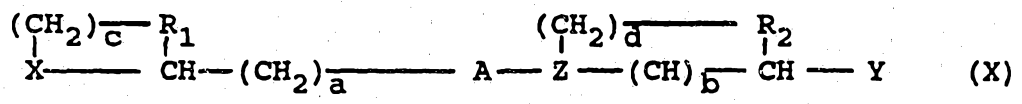
The invention also is a hapten of formula IX



(IX)

or a physiologically acceptable salt thereof, wherein a, b, c, d, X, Y, A, Z, R_1 and R_2 are as defined in formula IV above, provided that at least one of R_1, R_2, R_4 and R_6 is bound to at least one of the remaining substituents R_1, R_2, R_4 and R_6;

In yet another aspect, the invention is a hapten of formula X

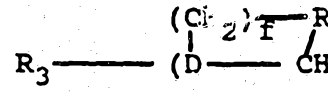


(X)

or a physiologically acceptable salt thereof, wherein a, b, c, d, X, Y, A, Z, R_1 and R_2 are defined as above in formula IV,

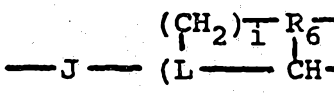
provided that when

(i) X is



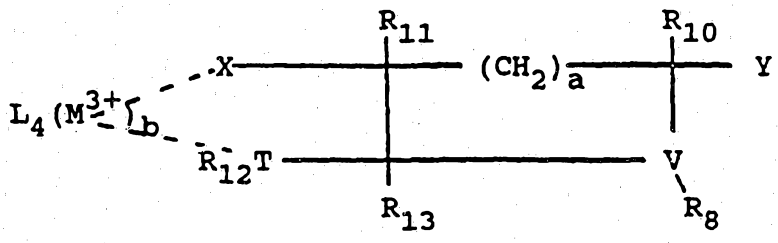
, then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

Another aspect of the invention is a hapten of formula (XI)

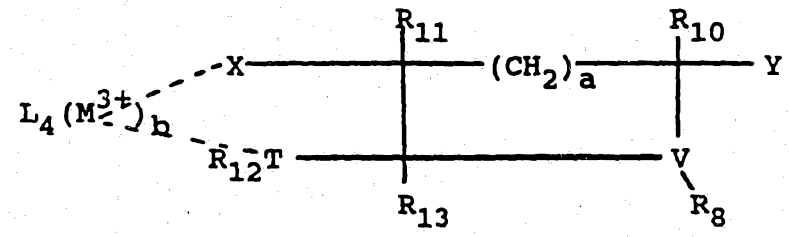


(XI)

wherein a, b, R_8, R_{10}, R_{11}, R_{12}, R_{13}, L, M, T, V, X and Y are defined as in formula V above provided that at least

one of R_4, R_6, R_9, R_{10} and R_{11} is bound to at least one of the remaining substituents R_4, R_6, R_9, R_{10} and R_{11}.

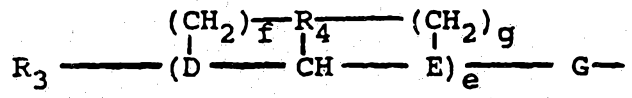
Another aspect of the invention is a hapten of formula XII



(XII)

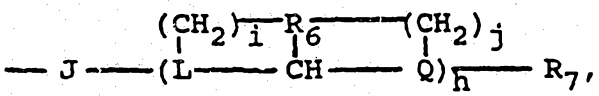
or a physiologically acceptable salt thereof, wherein a, b, R_8, R_{10}, R_{11}, R_{12}, R_{13}, L, M, T, V and Y are defined as in formula V above, provided that when

(i) X is



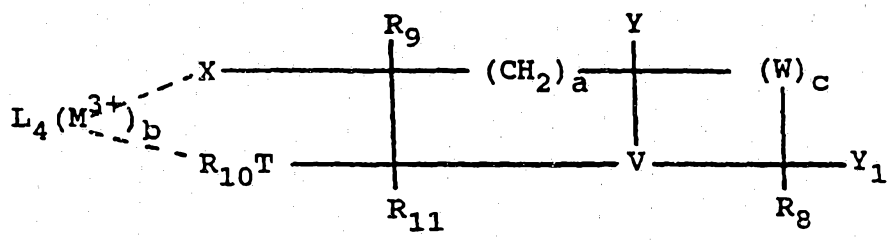
, then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

The invention is also a hapten of formula XIII

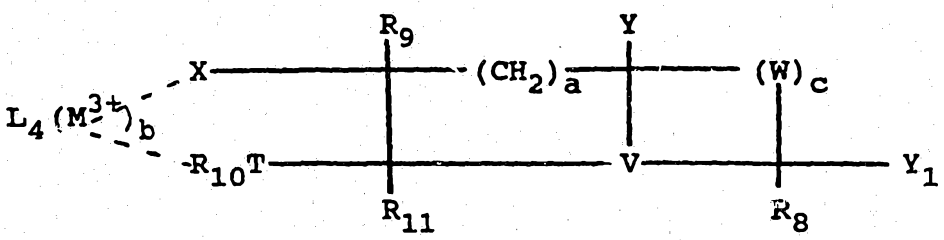


(XIII)

or a physiologically acceptable salt thereof, wherein a, b, c, R_8, R_9, R_{10}, R_{11}, L, M, T, V, W, X and Y are

defined as in formula VII above, provided that at least one of said substituents R_4, R_6, R_8 and R_9 is bound to at least one of said remaining substituents R_4, R_6, R_8 and R_9.

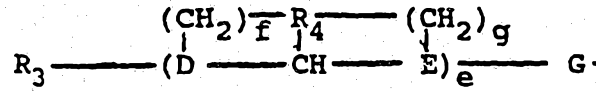
Another aspect of the invention is a hapten of formula XIV



(XIV)

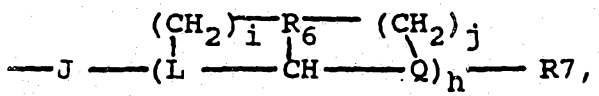
or a physiologically acceptable salt thereof, wherein a, b, c, R_8, R_9, R_{10}, R_{11}, L, M, T, V, X and Y are defined as in formula VI above, provided that when

(i) X is



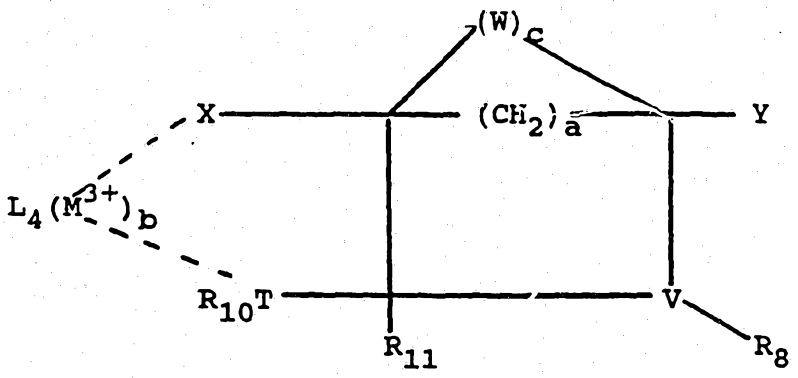
, then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

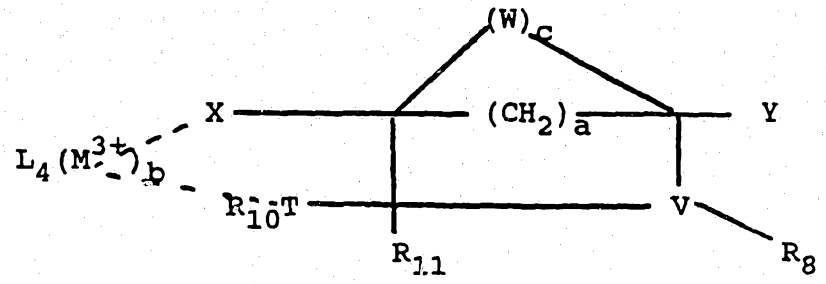
The invention is also directed to a hapten of formula XV



(XV)

or a physiologically acceptable salt thereof, wherein a, b, c, R_8, R_{10}, R_{11}, L, M, T, V, W, X and Y are defined as in formula VII above, provided at least one of said substituents R_4, R_6, R_9 and Y is bound to at least one of said remaining substituents R_4, R_6, R_9 and Y.

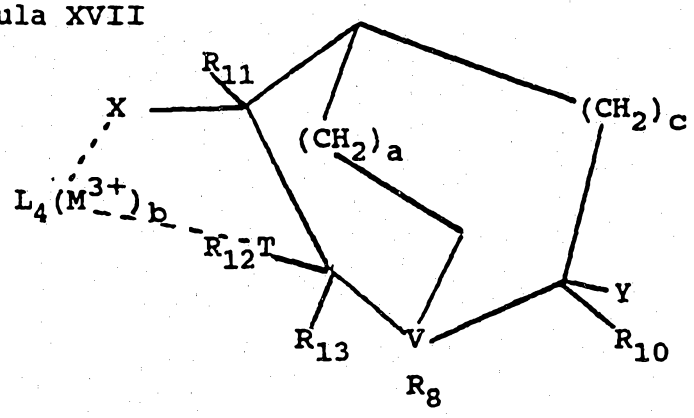
In another aspect the invention is a hapten of formula XVI



(XVI)

or a physiologically acceptable salt thereof, wherein a, b, c, R_8, R_{10}, and R_{11}, L, M, T, V, X and Y are defined as in formula VII above.

The invention is also directed to a hapten of formula XVII



(XVII)

or a physiologically acceptable salt thereof, wherein a, b, c, R_8, R_{12}, R_{13}, L, M, T, X and Y are defined as in formula VII above, R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain; and wherein

one or more of R_4, R_6, R_9, R_{10} and R_{11} are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9, R_{10} and R_{11} provided R_{14} is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety as defined above.

One of ordinary skill in the art will realize that in accordance with this invention, the above definitions of the X, Y, R_1 and R_2 substituents for the haptens of formulae I-III are interchangeable with the definitions of X, Y and those substituents defined as side chains of naturally occurring amino acids, or analogs thereof, for the haptens of formulae IV-XVII.

The foregoing haptens may be used as antigens for <u>in vitro</u> elicitation of catalytic antibodies. However, for purposes of <u>in vivo</u> elicitation, the haptens must be

coupled to a suitable carrier molecule in order to obtain an immunogen suitable for immunization. Therefore, the invention is also directed to immunogens capable of eliciting catalytic antibodies. Such immunogens comprise a hapten as hereinbeforedescribed coupled to a carrier molecule by a suitable coupling moiety.

In another aspect the invention is directed to catalytic antibodies which are elicited by antigens comprising the haptens of the invention as described above. Similarily, the invention is also directed to catalytic antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by antigens comprising haptens according to the invention as described above, wherein the antibodies have been prepared by exposing cells capable of producing antibodies to the antigens and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby producing a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest.

Alternatively, cells capable of producing catalytic antibodies can be stimulated to grow in culture and, therefore, can be immortalized using methodologies well known in the art. For example, lymphocytes can be so stimulated using a virus, a chemical agent or a nucleic acid (e.g., an oncogene).

In still another aspect, the invention is directed to a method for producing catalytic antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by antigens comprising the haptens according to the

invention as described above. The method comprises exposing cells capable of producing antibodies to the antigens and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest.

The invention is also directed to a method for catalyzing the cleavage or formation of an amide or ester bond in a molecule. The method comprises contacting the molecule with an effective amount of a catalytic antibody which has been elicited by antigens comprising haptens according to the invention.

In another aspect, the invention is directed to a method for catalyzing the cleavage or formation of a specific amide bond within a specific amino acid sequence of a molecule containing numerous amino acids joined by amide bonds. The method comprises contacting the molecule with an effective amount of a catalytic antibody which has been elicited by antigens comprising haptens according to the invention. The haptens have complimentarity with the specific amino acid sequence.

As noted earlier, the catalytic antibodies elicited by antigens comprising haptens according to the invention can be used, for example, to digest epitopes on viral proteins or tumor-derived growth factors on other peptides involved in health- or life- threatening situations.

Thus, in another aspect, the invention is directed to a method for treating acquired immune deficiency syndrome (AIDS) by inhibiting human immunodeficiency virus (HIV). The method comprises treating a patient

with an effective amount of a catalytic antibody elicited using a hapten of the invention.

The invention is also directed to a method for treating hypertension by inhibiting human renin activity. The method comprises treating a patient with an effective amount of a catalytic antibody elicited using a hapten of the invention.

Another aspect of the invention is a method for catalyzing the cleavage or formation of myohaemerythrin between the glycine residues at positions 85 and 86 of the myohaemerythrin molecule. For cleavage, the method comprises contacting myohaemerythrin with an amount of a monoclonal antibody effective to catalyze the cleavage, said antibody having been elicited with an antigen comprising a hapten of the invention, preferably a hapten of formulae V, VI, VII and XI-XVII. For formation myohaemerythrin fragments 1-85 and 86-118 are contacted with an amount of a monoclonal antibody effective to catalyze the formation, said antibody having been elicited with an antigen comprising a hapten of the invention, preferably a hapten of formulae V, VI, VII and XI-XVII.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention, as well as other objects, features and advantages thereof will be understood more clearly and fully from the following detailed description, when read with reference to the accompanying drawings, in which:

Fig. 1 shows the tetrahedral carbon transition state in the hydrolysis of a peptide bond;

Fig. 2 shows the tetrahedral carbon transition state in the hydrolysis of an ester bond;

Fig. 3 depicts pyradazinedione analogs;

Fig. 4 depicts bicyclic ß-turn and rigid tricyclic analogs;

Fig. 5 depicts macrocyclic ß-turn analogs;

Fig. 6 shows the reaction sequence for the synthesis of N-(N-phenylalanylsulfonylacetyl)glycine;

Fig. 7 shows the reaction sequence for the synthesis of 3-(N-Carbobenzyloxymethyl)sulfonyl-2-methylpropanoic acid;

Fig. 8 shows the reaction sequence for the synthesis of 3-Difluoro-5-(6-maleimidohexanoyl)amino-2-methyl-4-oxo-6-phenylhexanoic acid;

Fig. 9 shows the reaction sequence for the synthesis of 5-[N-Benzyloxycarbonylmethylamido]-3-3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid;

Fig. 10 depicts a type I ß-turn;

Fig. 11 depicts a ß-turn containing a N-amino-methylamidinium covalent link;

Fig. 12 depicts a phosphonamidate hapten according to the invention having the ß-turn conformation;

Fig. 13 shows Scheme 1 in the synthesis of the conformationally constrained hapten shown in Fig. 12;

Fig. 14 shows Scheme 2 in the synthesis of the conformationally constrained hapten shown in Fig. 12;

Fig. 15 shows Scheme 3 in the synthesis of the conformationally constrained hapten shown in Fig. 12;

Fig. 16 shows the reaction sequence for the synthesis of [(6-maleimido)hexanyl]amino](2-phenylethyl)hydroxyphosphinyl D-alanine;

Fig. 17 shows the reaction sequence for the synthesis of [(6-maleimido)hexanoyl]amino](2-phenylethyl)hydroxyphosphinyl D-alanine;

Fig. 18 shows the reaction sequence for the synthesis of [(6-maleimide)hexanoyl]amino]-2-phenylethyl(2-carboxy-1-propy)hydroxyphosphonous acid;

Fig. 19 shows the reaction sequence for the synthesis of (2-[(6-maleimido)butanoyl]amino]-3-phenylpropylimino-D-alanine;

Fig. 20 shows the reaction sequence for the synthesis of (S)-3-(Benzyloxycarbonyl)amino-2-Oxo-1-azetidineacetic acid;

Fig. 21 shows the reaction sequence for the synthesis of (3'S,2R)[3-Amino-2-Oxo-1-Azetidinyl]-3-Methylbutanoic Acid;

Fig. 22 shows the reaction sequence for the synthesis of *cis*-3-(Benzyloxycarbonyl)amino-4-Carboxy-2-Azetidinone;

Fig. 23 shows the reaction sequence for the synthesis of 3-(Benzyloxycarbonyl)amino-2-Oxo-1-Cyclobutaneacetic Acid and 3-(Benzyloxycarbonyl)amino-2-Hydroxy-1-Cyclobutaneacetic Acid;

Fig. 24 shows the reaction sequence for the synthesis of 2-(Benzyloxycarbonyl)amino-3-Hydroxycyclobutanecarboxylate and 2-(Benzyloxycarbonyl)amino-3-Oxocyclobutanecarboxylate;

Fig. 25 shows the reaction sequence for the synthesis of 3-*exo*-(Benzyloxycarbonyl)amino-2-*exo*-Hydroxynorbornyl-7-*anti*-carboxylate and 3-*exo*-(Benzyloxycarbonyl)amino-2-Oxonorbornyl-7-*anti*-carboxylate;

Fig. 26 shows the reaction sequence for the synthesis of 3-*endo*-(Benzyloxycarbonyl)amino-2-*endo*-Hydroxynorbornyl-7-*anti*-carboxylate and 3-*endo*-(Benzyloxycarbonyl)amino-2-Oxonorbornyl-7-*anti*-carboxylate;

Fig. 27 shows the dose dependent inhibition by clone AHIV 1.3 of HIV-I p 24 gag production in infected H9 cells; and

Fig. 28 shows the dose dependent inhibition by clones AHIV 1.3, AHIV 1.6 and AHIV 2.0 of HIV-1-induced cell-fusion.

DETAILED DESCRIPTION OF THE INVENTION

Broadly, the invention relates to antigens which are capable of eliciting through immunogenic methods antibodies which can catalyze the cleavage or formation of a peptide linkage or the cleavage or formation of an ester in a molecule. These antigens comprise a hapten or a hapten and a suitable carrier molecule. Because the antibodies so elicited can catalyze a chemical reaction, they are defined as "catalytic antibodies." Catalytic antibodies are identified and described in Schochetman and Massey, Application Serial No. 674,253 filed November 27, 1984, referred to above in the "Statement of the Invention."

During the course of a chemical reaction, the reactants undergo one or more transitions through structures which are energetically less favorable than either the reactant or product. In molecular terms, these transition states (or intermediate structures) reflect changes in bond lengths and bond angles as well as formation and breakage of bonds. The energy required to achieve a transition state is denoted as the activation energy, which may also be considered as the difference in energy between the energy of the transition state and the energy of the reactants.

Catalysts increase chemical reaction rates by lowering the activation energy of a reaction. Antibodies elicited to a hapten or immunogen of the invention, which antigens are chosen because, inter alia, they resemble the presumed transition state structure (i.e., a transition state analog or a strained

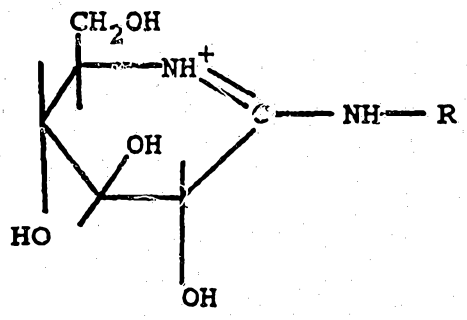
ground state structure or both), can catalyze reactions. The antibody thus produced should stabilize the energy of the transition state relative to reactants and products. This approach has been successfully demonstrated in the generation of several catalytic monoclonal antibodies.

Catalytic antibodies elicited with haptens according to the invention are "site specific" in that they are deliberately designed only to catalyze cleavage of peptide bonds having certain structural conformations at specific sites in a protein molecule. Likewise, these catalytic antibodies are designed only to catalyze the formation of peptide linkages from the N- and C-termini of amino acids having certain structural conformations at those terminals. Therefore, haptens according to the invention may be used to elicit a site specific catalytic antibody capable of cleaving peptide bonds at specific sites in a protein molecule to produce two or more cleaved protein strands. The same catalytic antibody can then catalyze the formation of peptide bonds wherein those cleaved strands having the right structural conformation are joined.

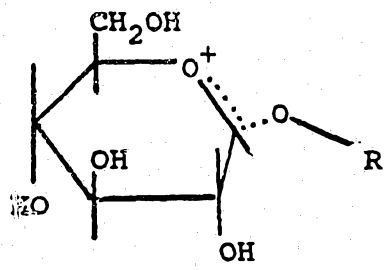
Thus, the haptens of the invention are designed to mimic the transition-states or strained ground states or both for a variety of chemical reactions. Preferably, though not exclusively, the reactions are the cleavage or formation of a peptide bond or an ester bond.

Certain haptens of the invention may also be able to mimic the transition states of other non-peptide, non-ester, types of chemical reactions. Thus, the invention contemplates, for example, a hapten of formula IV above wherein A is C=NH and Z is NH and both A and Z are part of a cyclic carbohydrate moiety and R_2 is any other chemical grouping. Such a hapten [1] would provide a

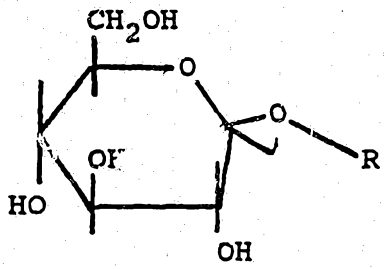
good mimic of the proposed transition state [2] (12) for hydrolysis of a glycosidic bond in a typical O-linked glycoside [3], as illustrated below:



[1]



[2]



[3]

In an embodiment of the invention, a method is provided for catalyzing the cleavage or formation of a specific amide or ester bond within a specific amino acid sequence contained in a molecule. The molecule is contacted with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of the amide or ester bond under conditions suitable for the cleavage or formation to take place. The monoclonal

antibody is elicited using an antigen comprising a hapten of the invention.

The term "amide bond" refers to a simple amide bond (e.g., an amide bond in a side chain of a naturally occurring amino acid) or an amide bond which joins two adjacent amino acid residues, i.e., a peptide bond. The term "peptide" includes dipeptides and polypeptides.

The term "analog of an amide bond" as used herein is defined as a normal amide bond (- CO - NH -) in which one or more moieties in the normal amide bond are replaced by one or more different moieties similar in charge and/or size to the normal moieties replaced. The same holds true for an "analog of an ester bond". "Moiety" is defined as a radical (e.g., an atom, CH_3, C_6H_5, OH, NH_2, etc.). For example, in one embodiment of the invention, an analog of an amide bond is - CO - CF_2 wherein the normal NH moiety is replaced by the CF_2 moiety.

In its broadest sense, the term "antigen" is defined as a molecule which induces the formation of an antibody. As used herein, the term "antigen" means a molecule which is inherently immunogenic, a hapten according to the invention or an immunogen which comprises a hapten according to the invention coupled to a carrier molecule by a suitable coupling moiety. Carrier molecules include, for example, keyhole limpet hemocyanin (KLH), thyroglobulin, chicken immunoglobulin, ovalbumin, bovine serum albumin (BSA), T-helper peptides, etc. "Coupling moieties" as used herein refer to biotechnological cross-linking reagents well known in the art (e.g., commercially available from Pierce, Rockford, Illinois) and include, for example, Trout's reagent, dissuccinyl suberate, etc.

The term "transition state analog" as used herein refers to an array of atoms which is designed to approximate or "mimic" the configuration of an amide bond or an ester bond as such bonds exist in a hydrolytic transition state. As an illustrative example, the groups "A" and "A-Z" in formulae I and IV, respectively, above represents a number of such arrays.

The term "strained ground state" as used herein refers to an array of atoms that is designed to approximate or "mimic" one or more high energy conformations of an amide or ester bond. As an example, formula V above represents a number of such arrays.

The term "dipeptide analog" as used herein refers to a structure which comprises a transition state analog or strained ground state analog or elements of both having side chains of two amino acids which are in positions analogous to those of the dipeptide being mimicked. In other words, in a dipeptide analog, the normal amide bond (i.e., -CO-NH-) between the two amino acids has been replaced by an array of atoms as defined above. Additional amino acid residues may be incorporated to surround the dipeptide analog to form a polypeptide. Thus, the dipeptide analog replaces the peptide bond "targeted" for cleavage in the substrate molecule. In one embodiment of the invention, the moieties surrounding the dipeptide analog contain peptide bond linkages which can be altered such that the naturally occurring C=O group is replaced by NH, O, S, CH_2, CF_2 or C=S and/or the naturally occurring NH group is replaced by O, S, CH_2, CF_2, C=O or C=S. For example, the moieties can be retropeptides in which the C=O and NH groups of the amide bonds are interchanged.

The terms "some or all" refer to a portion of the target molecule including at least the amide, ester or

glycosidic bond to be cleaved or all of the target molecule. For example, in an embodiment of the invention, haptens designed for the purpose of eliciting antibodies to catalyze the cleavage of a specific peptide bond in a protein molecule comprising a polypeptide of many amino acid residues, the dipeptide analog corresponding to the target peptide bond need only be surrounded by not more than about eight amino acid residues. However, if the target molecule is a relatively short peptide, it is advantageous to surround the peptide bond analog with all the amino acid residues of the target molecule. Of course, one of ordinary skill in the art will realize that the desired specificity, the nature of the target molecule and other factors will dictate the ideal number of amino acid residues needed to surround the dipeptide analog.

The term "substantially corresponds" refers to moieties which are similar in charge and/or size to moieties in the amide bond analog, dipeptide analog or naturally occurring amino acid side chain analog. Preferably, the moieties are identical in size and charge, although such identity is not necessary for the hapten of the invention.

The term "hapten" as used herein is defined as a molecule which can act as an epitope. Haptens according to the invention incorporate a dipeptide analog according to the invention.

Physiologically acceptable salts include salts of mineral acids, for example, hydrochloric acid, sulfuric acid, nitric acid and the like, salts of monobasic carboxylic acids such as, for example, acetic acid, propionic acid and the like, salts of dibasic carboxylic acids such as, for example, maleic acid, fumaric acid and

the like, and salts of tribasic carboxylic acids such as, for example, citric acid and the like.

The term "naturally occurring amino acid" as used herein includes the twenty essential alpha-amino acids and other alpha-amino acids which may or may not be found in proteins. These amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, 4-hydroxyproline, 5-hydroxylysine, epsilon-N-methyllysine, 3-methylhistidine, beta-alanine, gamma-aminobutyric acid, homocysteine, homoserine, citrulline, ornithine, canavanine, djenkolic acid and beta-cyanoalanine. An amino acid consists of a carbon atom to which is bonded an amino group, a carboxyl group, a hydrogen atom and a distinctive group referred to as a "side chain". Accordingly, the above-described haptens include a side chain of a naturally occurring amino acid as well as an "analog of said side chain." The term "analog of said side chain" as used herein is defined as a side chain of a naturally occurring amino acid in which one or more moieties of the naturally occurring side chain is replaced by one or more different moieties which substantially corresponds to the naturally occurring moiety. Those side chains containing a hydroxy group can be glycosylated, phosphorylated, sulphonylated or protected by a hydroxy protecting group. The hydroxy group of any of the side chains may be protected by any number of suitable hydroxy protecting groups well known in the art. These include, for example, a tertiary butyl group.

The term "terminal amino protecting group" means any group capable of protecting the terminal amino

moiety of a peptide or amino acid. Therefore, terminal amino protecting groups include acetyl, succinyl, biphenylcarbonyl, benzoyl, t-butyloxycarbonyl, carbobenzyloxy, tosyl, dansyl, isovaleryl, phthalyl, 1-adamantanesulphonyl, acetimido, benzimido, amidino, carbamyl and the functional equivalents thereof.

The term "terminal carboxyl protecting group" means any group capable of protecting the terminal carboxyl moiety of a peptide or amino acid. Terminal carboxyl protecting groups include (C_1-C_9)alkyl, phenyl, substituted methyl esters such as methoxymethyl and phenacyl esters, 2- substituted ethyl esters such as cyclohexyl and allyl, substituted benzyl esters such as para-methoxybenzyl and para-bromobenzyl, amides such as piperidinyl and hydrazide and functional equivalents thereof.

With regard to haptens of formulae IV-XVII, the value of 2 for the variable b (formula VIII), c, d (formulae IV, IX, X), f, g, i and j (formulae IV-XVII) provide for the possibility of a proline ring structure. However, it will be understood that when any of b, c, d, e, f, g, i or j is 0, there is no proline ring structure at the respective sites of those variables in the aforementioned haptens.

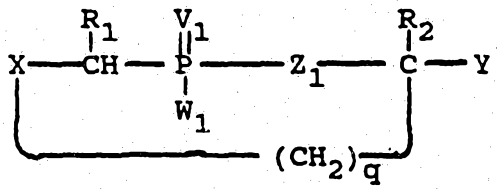
With regard to haptens of formulae V-VII and XI-XVII, L is a ligand and preferably L_4 is $4H_2O$, $4NH_3$, 2 ethylenediamine or triethylenetetraamine.

Haptens according to the invention contain one or more asymmetric centers and therefore exist in enantiomeric and/or diastereomeric forms. In general, the corresponding haptens according to the invention are obtained in the form of racemates or mixtures of diastereomers. If desired, techniques well known in the art for the separation of the mixtures into sterically

homogeneous constituents may be used. Preparation of the optical isomers in a pure state is also possible by using sterically homogeneous starting materials.

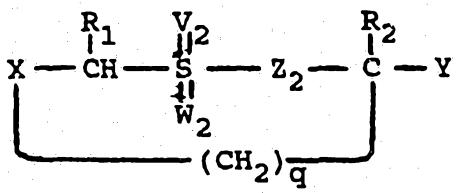
One of ordinary skill in the art will realize that arrays containing carbonyl adjacent to difluoromethylene or fluoromethine and boron will assume a tehrahedral like configuration upon reaction with water in an aqueous environment.

Preferred haptens of formula I containing a central phosphorus atom include the following cyclic structure



wherein R_1, R_2, X, Y, W_1, V_1, Z_1 and q are defined as in formula I above. Especially preferred are those in which V_1 is O, W_1 is OH, SH or H, Z_1 is O, NH or CH_2 and q is 0 or 1.

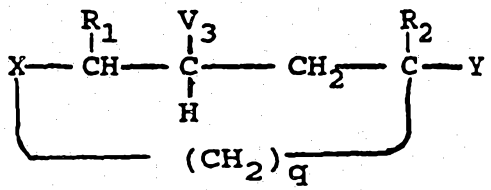
Preferred haptens of formula I containing a central sulphur atom include compounds in which Z_2 is NH. Such a hapten is aminomethanesulfonamidylalanyl acid. Other preferred haptens of formula I containing a central sulfur atom include the following cyclic structure



wherein R_1, R_2, X, Y, V_2, W_2, Z_2 and q are defined as in formula I above. Especially preferred are those in

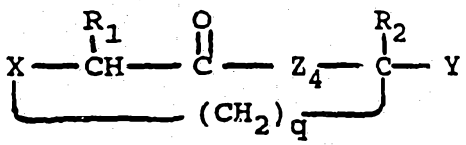
which V_2 and W_2 are O or a lone pair of electrons, Z_2 is O, CH_2 or NH and q is 0 or 1.

Preferred haptens of formula I containing a central carbon atom include compounds in which V_3 is OH and Z_3 is CH_2, V_3 is NH_2 and Z_3 is CH_2. Other preferred haptens of formula I containing a central carbon atom include the following cyclic structure



wherein R_1, R_2, X, Y, V_3 and q are defined as in formula I above. Especially preferred are those wherein V_3 is OH or NH_2 and q is 0 or 1.

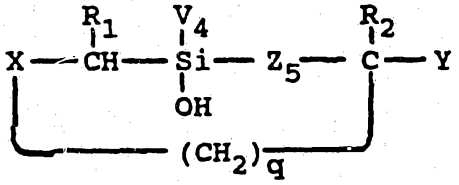
Preferred haptens of formula I containing a central carbonyl include the compound in which Z_4 is CF_2. Such a preferred hapten is 5- (serinyl)amino 3,3-difluoro 4-oxo 6-hydroxy heptanoic acid. Other preferred haptens of formula I containing a central carbonyl include the following cyclic structure



wherein R_1, R_2, X, Y, Z_4 and q are defined as in formula I above. Especially preferred are those in which Z_4 is CF_2 and q is 0 or 1.

Preferred haptens of formula I containing a central silicon atom include compounds in which V_4 is OH and Z_5 is O, and V_4 is OH and Z_5 is CH_2. A preferred hapten is 3-(aminomethyldihydroxysilyl) propionic acid.

Other preferred haptens of formula I containing a central silicon atom include the following cyclic structure

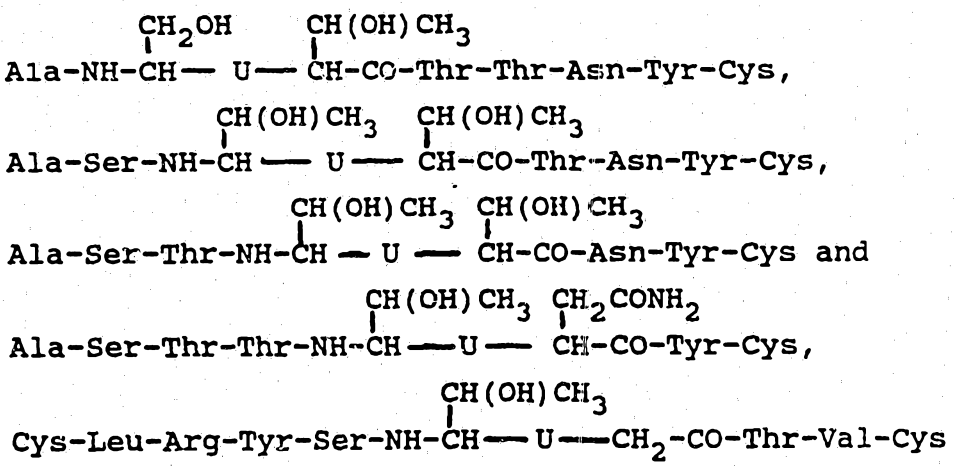


wherein R_1, R_2, X, Y, Z_5 and q are defined as above. Especially preferred are those in which V_4 is OH, Z_5 is CH_2 and q is 0 or 1.

Preferred haptens of formula I containing a central boron atom include compounds wherein Z_6 is O. Such a preferred hapten is (S)-lactate- 1-(R)-amino-2-phenylethane boronate.

A preferred hapten according to formula II is (R)-2- hydroxymethyl-2-hydroxy-propanoic acid diol 1-amino-2- phenylethaneboronate.

Other preferred haptens of the invention include



wherein U is A as in formula I or U is A-Z as in formula IV.

Other preferred haptens according to formulae I-IV include those in which R_1 is selected from the group consisting of CH_2OH and $CH(OH)CH_3$; R_2 is selected from the group consisting of $CH(OH)CH_3$ and CH_2CONH_2; X is selected from the group consisting of amino bonded to the C terminus of alanine, amino bonded to the C terminus of serine in the dipeptide Ala-Ser, amino bonded to the C terminus of threonine in the tripeptide Ala-Ser-Thr and amino bonded to the C terminus of theronine in the polypeptide Ala-Ser-Thr-Thr; and Y is selected from the group consisting of carbonyl bonded to the N terminus of threonine in the polypeptide Thr-Thr-Asn-Tyr-Cys, carbonyl bonded to the N terminus of threonine in the polypeptide Thr-Asn-Tyr-Cys, carbonyl bonded to the N terminus of asparagine in the tripeptide Asn-Tyr-Cys and carbonyl bonded to the N terminus of tyrosine in the dipeptide Tyr-Cys. Still other preferred haptens of formulae I-IV include those in which R_1 is $CH(OH)CH_3$, R_2 is H, X is amino bonded to the C terminus of serine in the polypeptide Cys-Leu-Arg-Tyr-Ser and Y is carbonyl bonded to the N terminus of threonine in the tripeptide Thr-Val-Cys. The foregoing preferred substituents are applicable to the other haptens of formulae V-XVII inasmuch as X and Y are common to all and R_1 and R_2 are defined as side chains of naturally occurring amino acids, or analogs thereof, as are various other substituents in the haptens of formulae V, VI, VII, XI-XVII.

One application of catalytic antibodies having site-specific proteolysis capabilities is in the immunotherapy of viral infection. Viruses utilize their external coat proteins to attach to cellular receptors and invade the cell after attachment. For example, human immunodeficiency virus (HIV) uses a portion of the

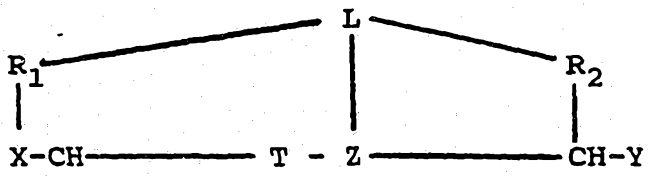
gp120 protein at its surface to attach to CD4 receptors on lymphocytes. The sequence for this cell attachment has been mapped to a region on the viral protein. With this information, antibodies can be generated by the methodology described in this invention to bind to this peptide sequence and cleave it in a site-specific manner.

However, such antibodies preferably bind to the "native" sequence in the protein as opposed to a linear sequence (which would occur in a denatured protein). Thus, the antigenic determinants or epitopes in the "native" protein are often conformational (i.e., three-dimensional) rather than random linear arrangements. Here again, knowledge of epitopes on the protein is important in the design of antibodies having paratopes that can induce modifications of such epitopes.

Therefore, haptens according to the invention are designed to have the same structural features of the epitopes, rather than random conformations. These structural features can be adopted by simple linear peptides, the lowest energy conformer being the preferred structure in solution. Secondary structural features may be introduced by cross-linking of amino-acid side-chains or the use of ß-turn mimetics. Conformationally constrained haptens incorporating structures which are compatible with the epitope in the native protein may be essential for inducing the correct motif within the tertiary structure of the catalytic antibody hyper-variable binding region. The advantages of conformationally constrained haptens are that they mimic the native structure in the protein and tend to mimic regions of the protein which are susceptible to cleavage. Accordingly, in the structural formula I shown above for the haptens of the invention, the substituents R_1, R_2, X and Y may be bound to one or more of the

remaining substituents R_1, R_2, X and Y by a covalent bond or a linker moiety as defined above. Likewise, in formulae I, the substituents Z_1-Z_6 may be covalently bonded to the linker moiety by substitution at an appropriate atom in the linker moiety.

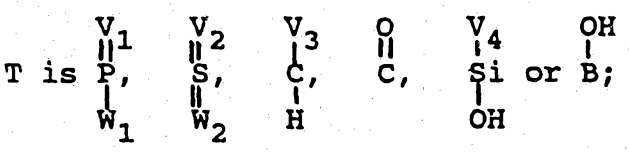
Preferred conformationally constrained haptens are represented by formula IA



(IA).

wherein

R_1, R_2, X and Y and are as defined with reference to formula I above;

T is  ;

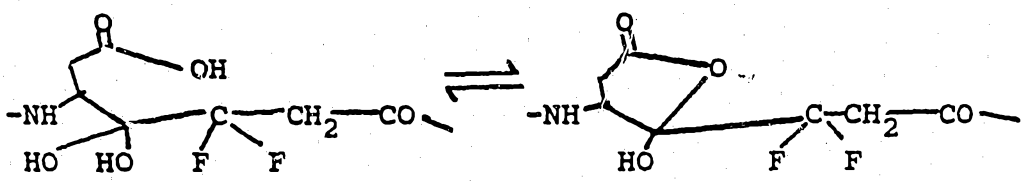
V_1, V_2, V_3, V_4, W_1 and W_2 are as defined with reference to formula I above;

Z is Z_1, Z_2, Z_3, Z_4 or Z_5 as defined with reference to formula I above; and

L is N or CH in the linker moiety as defined with reference to formula I above. These include pyradazinedione analogs, shown in Fig. 3, bicyclic ß-turn and rigid tricyclic analogs, shown in Fig. 4 and macrocyclic ß-turn analogs in Fig. 5. On comparison of the haptens set forth in Figs. 3, 4 and 5 and the general formula IA, the skilled artisan will readily appreciate that the side chains R_1 and R_2 are $-CH_2-$ or

$-CH_2CH_2-$ and these side chains R_1 and R_2 are joined by the linker moiety "L" which is $-CH_2-CO-N-CH_2-$, $-CH_2-N-CH_2-$, $-CH_2-CH-S-$ or -ortho-phenyl-CH- CH_2-. The nitrogen atom corresponding to the Z substituent is covalently bonded to the aforementioned linker moieties by substitution at an appropriate atom in the linker moiety, i.e., at the nitrogen atom in the first two linker moieties listed, at the carbon atom adjacent to the sulfur atom in the third linker moiety listed and at the carbon atom adjacent to the phenyl ring in the fourth linker moiety listed. Of course, the microcyclic-ß-turn analog shown in Fig. 11 corresponds to the generic formula I wherein the Z substituent is not bound to the linker moiety. While in Figs. 3, 4 and 5 phosphonamidate analogs are depicted, it is to be understood that any of the other tetrahedral carbon mimics according to the invention (e.g., $-SO_2-$, $-CHNH_2-$, CHOH-, $-Si(OH)_2-$, etc.) may also be used.

A further way in which conformational constraint can assist the cleavage reaction itself is where an amino acid side chain is constrained in a conformation which allows it to directly participate in peptide bond cleavage. For example, in one embodiment of the hapten of formula IV in which Z is CF_2, A is $C(OH)_2$, R_1 is the side chain of aspartic acid and R_2 is the side chain of any other amino acid, the linear conformation of the hapten is known to undergo a spontaneous tautomerism to a cyclic form, particularly when the hapten is part of a longer peptide sequence, thus:



Such a cyclization is formally equivalent to the process believed to occur during the hydrolysis of peptide bonds in which aspartic acid or asparagine is present on the N-terminal side of the bond to be cleaved. As a result of the assistance of the side chain during the cleavage, aspartic acid-X or asparagine-X bonds (where X is any amino acid but particularly glycine or proline) are known to undergo background hydrolysis at enhanced rates compared with other types of peptide bond. Indeed, such "metastable" bonds and their susceptibility to intramolecular assisted cleavage are well described in the literature (13,14). Thus, in the aforementioned formulation of hapten of formula I, the cyclic tautomer would represent an ideal mimic of the transition state for the intramolecular cleavage of aspartyl-X peptide bonds. Those skilled in the art will readily see that this concept can be extended to include the similar side chains of asparagine as well as the related side chains of glutamic acid and glutamine. It will also be apparent to one skilled in the art that similar cyclic forms to those described above may also be generated using hapten design in which the transition state is mimicked by phosphorus or sulfur analogs.

The haptens of the invention can also take on a configuration mimicking that of the native ß-turn or "hairpin" configuration of proteins by the formation of disulphide bridges between sulfur containing amino acid side chains which are incorporated into the hapten. Formation of disulphide bridges also promotes hydrogen bonding interactions. Disulphide bridge formation can be achieved by chemical methodology well known in the art.

It will be understood by the skilled artisan that the following syntheses may be modified to provide other haptens of the invention.

The synthesis of N-[N-(1-carboxy-2-phenyl)ethylaminosulfonylacetyl]glycine [6], a hapten of the general formula (IV) containing a central sulfur atom, is shown schematically in Fig. 6. Ethyl bromoacetate is converted to sodium ethyl sulfoacetate [1] by treating it with sodium sulfite at 50°C. Activation of [1] with phosphorus pentachloride followed by its reaction with phenylalanine t-butylester gives compound [3]. Hydrolysis of the ethyl ester of [3] with potassium hydroxide followed by the DCC promoted coupling of the product [4] with glycine benzylester yields compound [5]. Final product [6] is obtained by deprotection of [5] using catalytic hydrogenation with 10 % palladium on activated carbon followed by treatment with trifluoroacetic acid. The synthesis is set forth in more detail in the examples below.

The synthesis of 3-(N-carbobenzyloxymethyl)sulfonyl-2-methyl-propanoic acid [4], a hapten of the general formula IV containing a central sulfone group, is shown schematically in Fig. 7. The synthesis of both 3-mercapto-2-methylpropanoic acid [1] (15) and benzyl N-(bromomethyl)carbamate [2] (16) have been described in the literature. Reaction of the disodium salt of [1] with the bromoderivative [2] produces the sulfide compound [3]. Oxidation with periodate of [3] affords the desired sulfone product [4]. The synthesis is set forth in more detail in the examples below.

The synthesis of 3-difluoro-5-(6-maleimidohexanoyl)amino-2-methyl-4-oxo-6-phenylhexanoic acid [12], a difluoroketone hapten according to formula IV, is shown schematically in Fig. 8. The difluoro acid

[1] was prepared by reaction of crotyl alcohol with tetrafluoroethylene in the presence of catalytic amount of sodium hydride and the resultant intermediate was treated with n-butyllithium. The difluoro acid [1] was converted to its silver salt [2] with aqueous silver oxide. After drying the salt over phosphorus pentoxide, it was converted to the anhydride [3] by reacting with oxalyl chloride. The anhydride [3] was then treated with a p-phenylbenzoyl group to protect the N-terminus of amino acid.

The corresponding oxazolinone [5] was prepared by the reaction of D,L-phenylalanine with 4-biphenylcarbonylchloride and resulting protected phenylalanine [4] was reacted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Treatment of oxazolinone [5] with the anhydride [3] at 60°C for 40 h followed by the addition of oxalic acid at 110°C for 15 min produced the difluoroketone [6]. The ketone [6] was reduced to the corresponding alcohol [7] with sodium borohydride and treatment of [7] with excess 3 % sodium-amalgam in methanolic phosphate buffer at pH 6-7 gave the amine compound [8]. The liberated amine compound [8] was converted to benzyloxycarbonylamino compound [9] with N-(benzyloxy-carbonyloxy)succinimide. Compound [9] is oxidized with Dess-Martin periodinane and treatment of resulting difluoroketone [10] with ozone at -78°C followed by the addition of dimethylsulfide and then Jones oxidation yields the acid [11]. The final product [12] is prepared by the removal of the benzyloxycarbonyl group by catalytic hydrogenation followed by the reaction of the liberated amine compound with 6-maleimidohexanoic acid anhydride to give the compound [12]. The synthesis is described in more detail in the examples below.

The synthesis of 5-[N-Benzyloxycarbonyl-methyl)amido]-3,3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid [7], a retroamidodifluoroketone hapten according to formula IV, is shown schematically in Fig. 9. The hydrocinnamic acid ester [2] and difluoroacid ester [4] were prepared by the reaction of diazomethane with hydrocinnamic acid [1] and difluoro acid [3] respectively. Treatment of ester [2] with LDA followed by difluoroacid ester [4] gives corresponding difluoroketone [5]. After hydrolysis of ester [5] with K_2CO_3, compound [6] is obtained by coupling with glycine benzylester. Ozonolysis of compound [6] followed by Jones oxidation should produce a final product [7]. The synthesis is set forth in more detail in the examples below.

As noted earlier, the invention is also directed to conformationally constrained haptens which mimic the native configurations of proteins. Fig 10 is a type I ß-turn where the carbonyl group of the residue is hydrogen-bonded to the amino group of the residue C+3. It is possible with a change of conformation with the same hydrogen bond intact to form other types of ß-turns such as type II or type III (3.0_{10} helix). In all of the different types of ß-turns it is desirable to replace the hydrogen bond for a covalent link such as the N-amino- methylamidinium link as shown in Fig. 11. The molecule is now locked into a ß-turn. Therefore, replacing the amide bond between residues R_1 and R_2 by a transition state analog gives a conformationally constrained hapten as shown in Fig. 12 useful for eliciting catalytic antibodies.

The synthesis of the above mimetic of a ß-turn (Fig. 12) is in two parts: first, the preparation of the dithioester (7) as shown in Fig. 13 (Scheme 1); and

second, the formation of the ten membered ring as shown in Fig. 14 (Scheme 2). The method for preparing the dithioester (7) follows essentially a literature procedure (17).

The BOC(t-butyldicarbonate)-protected glycine (1) is first converted to the triethylamine salt by treatment with triethylamine and is then transformed to the corresponding piperidide (2) by the reaction of the BOC-protected glycine triethylamine salt with Lawesson's reagent (LR) and piperidine. Removal of the BOC group by trifluoroacetic acid followed by the addition of FMOC-Cl gives the FMOC-protected glycylpiperidide (4). Lawesson's reagent is next used as a thionation reagent to form the corresponding thiopiperidide (5). The thiopiperidide is S-methylated by reaction with an excess of methyl iodide in THF for 12 hrs. to give (6). Thiolysis using hydrogen sulphide at 0°C yields the dithioester (7).

The formation of the ten membered ring compound (Scheme 2) begins with the reaction of bromomethyl-phthalimide with methylamine to give the secondary amine (8). Addition of 2-[(t-butoxycarbonyloxyimino)-2-phenylacetonitrile] followed by removal of the phthalimide group with hydrazine yields the primary amine (10). Thioacetylation of (10) with the dithioester (7) from Scheme 1 followed by activation with methyl iodide and condensation with (L)-2-aminophosphon-amide acid (13) gives compound (14).

The polyamide gel resin (16) functionalized as a succinimide ester is prepared by reacting the polyamide gel resin functionalized as sarcosine methyl ester (commercially available as Pepsyn™ from CRB Ltd.) with ethylenediamine followed by Lomant's reagent as shown in Fig. 15 (Scheme 3). Compound (17) is prepared by

first removing the FMOC group from compound (14) with pyridine in DMF followed by reaction with the activated resin (16). Removal of the BOC group with trifluoroacetic acid followed by internal cyclization using IIDQ gives the final product (19). The presence of the disulphide linker in product (19) allows the release of the product into free solution by reductive cleavage using dithiothreitol under basic conditions. The product may then be purified and analyzed before attaching to a carrier protein using the bifunctional linker, sulfosuccinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate.

The utility of the antigens of this invention coupled with appropriate screening procedures and reiterative thermodynamic perturbation studies of transition-state structures and free-energies of interaction with catalytic groups provide a methodology for production of catalytic antibodies by a rational design approach.

The invention also is directed to catalytic antibodies which are elicited by antigens comprising haptens according to the invention. These antibodies may be monoclonal or polyclonal but are preferably monoclonal and may be in the form of purified immunoglobulins (IgG, IgM, IgA, IgD or IgE) or antibody fragments, such as Fab, $F(ab')_2$, F_v, etc., of immunoglobulins.

A catalytic antibody in accordance with the invention is a substance which is capable of changing the rate of a chemical reaction, all other conditions (e.g., temperature, reactant/substrate concentration, etc.) being the same and which does not enter into the chemical reaction and therefore is not consumed in the reaction. It is also a substance which exhibits the capability of

converting multiple moles of reactant/substrate per mole of catalytic antibody; which, from a mechanistic viewpoint, binds the reactant/substrate, effects the accelerated conversion of the reactant/substrate to the product and then releases the product; and which changes the rate of the chemical reaction without shifting the position of the equilibrium. The aforementioned definitions are characteristics of ideal catalysts. However, in practice, even the best of catalysts become poisoned or deactivated by contamination in the reaction system or as a result of chemical or physical destruction during the reaction process. For reasons well known in the art, the true operation of a catalyst may be obscured by components of the reaction system or by the condition of the reaction environment.

The art has adopted certain working definitions to express catalytic activity. These expressions are [1] k_{cat}, or "turnover" and [2] k_{cat}/k_{uncat}, the "rate enhancement factor". Turnover indicates the number of molecules of reactant/substrate which can be converted to product per mole of catalytic antibody per unit time. For example, if a molecule exhibits a turnover of 10^3 molecules of substrate per minute and the molecule maintains its catalytic activity for 24 hours at room temperature and at its optimal pH, each molecule of catalyst would then make a total of 1.4×10^6 conversions, indicating its catalytic behavior. This total conversion is to be distinguished from the total conversion in a stoichiometric reaction, which will never exceed 1.0, no matter how long the reaction is carried out. The rate enhancement factor is a dimensionless number which expresses the rate of reaction in the presence of catalyst to the rate of reaction in the

absence of catalyst, all other reaction conditions (e.g., reactant concentration, temperature, etc.) being equal.

Catalytic antibodies according to the invention may be elicited through both in vitro and in vivo techniques. The term "elicited" as used herein means elicitation of catalytic antibodies by antigens according to the invention through both in vitro and in vivo techniques. However, the skilled artisan will readily appreciate that when in vitro elicitation is involved, the haptens according to the invention, by themselves, may be used to elicit the catalytic antibodies. However, when elicitation is achieved through in vivo techniques, it is understood that immunogens comprising haptens complexed to a suitable carrier molecule are used to elicit the catalytic antibodies. Another aspect of the invention is directed to a method for producing antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by an antigen. The antigen comprises a hapten according to the invention. The haptens are designed to elicit the appropriate hypervariable binding region in an antibody molecule to express intrinsic binding energy for the transition- state of a chemical reaction, particularly a hydrolytic reaction. Arrangement of amino-acid side chains generated in the combining-site will be appropriate for performing chemical modification of an epitope of interest. Additional improvements in catalytic efficiency can be achieved by site-directed mutagenesis.

Broadly, the method comprises exposing cells capable of producing antibodies to the antigen and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and

thereby producing a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest. The monoclonal antibody so identified may then be replicated, again by either in vivo or in vitro techniques, to obtain a quantity sufficient to catalyze the chemical reaction of interest.

The detection of antibodies with the desired catalytic activity and specificity is achieved by screening the hybridomas once they have been elicited. For example, screening may be achieved by high performance liquid chromatography (HPLC) or spectrophotometric methods (ELISA). Catalytic monoclonal antibodies are elicited "in vivo" by modification of the technique disclosed by Koprowski et al. in U.S. Patent No. 4,196,265, issued April 1, 1980, which is hereby incorporated by reference. The details of that process are known in the art. A series of monoclonal antibodies directed to a specific molecule are prepared under suitable conditions. This involves first immunizing BALB/C mice with an appropriate antigen. The antigen comprises a hapten according to the invention bound to a peptide or other carrier molecule.

Antibody-producing lymphocytes are then removed from the spleens of the immunized mice and hybridized with myeloma cells such as SP2/0 cells to produce hybridoma cells. These hybridoma cells are then plated in the wells of microtiter plates. The series of monoclonal antibodies being produced by the hybridoma cells is screened under appropriate conditions to identify monoclonal antibodies which catalyze the desired reaction under appropriate conditions. Alternatively, the medium may be tested for antibodies

that bind to the immunogen and the hybridomas producing these antibodies then expanded in tissue culture or grown in vivo. Screening may be conveniently accomplished by treating a standardized solution of the reactant with an aliquot of medium withdrawn from a microtiter well and measuring the presence of the desired product by conventional instrumental methods. This measurement may be readily conducted, for example by spectrophotometric methods or by gas-liquid or high pressure liquid chromatography. By comparison with standardized samples of the desired product or reactant, rates of reaction may be quantified. In this manner, wells containing hybridoma cells producing catalytic monoclonal antibodies are identified. The selected hybridoma cells are then cultured to yield colonies.

These colonies may be further propagated in in vitro or in vivo systems. In the latter case, mice such as syngeneic BALB/C mice are inoculated intraperitoneally with the selected hybridoma cells and produce tumors, generally within two or three weeks. These tumors are accompanied by the production of ascites fluid which contains the desired monoclonal antibodies. The monoclonal antibodies are then separately recovered from the ascites fluid by conventional methods such as ultrafiltration, ultracentrifugation, dialysis and immunoffinity chromatography.

The invention is also a method for catalyzing the cleavage or formation of an amide (peptide) or ester bond in a molecule. The molecule can be a natural or synthetic peptide or protein. Target molecules include bimolecules which are defined as any molecule which affects a biological system in vivo or in vitro. Biomolecules may be synthesized by cells or chemically

synthesized. Examples of biomolecules include proteins, glycoproteins, peptides, steroids, and maleic acid. Also included are synthetic organic analogs of peptides, steroids, maleic acid, etc. Pharmaceutically active compounds such as theophylline, caproin, cyclosporin, etc., are also considered to be biomolecules. The method comprises contacting a molecule containing one or more amide (peptide) or ester bonds with an effective amount of a catalytic antibody which has been elicited by an antigen comprising a hapten of the invention.

In accordance with the invention, the separately recovered monoclonal antibodies are contacted with a molecule under suitable conditions permitting the formation of a complex between the monoclonal antibody and the molecule. In general, the concentration of the catalytic antibodies used is less than the equivalent concentration of the target molecule and may be in the picomolar range. The antibodies should function under normal physiologic conditions *in vivo*. The skilled artisan will appreciate that the conditions suitable for complex formation may vary depending on the particular molecule and monoclonal antibody under consideration.

Accordingly, the methods of this invention may be practiced under a variety of reaction conditions, *in vivo* and *in vitro*, as long as the monoclonal antibodies are not prevented from complexing with the molecules or otherwise rendered inactive. More specifically, suitable conditions for complex formation encompass solution phase and emulsion reaction systems including a protic solvent, preferably water, maintained at a pH value between about 6.0 and about 8.0, preferably between about 6.0 and about 7.5 and at a temperature from about 4°C to about 50°C, preferably from about 20°C to about 45°C. The ionic strength, $\mu = 1/2 \Sigma c_i z_i^2$,

where c is the concentration and z is the electronic charge of an ionic solute, should be maintained at a value below about 2.0 moles/liter, preferably between 0.1 and 1.5 moles/liter. The method of this invention may be carried out at reduced or elevated pressure, but preferably is practiced at ambient pressure. In addition to solution phase and emulsion reaction systems, suitable conditions also include the use of support materials to which the monoclonal antibody is attached. Such support materials are well-known to those of ordinary skill in the art as are methods for attaching monoclonal antibodies to them.

Catalytic antibodies elicited with the antigens of the invention may be useful in the treatment of autoimmune disease, cancer and thrombolytic disease. The catalytic antibodies may also be useful for treatment of cardiovascular disease eliminating high density lipoproteins and for the detoxification of bacterial endotoxins. Vaccines comprising synthetic peptides optionally linked to carrier proteins, for example, against foot and mouth disease (FMD) and E. coli enterotoxin, have been proven efficacious in recent years.

Oligo peptides having variable lengths with sequences from the receptor-binding regions of viruses which employ a specific cellular receptor for penetration of the host cell and having a transition state analog dipeptide isostere in a critical region of the sequence induce on immunization, optionally after coupling to a suitable carrier protein, catalytic antibodies that cleave the viral coat protein and prevent virus penetrating the cell. The dimensional structure of Rhino 14 and Polio 1 virus particles has been charted by X-ray scattering. Regions have been

identified which are binding sites to cellular receptors. The region of the human immunodeficiency virus type 1 (HIV I) critical for interaction with the CD4 receptor on T-lymphocytes has been located and mapped to sequences in the gp120 goat protein. Thus, in one embodiment of the invention, oligo peptides are used which contain partial sequences from the envelope proteins of viruses critical for host cell attachment and a transition-state dipeptide isostere selected from the haptens according to the invention. The resultant peptide analogs are used to induce catalytic antibodies that inactivate viruses by proteolysing segments (epitopes) of the viral coat protein critical for infectivity. Preferably, oligopeptides are used having sequences from the receptor binding region of retroviruses e.g., HIV I, HIVII and picorna viruses, e.g., Rhino 14, viral polypeptides, inflammatory proteins, anaphylactic proteins, lymphokines, cytakines and other polypeptide mediators of host infection or toxic syndromes.

The invention will be more fully described and understood with reference to the following illustrative examples.

Example 1

Synthesis of N-[N-(1-carboxy-2-phenyl)ethylaminosulfonylacetyl]glycine

The reaction sequence is shown in Fig. 6.

Sodium ethyl sulfoacetate [1]

A solution of sodium sulfite (126.0g, 1.00 mol) in water (400 mL) was cooled and stirred while a solution of ethyl bromoacetate (167.0g, 1.00 mol) in ethyl alcohol (200 mL) was added dropwise. After the addition was

complete the mixture was heated briefly to 50°C, then concentrated to dryness (two portions of ethyl alcohol/benzene were added and stripped to aid in the removal of water). The solid residue was extracted with boiling 2:1 acetic acid/ethyl acetate (total 900 mL) and the hot solution was filtered through Celite and chilled overnight. The crystallized product was filtrated as a white solid and subsequent crops were obtained by diluting the mother liquors with additional ethyl acetate. The combined products gave 152g (80 %) of [1] as a white solid. (mp. ca. 158°C (dec)).

Ethyl chlorosulfoacetate [2]

The sodium salt [1], (19.0g, 100 mmol) and phosphorus pentachloride (23.0g, 110 mmol) were separately pulverized then combined in a flask equipped with a condenser and drying tube. After swirling a few minutes, a reaction was occurred: after the exothermic reaction subsided the flask was warmed on a steam bath for 45 min, then the phosphoryl chloride was stripped in vacuo. A portion of benzene (50 mL) was added and the resulting solution was filtered through Celite and evaporated to give 15.9g (85 %) of [2] as a clear oil. This compound was used for next reaction without further purification.

Ethyl N-(1-t-butoxycarbonyl-2-phenyl)ethylaminosulfonyl-acetate [3]

To a solution of [2], (15.9g, 85.0 mmol) in benzene (65 mL) is added a solution of L-phenylalanine t-butylester (20.1g, 91.0 mmol) and triethylamine (15 mL, 108 mmol) in benzene (50 mL) dropwise at 0°C. After addition is complete, the mixture is warmed gently for 5 min, then is cooled and filtered, and the filtrate is washed with water (50 mL), dilute hydrochloric acid (2x50

mL), aqueous sodium hydrogen carbonate (2x50 mL), and aqueous sodium chloride (50 mL). After drying ($MgSO_4$), the solvent is removed in vacuo to give crude [3] as an oil. Crystallization from benzene/n-hexane followed by recrystallization from the same solvent gives pure [3] as a solid.

N-(1-t-butoxycarbonyl-2-phenyl)ethylaminosulfonylacetic acid [4]

The ester [3], (18.5g, 50.0 mmol) is refluxed in a solution of potassium hydroxide (7.0g, 125 mmol) in water (50 mL) and ethyl alcohol (20 mL) for 2.5 h. Charcoal is added, the solution is heated to boiling for 5 min, filtered through Celite, washed with ether (100 mL), acidified with hydrochloric acid, and extracted with ether (3x100 mL). The ether extract is washed with water (3x100 mL), dried ($MgSO_4$), and concentrated to give crude [4] which is crystallized from benzene to give pure [4] as a solid.

N-[N-(1-t-butoxycarbonyl-2-phenyl)ethylaminosulfonyl-acetyl]glycine benzylester [5]

To a solution of [4], (5.00g 14.6mmol) in dichloromethane (20 mL) is added a solution of 1,3-dicyclohexylcarbodiimide (1.50g, 7.30mmol) in dichloromethane (20 mL) at room temperature under argon. It is stirred for 30 min and the resulting precipitate is filtered and the filtrate is concentrated in vacuo. The resulting solid is dissolved in DMF (20 mL) and a solution of glycine benzylester (1.19g, 7.30 mmol) in DMF (10 mL) followed by a solution of N-methylmorpholine (0.88 mL, 8.00 mmol) in DMF (5 mL) are added dropwise. The reaction mixture is stirred for an hour and diluted with ethyl acetate (100 mL). The organic layer is washed with 1 N aqueous hydrochloric acid (2x50 mL), saturated

sodium bicarbonate (2x50 mL), and water (2x50 mL), dried (MgSO4), and concentrated in vacuo to give [5] as an impure solid. Pure product [5] is obtained by chromatography of the crude mixture with 100 g of silica gel and eluting with methyl alcohol/chloroform.

N-[N-(1-carboxy-2-phenyl)ethylaminosulfonylacetyl]glycine [6]

Into a Parr flask flushed with argon containing 0.30g of 10% palladium on charcoal catalyst is added a solution of [5], (3.00g, 6.12mmol) in ethyl acetate (30 mL). The mixture is shaken under 30 psi of hydrogen at room temperature over a period of 2 h. The mixture is filtered through Celite and trifluoroacetic acid (2 mL) is added into the filtrate. It is stirred at room temperature for 6 h. Excess solvents are evaporated in vacuo and the crude mixture is purified by chromatography using 50g of silica gel and eluting with ethyl alcohol/chloroform to give product [6] as a solid.

Example 2

Synthesis of 3-(N-Carbobenzyloxyaminomethyl)sulfonyl-2-methyl-propanoic acid

The reaction sequence is shown in Fig. 7.

3-(N-Carbobenzyloxyaminomethyl)thio-2-methylpropanoic acid [3] To a solution of 3-mercapto-2-methylpropanoic acid [1] (0.24 g , 2 mmol) in methanol (5 mL) is added sodium methoxide (0.216 g . 4 mmol) followed by benzyl N-bromomethylcarbamate [2] (0.488 g , 2 mmol). When the reaction is complete, CH_2Cl_2 (50 mL) is added and the solution is washed with aqueous 0.05 M HCl (50 mL). The organic layer is dried ($MgSO_4$), evaporated and purified by silica gel chromatography, using methyl

alcohol/dichloro- methane to give the sulfide product [3].

3-(N-Carbobenzyloxyaminomethyl)sulfonyl-2-methylpropanoic acid [4]

To a stirred solution of [3] (0.151g , 0.535 mmol) in methanol (20 ml) is added a solution of $NaIO_4$ (1.145g, 5.35 mmol) in water (5 mL). The reaction mixture is stirred at room temperature for 60 h. Over the course of the reaction, additional $NaIO_4$ (3.45g , 6.1 mmol) is added periodically until no starting sulfide [3] is present. The reaction mixture is filtered and the filtrate is concentrated to give a solid. This impure product is purified using silica gel chromatography with methyl alcohol/dichloromethane to give the final product [4].

Example 3

Synthesis of [(6-Maleimido)hexanoyl]amino](2-phenylethyl)hydroxyphosphinyl D-alanine

The reaction sequence is shown in Fig. 16.

Diphenyl (benzyloxycarbonylamino)-2-phenylethyl phosphonate. [1]

To a solution of phenylacetaldehyde (35 ml, 300mmol) in glacial acetic acid (30ml), was added triphenylphosphite (52.4g, 200mmol) and benzyl carbamate (30.2g, 200mmol). The mixture was stirred at room temperature for 1h, and then heated at 85° for 2h. Volatiles were removed *in vacuo*, and the oily residue was dissolved in methanol (350ml) and allowed to crystallize at -20° overnight. The resulting crystals were collected by filtration and recrystallized by dissolving in hot chloroform (100ml) and adding methanol (300ml). After cooling to -20° for 3h, the resultant crystals were

filtered from the mother liquors and dried. Yield: 34g (34.8%). N.M.R. and I.R. data were in accord with the assigned structure.

Dimethyl (benzyloxycarbonylamino)-2-phenylethyl phosphonate. [2]

To a solution of diphenyl-2(benzyloxycarbonylamino)-3-phenyl phosphonate (8.2g, 15 mmol) in dry methanol (450ml) was added potassium fluoride dihydrate (4.02g, 180mmol) and 18-crown-6 (0.1g). The solution was heated under reflux for 30min. After cooling volatiles were removed *in vacuo* and the residue dissolved in dichloromethane (300ml). The solution was washed with sodium hydroxide (50ml of a 3M solution) and the organic layer dried ($MgSO_4$) and evaporated to yield an oil which solidified upon standing. Yield: 6.1g (100%). N.M.R. and I.R. data were in accord with the assigned structure.

Methyl hydrogen (benzyloxycarbonylamino)-2-phenylethyl phosphonate [3]

To a solution of dimethyl-2(benzyloxycarbonylamino)-3-phenyl phosphonate (2.65 g, 7.3 mmol) in dioxan (10ml) was added sodium hydroxide solution (8 ml of a 1M solution, 8 mmol) and the solution stirred overnight. Solvent was removed *in vacuo* and the residue redissolved in water (5 ml) and acidified to pH 1 with 1M hydrochloric acid. The white precipitate was obtained by filtration and dried *in vacuo*. Yield: 1.86 g (73%). N.M.R. and I.R. data were in accord with the assigned structure.

[(benzyloxycarbonylamino)-2-phenylethyl methoxyphosphinyl] D-alanine methyl ester [4]

To a solution of methyl hydrogen 2-(benzyloxycarbonylamino)-3-phenylpropyl phosphonate (4.009g, 11.48 mmol) in dry $CHCL_3$ (15 ml) cooled to 0^o, was added $SOCl_2$ (0.92ml, 12.63 mmol) dropwise. After stirring for 3h, volatiles were removed *in vacuo*, and the resulting viscous oil was redissolved in dry $CHCL_3$ (10ml) and cooled to -30^o. To a suspension of D-alanine methyl ester in dry chloroform (2 ml) was added Et_3N (3.54 ml, 25.2mmol). The resultant suspension was added to the phosphochloridate solution with the aid of additional $CHCL_3$ (5 ml). The mixture was allowed to warm slowly to room temperature, stirred for 2h, diluted with EtOAc (200ml), washed with 1M HCl (2x 25 ml), water, and brine. The organic layer was dried and evaporated, and the resultant oil chromatographed on silica gel (EtOAc) to afford the phosphonamidate as a clear oil. Yield: 3.43g (69%). N.M.R. and I.R. data were in accord with the assigned structure.

[(6-maleimido)hexanoyl] amino] (2-henylethyl)methoxy-phosphinoyl D-alanine [5]

To a solution of the phosphonamidate [4] (318 mg, 0.732 mmol) in EtOAc (5 ml) was added 5% Pd on charcoal (30 mg) and the mixture stirred under an atmosphere of hydrogen until T.L.C. (silica plates, 5% $MeOH/CH_2Cl_2$) indicated absence of starting material. The catalyst was removed by filtration and the solvent evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 ml) and Et_3N (0.103 ml, 0.732 mmol) was added, followed by 6-maleimido hexanoic acid anhydride (296 mg, 0.732 mmol). The solution was stirred for 3h. Solvent was removed *in vacuo* to yield a thick oil which was purified by

reversed-phase HPLC to give the title compound. Yield: 90 mg (24%). N.M.R. and I.R. data were in accord with the assigned structure.

[(6-maleimido)hexanoyl] amino] (2-phenylethyl)hydroxy-phosphinyl D-alanine [6]

To a cooled (0°) solution of [(6-maleimido)hexanoyl] amino] (2-phenylethyl)methoxyphosphinoyl D alanine (20 mg, 0.041 mmol) in CH_2Cl_2 (1 ml) was added 1,4-cyclohexadiene (780 ml), followed by trimethylsilyl iodide (12 ml, 0.08 mmol). After 2 min., solvent was removed *in vacuo*, and the residue dissolved in 5 ml of 0.5M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.4 containing 2% CH_3CN. This solution was forced through 2 Waters "Sep-Paks". The Sep Paks were washed with 20 ml of 0.05M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.4 containing 2% CH_3CN, and the product eluted with 0.005M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.4 containing 50% CH_3CN (10 ml). Lyophilization afforded a white powder containing the desired title compound (confirmed by N.M.R.) admixed with inorganic buffer. This powder was used directly for conjugation to carrier protein. (See Example 19).

Example 4

Synthesis of O-[(6-maleimido)hexanyl]amino](2-phenyl-ethyl)phosphinyl lactic acid disodium salt

The reaction sequence is shown in Fig. 17.

Methyl O-[-(benzyloxycarbonyl)amino-2-phenylethyl methoxyphosphinyl] lactate. [7]

To a solution of methyl hydrogen 2-(benzyloxycarbonylamino)-3-phenylpropyl phosphonate (777mg, 2.22 mmol) in dry $CHCL_3$ (10ml) cooled to 0°, was added $SOCl_2$ (0.15ml, 2.66 mmol) dropwise. After stirring

for 3h, volatiles were removed *in vacuo*, and the resulting viscous oil was redissolved in dry $CHCL_3$ (3ml) and cooled to 0°. N-methylmorpholine (0.537 ml, 4.88 mmol) was added, followed by a solution of methyl (D) lactate (231 mg, 2.22 mmol) in dry $CHCl_3$. The mixture was stirred for 30 min, and then diluted with $CHCl_3$ (50ml) , washed with 1M HCl (15ml), dried ($MgSO_4$) and evaporated. The resultant oil was chromatographed (SiO_2) using 5% CH_2Cl_2/MeOH as eluant to give the title compound as a yellow oil. Yield: 0.54g (56%). n.m.r and i.r. data were in accord with the assigned structure.

Methyl O-[-(benzyloxycarbonyl)amino-2-phenylethyl hydroxyphosphinyl] lactate, triethylammonium salt. [8]

The starting phosphonate (253 mg, 0.58 mmol) was dissolved in a solution of dioxan/thiophenol/ triethylamine (2:1:1, 5 ml), and stirred overnight. The solution was added dropwise to pentane (50 ml) affording an oily precipitate. The pentane layer was decanted and the oil redissolved in CH2Cl2 (2 ml). Pentane (50 ml) was added to again afford an oily precipitate. The pentane was decanted and the oil dried under vacuum to afford the title compound as an oil. Yield: 347 mg (100%). n.m.r and i.r. data were in accord with the assigned structure.

O-[-(benzyloxycarbonyl)amino-2-phenylethyl hydroxyphosphinyl] lactic acid [9]

Methyl O-[2-(benzyloxycarbonyl)amino-3-phenyl phosphinyl] lactate, triethylammonium salt (1.443 g, 2.41 mmol) was dissolved in aq. LiOH (7.2 ml of a 1.5M solution, 10.8 mmol) and the solution stirred at room temperature. Progress of the reaction was monitored by reversed-phase HPLC. When reaction was complete, the

mixture was lyophilized. The resultant powder was dissolved in 200 ml of 0.05M $Na_2CO_3/NaHCO_3$ buffer, pH 8.65, and applied in two batches to a 28x2cm column of DEAE Trisacryl M. Each batch was eluted with a gradient of 0.05 to 0.25 M $Na_2CO_3/NaHCO_3$ buffer, pH 8.65 and fractions monitored by U.V. Fractions containing UV absorbing material were pooled and lyophilized to afford O-[-(benzyloxycarbonyl)amino-2-phenylethyl hydroxyphosphinyl] lactic acid as a white powder (555mg, 57%). 386mg (0.95 mmol) of the powder was dissolved in a minimum quantity of deionized water and applied to a column of 15g (dry weight) of SP Sephadex (Sigma SP-C25-120, previously swollen with deionized water for 3h and washed with 10% HCl w/v (250 ml), deionized water (800 ml),, 12% aq. NaCl w/v (250 ml), and deionized water (800 ml)). Product was eluted with 300 ml of deionized water, collecting all fractions.Yield: 427mg (100%). n.m.r and i.r. data were in accord with the assigned structure.

<u>O-[(6-maleimido)hexanoyl] amino] (2-phenyl-ethyl)phosphinyl lactic acid disodium salt. [10]</u>

The phosphonate disodium salt (167 mg, 0.37 mmol) was dissolved in H_2O (5 ml) and added to a suspension of previously hydrogenated 10% Pd/C (50 mg) in H_2O (5 ml) . The mixture was stirred under an atmosphere of hydrogen until HPLC indicated absence of starting material. The solution was filtered through Celite with the aid of extra MeOH (10 ml) and evaporated to yield an oil. This oil was dissolved in DMSO (1 ml) and H_2O (1 ml). To this solution was added a solution of 6-maleimido hexanoic acid anhydride (168 mg, 0.416 mmol). The initially cloudy solution was stirred for 30 min. during which it became clear. The solution was applied directly to a reverse phase HPLC column for purification. Separation of the

expected two diastereomers of the product was achieved. After lyophilization of appropriate fractions, the purified products were oils. Each oil was converted separately to the disodium salt via ion-exchange chromatography, as described above, to yield the pure diastereomers of the title compound as white powders. Combined yield: 43 mg (23% overall). n.r.r. and i.r. data were in accord with the assigned structure.

Example 5

Synthesis of [(6-Maleimido)hexanoyl]amino]-2-phenylethyl (2-carboxy-1-propyl)hydroxyphosphonous acid

The reaction sequence is shown in Fig. 18.

[1-(Benzyloxycarbonyl)amino]-2-phenylethyl phosphinic acid [11] was prepared by the method of Baylis *et al.* (18).

Methyl [1-(benzyloxycarbonyl)amino]-2-phenylethyl phosphinate. [12]

To a solution of [1-(benzyloxycarbonyl)amino]-2-phenylethyl phosphonous acid in EtOAc/ MeOH (10:1, 100 ml) was added ethereal diazomethane until the yellow color persisted. After standing for 10 min. excess diazomethane was destroyed by addition of glacial acetic acid. Solvent was removed *in vacuo* and the residue purified by chromatography on silica gel (5%MeOH/CH_2Cl_2 eluant) to afford the product as a clear oil which solidified on standing. Yield: 2.985 g (87%). N.M.R. and I.R. data were in accord with the assigned structure.

Methyl [(1-(benzyloxycarbonyl)amino]-2-phenylethyl)(2-carbomethoxy-1-propyl) phosphinate. [13]

To a solution of methyl [1-(benzyloxycarbonyl)amino]-2-phenylethyl phosphinate (936

mg, 2.81 mmol) in dry MeOH (5 ml) cooled to 0°, was added NaOMe (1.54 ml of a 2M solution) dropwise. After completion of addition, the solution was stirred for 5 min. and methyl methacrylate (300 ml, 2.82 mmol) was added in one portion. The solution was stirred at room temperature for 5h and poured into 1M HCl (10 ml). The solution was extracted with EtOAc (2x 100 ml) and the organic layers combined, washed with brine, dried ($MgSO_4$) and evaporated. Chromatography on SiO_2 afforded the title compound as a clear oil. Yield: 917 mg, (75%). N.M.R. and I.R. data were in accord with the assigned structure.

([1-(benzyloxycarbonyl)amino]-2-phenylethyl)(2-carboxy-1-propyl) hydroxyphosphonous acid [14]

To a solution of methyl [(1-(benzyloxycarbonyl)amino]-2-phenylethyl)(2-carbomethoxy-1-propyl) phosphinate (720 mg, 1.66 mmol) dioxan (3 ml) and water (2 ml) was added aq. LiOH (1.16 ml of a 3M solution, 3.48 mmol). The resultant solution was stirred at room temperature and progress of the reaction was monitored by HPLC. Upon completion of reaction, the mixture was diluted with 200 ml of 0.05M $Na_2CO_3/NaHCO_3$ buffer, pH 8.65, and applied to a 28x2cm column of DEAE Trisacryl M. The column was eluted with a gradient of 0.05 to 0.25 M $Na_2CO_3/NaHCO_3$ buffer, pH 8.65 and fractions monitored by U.V. Fractions containing U.V. absorbing material were pooled and lyophilized to afford the title compound as a white powder. Yield: 496 mg, 74% . N.M.R. and I.R. data were in accord with the assigned structure.

[(6-Maleimido)hexanoyl] amino]-2-phenylethyl(2-carboxy-1-propyl) hydroxyphosphonous acid [15]

To a previously hydrogenated suspension of 10% Pd/C (20 mg) in MeOH (0.5 ml) was added a solution of methyl [(1-(benzyloxycarbonyl)amino]-2-phenylethyl)(2-carbomethoxy-1-propyl) phosphinate (75 mg, 0.185 mmol) in MeOH (1 ml). The mixture was stirred under an atmosphere of hydrogen for 2h. The catalyst was removed by centrifugation and the supernatant evaporated to give a white solid. This solid was dissolved in DMSO (250 ml) and Et_3N (3 drops) were added. To this mixture was added a solution of 6-maleimido hexanoic acid anhydride (87 mg, 0.222 mmol) in DMSO (250 ml). After 2h, the product was isolated directly by reversed-phase HPLC. The product was repeatedly lyophilized to afford a white powder. Yield: 31 mg (36%). N.M.R. and I.R. data were in accord with the assigned structure.

Example 6

Synthesis of (2-[(6-maleimido)butanoyl]amino]-3-phenylpropylimino-D-alanine

The reaction sequence is shown in Fig. 19.

2-amino-3-phenylpropanonitrile. [16]

To a solution of freshly distilled phenylacetaldehyde (29.25 ml, 0.25 mol) in MeOH (100 ml) was added a solution of NaCN (12.25 g, 0.25 mol) and NH_4Cl (26.75 g, 0.5 mol) in H_2O (100 ml). The mixture was stirred for 18h, and MeOH was removed *in vacuo*. The resulting aqueous solution was extracted with CH_2Cl_2 and the organic layer dried and evaporated to yield the product as a yellow oil which was used without further purification. Yield: 34.46g (94%). N.M.R. and I.R. data were in accord with the assigned structure.

2-(benzyloxycarbonyl)amino-3-phenylpropanonitrile. [17]

To a solution of N-benzyloxycarbonyloxy succinimide (12.46g, 0.05 mol) in CH_2Cl_2 (25 ml) was added a solution of 2-amino-3-phenylpropanonitrile (7.31 g, 0.05 mol), followed by Et_3N (7 ml, 0.05 ml). The exothermic reaction was cooled in an ice bath for 10 min, and then allowed to stir at room temperature for a further 10 min. The solution was poured into 0.05M NaOH solution (50 ml) and extracted with CH_2Cl_2. The organic layer was dried, evaporated and chromatographed (SiO_2, 5% MeOH/CH_2Cl_2) to afford the title compound as a white solid. Yield: 8.93g, (64%). N.M.R. and I.R. data were in accord with the assigned structure.

2-(benzyloxycarbonyl)amino-3-phenylpropylimino-D-alanine. [18]

a) 2-(benzyloxycarbonyl)amino-3-phenylpropanonitrile (112 mg, 0.40 mmol) was dissolved in dry CH_2Cl_2 (3 ml) and MeOH (12.82 mg, 0.4 mol). The mixture was cooled to 0^o and saturated with dry HCl gas. The mixture was stirred at 0^o for 48h affording a white precipitate of imino ester hydrochloride. The precipitate was filtered off and dried. Yield: 98 mg (70%).

b) The imino ester hydrochloride (446 mg, 1.28 mmol) was converted to the free base by extracting once with $CHCl_3$ (10 ml) and aq. NaOH (10 ml of a 1M solution). The organic layer was dried and evaporated to afford the free base as an oil (361 mg, 91 %). This was dissolved in dry MeOH (8 ml) and D-alanine (103 mg, 1.16 mmol, dried over P_2O_5) was added. The mixture was heated to reflux for 1.5 h. The solution was filtered and diluted with Et_2O to afford the title compound as a white solid, obtained by filtration. Yield: 252 mg (59 %). N.M.R. and I.R. data were in accord with the assigned structure.

(2-[(6-maleimido)butanoyl] amino])-3-phenylpropylimino-D-alanine [19]

A solution of 2-(benzyloxycarbonyl)amino-3-phenylpropylimino-D-alanine (30.4 mg, 0.082 mmol) in MeOH (5 ml) was hydrogenated over 10% Pd/C (6.5 mg) for 1.5h. The catalyst was removed by filtering through a pad of Celite and the filtrate evaporated *in vacuo*. The residue was dissolved in 0.5M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.0 and added to a solution of N-(4-maleimido)butanoyl succinimide (27.6 mg, 0.098 mmol) in DMSO (0.1 ml). The mixture was stirred overnight, and purified directly by reversed-phase HPLC (H_2O/CH_3CN) to afford the title compound as a white solid. Yield: 3.9 mg (12%). N.M.R. and I.R. data were in accord with the assigned structure.

Example 7

Synthesis of 3-Difluoro-5-(6-maleimidohexanoyl)amino-2-methyl-4-oxo-6-phenylhexanoic acid

The reaction sequence is shown in Fig. 8.

2,2-Difluoro-3-methyl-4-pentenoic acid [1]

A 250 mL three neck flask equipped with a magnetic stir bar, a low temperature thermometer, a condenser filled with dry ice, and a balloon was charged with dry tetrahydrofuran (100 mL). The flask was cooled to approximately -50°C, and tetrafluoroethylene was passed through a tube of silica and then through a needle into tetrahydrofuran until saturation was reached (as judged by inflation of the balloon). The flask was allowed to warm to -5°C to -10°C which caused the balloon to inflate slightly under acetone-dry ice bath. To a separate 50 mL flask was added sodium hydride (100 mg, 4.20 mmol, 60 % suspension in oil), and the oil was removed by repeated

washing with dry tetrahydrofuran under argon. A solution of crotyl alcohol (3.74 g, 52.0 mmol) in dry tetrahydrofuran (2.0 mL) was slowly added by syringe with stirring, and the mixture was allowed to stir for an additional 10 min. The solution was then transferred dropwise by syringe to the three-neck flask with stirring at -5°C to -10°C. After approximately 3 h, additional tetrafluoroethylene was bubbled into the solution for about 15 min. After 6 h, the reaction was placed under argon and cooled to -60°C and n-butyllithium (32.5 mL, 52.0 mmol, 1.6M in n-hexane) was added dropwise over an hour from an additional syringe with the temperature kept constant at -60°C. The mixture was cooled to -78°C and water (2.0 mL) was added. The cooling bath was removed, and the solution was stirred overnight. The solution was basified with aqueous sodium hydroxide to pH 10 and the solvent was removed in vacuo. The residue was acidified with 1N hydrochloric acid to pH 1 and extracted with diethyl ether (3x100 mL). The organic layers were combined and extracted with saturated aqueous sodium bicarbonate (3x200 mL). The aqueous extract was acidified to pH 1 with hydrochloric acid and extracted three times with diethyl ether, dried ($MgSO_4$), concentrated *in vacuo* to give 1.50g (20 %) of product [1] as a pale yellow oil. The N.M.R., I.R. and mass spectra were in accord with the assigned structure.

Silver 2,2-difluoro-3-methyl-4-pentenoate [2]

A suspension of silver oxide in water was prepared by adding a solution of NaOH (346 mg, 8.67 mmol) in water (15 mL) to an aqueous solution of silver nitrate (1.47g, 8.67 mmol) in water (15 mL), decanting the supernatant liquid, washing the residue with water (3x20 mL) and adding 20 mL of water. To this vigorously stirred

suspension was added a solution of 2,2-difluoro-3-methyl-4-pentenoic acid [1], (1.30g, 8.67 mmol) in water (20 mL). After 30 min, the mixture was filtered and the filtrate concentrated to afford a solid residue which was dried over phosphorous pentoxide (0.05 Torr, 50°C, 24 hours) to give 1.38 g (62 %) of product [2] as a white amorphous powder. The N.M.R., I.R. and mass spectra were in accord with the assigned structure.

2,2-Difluoro-3-methyl-4-pentenoic acid anhydride [3]

A suspension of the silver salt [2], (5.09 g, 19.8 mmol) in dichloromethane (30 mL) was stirred under argon, cooled to 0°C and then oxalyl chloride (1.25 g, 9.86 mmol) was added slowly. The cooling bath was removed and the reaction mixture allowed to warm up to room temperature. Warming up to reflux for 30 minutes completed the reaction. Cooled again to room temperature, the supernatant liquid was decanted and the residue washed with dichloromethane (10 mL). The organic layers were combined and the solvents removed in vacuo to give a pale yellow oil. This extremely hygroscopic product [3] was used for a next reaction without further purification.

N-p-Phenylbenzoyl-DL-phenylalanine [4]

To a solution of D,L-phenylalanine (7.00g, 42.4 mmol) in 1N NaOH (700 mL) was added 4-biphenylcarbonylchloride (13.8g, 63.6 mmol) in a small portion over a period of 2 h. The mixture was stirred at room temperature for additional 3 h. It was slowly acidified with concentrated HCl to pH 3. The resulting solid was filtered and washed with cold water. The precipitate was dried in a desiccator under vacuum. The crude products was chromatographed with 150 g of silica gel (methyl

alcohol/dichloromethane (1:20)) to give 10.5g (72 %) of product [4] as a pale yellow solid. The nmr and ir spectra were accord with the assigned structure.

2-p-biphenyl-4-phenylmethyl-5(4H)-oxazolinone [5]

To a solution of N-p-phenylbenzoyl-DL-phenylalanine [4], (2.00g, 5.80 mmol) in dichloromethane(100 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.00g, 5.19 mmol) in a single portion. The resulting mixture was stirred 0°C for 30 minutes. It was diluted with dichloromethane (100 mL) and washed with cold water (2x200 mL), cold aqueous 1M $NaHCO_3$ (2x200 mL), cold saturated aqueous NaCl solution (2x100 mL), and cold water (100 mL). It was dried ($MgSO_4$) and concentrated *in vacuo* to give 1.90g of product [5] as a white solid in quantitative yield. The N.M.R. and I.R. spectra were in accord with the assigned structure.

2-p-phenylbenzoylamino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-one [6]

A mixture of 2,2-difluoro-3-methyl-4-pentenoic acid anhydride [3], (2.79g, 9.90 mmol) and 2-p-biphenyl-4-phenylmethyl-5(4H)-oxazolinone [5], (2.70g, 8.26 mmol) was stirred under argon for 40 h at 60°C (oil bath temperature) to give a lightly red viscous oil. Anhydrous oxalic acid (0.744 g, 8.28 mmol) was then added and the mixture was heated for 15 minutes (110-120°C, oil bath temperature). After the violent gas evolution had ceased, the oil was allowed to cool to room temperature and then diluted with ethyl acetate (100 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (3x100 mL), brine (2x100 mL), water (100 mL), and dried ($MgSO_4$). The filtered mixture was concentrated and chromatographed with 100 g of silica gel (ethyl

acetate/hexane (1:9)) to give 1.48g (42 %) of product (6) as a white solid. The N.M.R., I.R. and mass spectra were in accord with the assigned structure.

2-p-phenylbenzoylamino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-ol [7]

To a solution of 2-p-phenylbenzoylamino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-one [6], (0.84g, 1.94 mmol) in ethyl alcohol (50 mL) was added sodium borohydride (294 mg, 7.76 mmol) in a single portion and stirred at room temperature for 5 minutes. The resulting mixture was poured into water (50 mL) and excess ethyl alcohol was removed *in vacuo*. The resulting white precipitate was filtered and remaining filtrate was extracted with ethyl acetate (4x100 mL) and dried ($MgSO_4$). The resulting solid after concentration was combined with the precipitate to give 0.70 g (94 %) of product [7] as a white solid. It was pure enough for a next reaction without further purification. The N.M.R., I.R. and mass spectra were in accord with the assigned structure.

2-Amino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-ol hydrochloride [8]

To a solution of 2-p-phenylbenzoylamino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-ol [7], (786mg, 1.81 mmol) in methyl alcohol (50 mL) was added $NaH_2PO_4.H_2O$ (2.50g, 18.1 mmol) in a single portion. 3 % Na-Hg (16.7g, 21.7 mmol) was added at room temperature and stirred for 1 h. The mixture was filtered into a solution of 1N HCl (11.0 mL, 11.0 mmol) and was washed with additional methyl alcohol (20 mL). The excess methyl alcohol was removed in vacuo to give white precipitate. The resulting precipitate was filtered and washed with water (20 mL).

The combined aqueous layers were lyophilized to give a white solid. It was dissolved in ethyl acetate (100 mL) and filtered. The filtrate was evaporated to give 420 mg (80 %) of product [8] as a white solid. The N.M.R., I.R. and mass spectra were in accord with the assigned structure.

2-Benzyloxycarbonylamino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-ol [9]

To a solution of 2-amino-1-phenyl-4,4-difluoro-5-methyl-6-hepten-3-ol hydrochloride [8], (420 mg, 1.44 mmol) in 1:1 mixture of water and methyl alcohol (25 mL) was added triethylamine (0.2 mL, 1.44 mmol) in a single portion followed by N-(benzyloxycarbonyloxy)succinimide (476 mg, 1.87 mmol) at room temperature. The reaction was maintained at pH 8 by the addition of additional triethylamine (Ca 0.2 mL). It was stirred at room temperature for an hour, and acidified with aqueous 1N HCl to pH 5. The excess methyl alcohol was removed in vacuo and the aqueous layer was lyophilized to give a colorless oil. This was chromatographed with 50g of silica gel (ethyl acetate/hexane (1:9)) to give 550 mg (98 %) of product [9] as a colorless yellow oil. The N.M.R., I.R. and mass spectra were consistent with the assigned structure.

2-Benzyloxycarbonylamino-4,4-difluoro-5-methyl-1-phenyl-6-hepten-3-one [10]

To a solution of Dess-Martin periodinane (1.24g, 2.92 mmol) in dichloromethane (18 mL) is added the difluoro alcohol [9], (307mg, 0.790 mmol) in a single portion, and the mixture is stirred at room temperature for 3 h. The reaction mixture is then diluted with 20 mL of ether and poured into a 0.26 M solution of sodium

thiosulfate in saturated aqueous sodium bicarbonate (60 mL). The layers are separated, and the organic phase is washed with saturated aqueous sodium bicarbonate (2x25 mL) and water (2x25 mL). The combined aqueous layers are then back extracted with ether (2x50 mL). The combined organic phases are dried ($MgSO_4$) and filtered. Excess solvents are then removed in vacuo and the crude mixture is purified by chromatography using 50 g of silica gel and eluting with ethyl acetate/n-hexane to give the product [10] as a solid.

5-Benzyloxycarbonylamino-3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid [11]

A solution of [10] (194mg, 0.50 mmol) in dichloromethane (20 mL) is treated with O_3 at -78°C for 5 min (about 2.5 mmol of O_3). Dimethylsulfide (0.2 mL, 2.7 mmol) is added and the solution allowed to warm to room temperature. After removal of solvents, the resulting mixture is dissolved with acetone (3.0 mL) and is treated with a Jones reagent (2 mL, 1 M CrO_3/H_2SO_4) overnight. The resulting solution is filtered through Celite and evaporated in vacuo. The crude product is diluted with water (50 mL), and the aqueous phase is extracted with ethyl acetate (4x20 mL). The combined organic layers are washed with brine (2x50 mL) and water (50 mL), dried ($MgSO_4$) and concentrated in vacuo and the resulting mixture is purified by chromatography using 50g of silica gel and eluting with methyl alcohol/dichloromethane to give the product [11] as a solid.

3-Difluoro-5-(6-maleimidohexanoyl)amino-2-methyl-4-oxo-6-phenylhexanoic acid [12]

To a solution of [12], (203mg, 0.50 mmol) in ethyl acetate (5 mL) is added 10% palladium on charcoal catalyst (20 mg) and the mixture is stirred under an

atmosphere of hydrogen for 3 h. The mixture is filtered through Celite and the solvent evaporated in vacuo. The residue is dissolved in dichloromethane (10 mL) and triethylamine (0.070mL, 0.50 mmol) is added, followed by 6-maleimidohexanoic acid anhydride (202mg, 0.50 mmol). The reaction mixture is stirred for 3 h at room temperature. Solvent is removed in vacuo to give a thick oil which is purified by reverse-phase HPLC to give the product [12] as a solid.

Example 8

Synthesis of 5-[N-Benzyloxycarbonylmethylamido]-3,3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid

The reaction sequence is shown in Fig. 9.

Hydrocinnamic acid methyl ester [2]

A separatory funnel (with clear seal joint) was placed over the reaction vessel and charged with a solution of Diazald® (5.0g, 23 mmol) in ether (45 mL). The reaction vessel was warmed to 65°C with a water bath and the Diazald® solution was added over a period of 20 min. The rate of distillation approximated the rate of addition. When all the Diazald® had been used, additional ether (10 mL) was added slowly and the distillation was continued until the distillate was colorless. The ethereal distillate contained about 700mg (16.6 mmol) of diazomethane. The resulting diazomethane was poured into a solution of hydrocinnamic acid [1], (2.0g, 3.3 mmol) in ether (20 mL) in an ice bath. Acetic acid was then added dropwise into the reaction mixture until the yellow color and the evolution of gas had disappeared. The excess solvent was removed in vacuo to give 2.0g (90 %) of product [2] as a yellow oil.

2,2-Difluoro-3-methyl-4-pentanoic acid methyl ester [4]

Diazomethane was made in the same way described above for compound [2] on the same scale. The excess diazomethane was added to a cooled (0°C) solution of acid [3], (2.0g, 3.3 mmol) in ether (20 mL). Acetic acid was then added to the reaction mixture dropwise until the yellow color was discharged. Solvent was removed in vacuo and the residue distilled to give 1.75g (80 %) of ester [4] as a colorless oil (bp. 112°C).

4,4-Difluoro-5-methyl-3-oxo-2-phenylmethyl-6-heptenoic acid methyl ester [5]

A THF solution (10 mL) of methyl ester [2] , (1.64g, 10 mmol) is added dropwise to a solution of lithium diisopropylamide (15 mmol) in 30 mL of THF at -78°C . The mixture is stirred for 30 min at -78°C prior to the addition of a solution of difluoro acid methyl ester (3.28g, 20 mmol) in THF (10 mL). The reaction mixture is allowed to warm gradually to room temperature and stirred for a period of 26 h. The reaction is then quenched at room temperature by the addition of 5 % aqueous hydrochloric acid (40 mL). The layers are then separated, and the organic phase is washed with water (2x20 mL) and saturated aqueous sodium chloride (2x20 mL). The combined aqueous layers are then back extracted with ether (25 mL). The combined organic phases are dried over anhydrous $MgSO_4$ and filtered, and the solvent is removed under reduced pressure to give product [5] as an oil. Pure product [5] is obtained by chromatography of the crude mixture using 100g of silica gel and eluting with ethyl acetate and n-hexane.

2-[N-(Benzyloxycarbonylmethyl)amido]-4,4-Difluoro-5-methyl-1-phenylmethyl-6-hepten-3-one [6]

To a solution of ester [5], (858mg, 2.0 mmol) in methanol (10 mL) is added a solution of potassium carbonate (2.76g, 20 mmol) in water (10 mL) at room temperature. The resulting solution is warmed under gentle reflux for 3 h. Excess methyl alcohol is removed in vacuo and the reaction mixture is acidified by the addition of concentrated hydrochloric acid dropwise to pH 6. The mixture is lyophilized and the resulting white solid is dissolved with ethyl acetate (50 mL) and filtered. The filtrate is concentrated in vacuo and is dissolved in dichloromethane (20 mL). A solution of 1,3-dicyclohexylcarbodiimide (205mg, 10 mmol) in dichloromethane (10 mL) is added at room temperature under argon and the mixture stirred for 30 min. The undissolved precipitate is filtered and the filtrate is concentrated in vacuo. The resulting solid is dissolved in DMF (10 mL) and a solution of glycine benzylester (165 mg, 1.0 mmol) in DMF (10 mL) followed by a solution of N-methylmorpholine (0.13 mL, 1.2 mmol) in DMF (5 mL) is added dropwise. The reaction mixture is stirred for an hour and then diluted with ethyl acetate (100 mL). The organic layer is washed with 1 N aqueous hydrochloric acid (2x50 mL), saturated sodium bicarbonate (2x50 mL), and water (2x50 mL), dried ($MgSO_4$), and concentrated in vacuo to give [6] as an impure solid. Pure product [6] is obtained by chromatography of the crude mixture using 100g of silica gel and eluting with ethyl acetate/n-hexane.

5-[N-(Benzyloxycarbonylmethyl)amido]-3,3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid [7]

A solution of [6] (429mg, 1.0 mmol) in dichloromethane (20 mL) is treated with O_3 at -78°C for 12 min (about 6 mmol of O_3). Dimethylsulfide (0.4mL, 5.4

mmol) is added and the solution allowed to warm to room temperature. After removal of solvents, the resulting mixture is dissolved in acetone (6.0 mL) and treated with Jones reagent (4.0mL, 1 M CrO_3/H_2SO_4) overnight. The resulting solution is filtered through Celite and evaporated in vacuo. The crude product is diluted with water (50 mL), and the aqueous phase is extracted with ethyl acetate (4x20 mL). The combined organic layers are washed with brine (2x50 mL) and water (50 mL), dried ($MgSO_4$) and concentrated in vacuo and the resulting mixture is purified by chromatography using 50g of silica gel and eluting with methyl alcohol/dichloromethane to give the product [7] as a solid.

Example 9

Preparation of (S)-3-(Benzyloxycarbonyl)amino-2-Oxo-1-Azetidineacetic acid [5]

The reaction sequence is shown in Fig. 20. The ß-lactam derivative (S)-3-(*tert*-butoxycarbonyl)amino-2-azetidinone [1] was prepared by a published procedure (19).

Preparation of (S)-Benzyl 3-*tert*-(Butoxycarbonyl)amino-2-Oxo-1-Azetidineacetic acid [2]

1.5 g (8.2 mmol) of (S)-3-(*tert*-butoxycarbonyl)amino-2-azetidinone and 1.98 g (8.2 mmol) of benzyl 2-bromoacetate were dissolved in 40 ml of anhydrous DMF and stirred at 0°C under a nitrogen atmosphere. Sodium hydride (60% oily suspension), 0.394 g (16 mmol), was added quickly, and the reaction mixture was stirred for 2 hr at 0°C. At this time 175 ml of ethyl acetate was added to the mixture, and the solution was washed with water (150 ml), saturated aqueous $NaHCO_3$ (150 ml), and water (150 ml) respectively, and then dried

by filtration over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure, and the product was purified by chromatography on silica gel (eluted with 5:4 methylene chloride:diethyl ether) to yield 0.283 g (0.82 mmol) of (S)-benzyl 3-(*tert*-butoxycarbonyl)amino-2-oxo-1-azetidineacetic acid [2]. ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 9H), 3.4 (m, 2H), 3.7 (t, 1H), 4.06 (s, 2H), 4.4 (bs, 1H), 5.2 (s, 2H), 7.3 (s, 5H).

Preparation of (S)-Benzyl 3-Amino-2-Oxo-1-Azetidineacetic acid [3]

(S)-benzyl 3-(*tert*-butoxycarbonyl)amino-2-oxo-1-azetidineacetic acid 0.025 g (0.075 mmol), and 0.014 g of *p*-toluenesulfonic acid monohydrate were dissolved in 12 ml of ethyl acetate, and the resulting solution was refluxed for 2-1/2 hr. Then 10 ml of 5% aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with ethyl acetate (5 x 10 ml). The combined organic layers were dried by filtration over anhydrous magnesium sulfate and the solvent was removed by evaporation under reduced pressure to yield 0.0081 g (0.035 mmol) of (S)-benzyl 3-amino-2-oxo-1-azetidineacetic acid [3]. ^{1}H NMR (CD_3COCD_3): δ 3.1 (bs, 1H), 3.5 (dd, 1H), 3.8 (t, 1H), 4.1 (s, 2H), 4.9 (m, 1H), 5.1 (s, 2H), 7.3 (s, 5H).

Preparation of (S)-3-Amino-2-Oxo-1-Azetidineacetic acid [4]

(S)-benzyl 3-amino-2-oxo-1-azetidineacetic acid, 0.0081g (0.035 mmol), was dissolved in 3 ml of methanol and 0.01 g acetic acid was added. Palladium, 0.10 g 10%, on activated carbon was added, and the mixture was stirred under one atmosphere of hydrogen gas for 1 hr. The catalyst was removed by filtration, and the solvent

was removed by evaporation under reduced pressure to yield 0.004 g (0.028 mmol) of (S)-3-amino-2-oxo-1-azetidineacetic acid [4]. ^{1}H NMR (D_2O): δ 3.5 (m, 2H), 3.7 (s, 2H), 4.4 (dd, 1H); ^{13}C NMR (D_2O): δ 43.4, 47.2, 53.1, 167.0, 175.6.

Preparation of (S)-3-(Benzyloxycarbonyl)amino-2-Oxo-1-Azetidineacetic acid [5]

0.01 g (0.07 mmol) (S)-3-amino-2-oxo-1-azetidineacetic acid is dissolved in 1 ml H_2O. Carbobenzyloxy chloroformate 0.025 g (0.1 mmol) is in 0.5 ml of acetone is added slowly and the resulting solution is stirred for 2 hr. At this time 10 ml of water is added and the aqueous mixture is washed with methylene chloride (2 x 10 ml). The aqueous layer is then acidified to pH 3 with 1M HCl and extracted with methylene chloride (3 x 10 ml). The combined organic layers are dried by filtration over anhydrous magnesium sulfate and the solvent is removed by evaporation under reduced pressure to yield 3-(benzyloxycarbonyl)amino-2-oxo-1-azetidineacetic acid [5].

Example 10

Preparation of (3'S,2R)-2-[3-Amino-2-Oxo-1-Azetidininyl]-3-Methylbutanoic Acid [14]

This material was prepared exactly as described in a published procedure (20) by the reaction sequence shown in Fig. 21.

Treatment of 6-aminopenicillanic acid [11] with Raney nickel yielded a 2:1 mixture of the ß-lactam derivatives (3'S,2R)-2-[3-(benzyloxycarbonyl)amino-2-oxo-1-azetidininyl]-3-methyl-3-butenoic acid [12] [^{1}H NMR (CD_3COCD_3): δ 1.0 (d, 6H), 2.1 (m, 1H), 3.5 (dd, 1H), 3.8 (t, 1H), 4.1 (d, 1H), 4.8 (m, 1H), 5.1 (s, 2H), 7.3 (s,

5H)] and (3'S,2R)-2-[3-(benzyloxycarbonyl)amino-2-oxo-1-azetidininyl]-3-methylbutanoic acid [13] [^{1}H NMR (CD_3COCD_3): δ 1.9 (s, 3H), 3.4 (dd, 1H), 3.9 (t, 1H), 4.8 (m, 1H), 4.9 (s, 1H), 5.0 (s, 1H), 5.05 (s, 1H), 5.1 (s, 2H), 7.3 (s, 5H)]. Hydrogenolysis of this mixture over palladium on activated carbon yielded (3'S,2R)-2-[3-amino-2-oxo-1-azetidininyl]-3-methylbutanoic acid [14] [^{1}H NMR (D_2O): δ 0.9 (d, 6H), 2.1 (m, 1H), 3.3 (dd, 1H), 3.8 (d, 1H), 3.9 (t, 1H), 4.3 (m, 1H)].

Example 11

Preparation of *cis*-3-(Benzyloxycarbonyl)amino-4-Carboxy-2-Azetidinone [22]

This material was prepared by a modification of a published procedure (21) as is shown in the reaction sequence in Fig. 22. (The (3)-lactam derivative *cis*-3-(benzyloxycarbonyl)amino-4-carbomethoxy-2-azetidinone [21] was prepared by a published procedure (22).

To a stirred solution 0.1 g (0.36 mmol) of *cis*-3-(benzyloxycarbonyl)amino-4-carbomethoxy-2-azetidinone in 5 ml of methanol at 0°C was slowly added 0.1 g K_2CO_3 in 3 ml of H_2O at 0°C. The solution was stirred for 1 hr at room temperature and then neutralized to pH 7 with phosphoric acid at 0°C and filtered. The methanol was removed by evaporation under reduced pressure, and the remaining aqueous solution was extracted with ethyl acetate (2 ml). The aqueous layer was acidified to pH 2 with phosphoric acid and then saturated with NaCl. The resulting solution was extracted with ethyl acetate (5 x 5 ml), and the combined organic layers are washed with saturated NaCl (5 ml) and dried by filtration over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure to yield 0.082 g (0.31

mmol) of cis-3-(benzyloxycarbonyl)amino-4-carboxy-2-azetidinone [22]. ^{1}H NMR (CD_3SOCD_3): δ 4.2 (d, 1H), 5.0 (s, 2H), 5.0 (dd, 1H), 7.2 (s, 5H), 8.1 (d, 1H), 8.6 (d, 1H).

Example 12

Preparation of 3-(Benzyloxycarbonyl)amino-2-Oxo-1-Cyclobutaneacetic Acid [34] (as a mixture of diastereomers) and 3-(Benzyloxycarbonyl)amino-2-Hydroxy-1-Cyclobutaneacetic Acid [35] (as a mixture of diastereomers)

The reaction sequence is shown in Figure 23. The derivative 2-(dibenzylamino)cyclobutanone [31] was prepared by a published procedure (23).

Preparation of Benzyl 3-Dibenzylamino-2-Oxo-1-Cyclobutaneacetic Acid [32]

To a stirred solution of 2.9 mmol lithium diisopropylamide in 5 ml THF is added dropwise 0.66 g (2.5 mmol) of 2-(dibenzylamino)cyclobutanone in 4 ml of THF at -78°C and the mixture is stirred for 15 min at -78°C and then for 15 min at 0°C. The solution is then cooled to -78°C and 0.53 ml (2.9 mmol) of HMPA is added. After five min, 0.70 g (2.9 mmol) of benzyl 2-bromoacetate in 10 ml of diethyl ether is added and the mixture is stirred at -78°C for 1 hr. The solvents are removed by evaporation under reduced pressure, and the product benzyl 3-dibenzylamino-2-oxo-1-cyclobutaneacetic acid [32] (as a mixture of diastereomers) is purified by chromatography on silica gel.

Preparation of 3-Amino-2-Oxo-1-Cyclobutaneacetic Acid [33]

Benzyl 3-dibenzylamino-2-oxo-1-cyclobutaneacetic acid, 0.10 g (0.25 mmol) is dissolved in 3 ml of methanol

and 0.01 g acetic acid is added. Palladium, 0.10 g 10% on activated carbon is added, and the mixture is stirred under one atmosphere of hydrogen gas for 24 hr. The catalyst is removed by filtration, and the solvent is removed by evaporation under reduced pressure to yield 3-amino-2-oxo-1-cyclobutaneacetic acid [33] (as a mixture of diastereomers).

Preparation of 3-(Benzyloxycarbonyl)amino-2-Oxo-1-Cyclobutaneacetic Acid [34]

0.01 g (0.07 mmol) of 3-amino-2-oxo-1-cyclobutaneacetic acid is dissolved in 1 ml H_2O. 0.025 g (0.1 mmol) of carbobenzyloxy chloroformate in 0.5 ml of acetone is then added slowly, and the mixture is stirred for 2 hr. At this time 10 ml of water is added, and the solution is washed with methylene chloride (2 x 10 ml). The aqueous layer is then acidified to pH 3 with 1M HCl and is extracted with methylene chloride (3 x 10 ml). The combined organic layers are dried by filtration over anhydrous magnesium sulfate, and the solvent removed by evaporation under reduced pressure to yield 3-(benzyloxycarbonyl)amino-2-oxo-1-cyclobutaneacetic acid [34] (as a mixture of diastereomers).

Preparation of 3-(Benzyloxycarbonyl)amino-2-Hydroxy-1-Cyclobutaneacetic Acid [35]

First, 0.01 g (0.036 mmol) of 3-(benzyloxy-carbonyl)amino-2-oxo-1-cyclobutaneacetic acid is dissolved in 10 ml of methanol and 0.01 g (0.26 mmol) of $NaBH_4$ is added. The solution is stirred for 1 hr at room temperature and the solvent is removed by evaporation under reduced pressure. Additional methanol is added and removed by evaporation under reduced pressure (4 x 10 ml). At this time 10 ml of methanol and 10 ml of 1M HCl

are added and the methanol is removed by evaporation under reduced pressure. The resulting solution is extracted with methylene chloride (5 x 10 ml), and the combined organic layers are washed with saturated NaCl (5 ml) and dried by filtration over anhydrous magnesium sulfate. The solvent is removed by evaporation under reduced pressure, and the product is purified by chromatography on silica gel to yield 3-(benzyloxy-carbonyl)amino-2-hydroxy-1-cyclobutaneacetic acid [35] (as a mixture of diastereomers).

Example 13

Preparation of 2-(Benzyloxycarbonyl)amino-3-Hydroxycyclobutanecarboxylate [44] (as a mixture of diastereomers) and 2-(Benzyloxycarbonyl)amino-3-Oxocyclobutanecarboxylate [45] (as a mixture of diastereomers)

The reaction sequence is shown in Fig. 24. Methyl 3-hydroxycyclobutanecarboxylate (as a mixture of diastereomers) was prepared by a published procedure (24).

Preparation of 3-Carboxymethylcyclobutanyl Azidoformate [42]

To a stirred solution of 0.60 g (4.62 mmol) of methyl 3-hydroxycyclobutanecarboxylate and 0.89 ml (11 mmol) of pyridine in 74 ml benzene at room temperature was added 0.91 g (3.1 mmol) triphosgene, and the resulting solution was stirred for 1 1/2 hr. The solution was then quickly washed with saturated NaCl (3 x 6 ml), and the organic layer was dried by filtration over anhydrous magnesium sulfate, and the solvent was removed by evaporation under reduced pressure. The resulting product was dissolved in 22 ml of dry DMF and 1.03 g (15.9 mmol) of sodium azide was added. The solution was

stirred at room temperature for 2 hr, 72 ml of water was added, and the solution was extracted with diethyl ether (5 x 30 ml). The organic extracts were combined, washed with saturated NaCl (3 x 32 ml), and dried by filtration over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure, and the product was purified by chromatography on silica gel (eluted with 1:1 ethyl acetate:hexanes) to yield 0.078 g (0.39 mmol) of 3-carboxymethylcyclobutanyl azidoformate [42] (as a mixture of diastereomers). ^{1}H NMR ($CDCl_3$): δ 2.6-3.2 (m, 5H), 3.8 (s, 3H), 4.9-5.3 (m, 1H); IR ($CDCl_3$): μ 2150 cm^{-1} (azide).

Preparation of 3-Carboxymethylcyclobutanyl-1,2-Cyclic Carbamate [43]

First, 3-carboxymethylcyclobutanyl azidoformate, 0.078 g (0.39 mmol), was dissolved in 5 ml dry methylene chloride, and the solution was sealed in a glass reaction tube and heated to 135°C for 1 hr. The solvent was removed by evaporation under reduced pressure, and the product was purified by chromatography on silica gel (eluted with 1:1 ethyl acetate:hexanes) to yield 0.029 g (0.17 mmol) of 3-carboxymethylcyclobutanyl-1,2-cyclic carbamate [43] (as a mixture of diastereomers). ^{1}H NMR ($CDCl_3$): δ 2.8 (m, 2H), 3.2 (m, 1H), 3.8 (m, 3H), 4.4 (m, 1H), 5.1 (dd, 1H), 6.2 (bs, 1H).

Preparation of 2-(Benzyloxycarbonyl)amino-3-Hydroxycyclobutanecarboxylate [44]

First, 3-carboxymethylcyclobutanyl-1,2-cyclic carbamate, 0.118 g (0.68 mmol) of and 0.384 g (6.88 mmol) of potassium hydroxide are dissolved in 20 ml of 1:1 (v:v) dioxane:water. The solution is refluxed for 23 hours. The resulting solution is extracted with diethyl

ether (2 x 20 ml) and 5 ml of water is added. The solution is cooled to 0°C and is vigorously stirred. 0.25 g (1.0 mmol) of carbobenzyloxy chloroformate is added, and the solution is stirred at room temperature for 1 hr. The mixture is acidified to pH 1 with 1M HCl and is extracted with methylene chloride (3 x 30 ml). The combined organic layers are washed with saturated NaCl (30 ml) and are dried by filtration over anhydrous magnesium sulfate. The solvent is removed by evaporation under reduced pressure, and the product 2-(benzyloxycarbonyl)amino-3-hydroxycyclobutanecarboxylate [44] (as a mixture of diastereomers) is purified by chromatography on silica gel.

Preparation of 2-(Benzyloxycarbonyl)amino-3-Oxocyclobutanecarboxylate [45]

To a stirred solution of 0.10 g (0.39 mmol) of 2-(benzyloxycarbonyl)amino-3-hydroxycyclobutanecarboxylate in 20 ml acetone is added 0.235 ml (0.591 mmol) of Jones' reagent (prepared by dissolving 2.5 g chromium(VI) oxide in 2.15 ml concentrated sulfuric acid and diluting to 10 ml with water) at room temperature over a period of 15 sec. After an additional 30 s, 2 ml of 2-propanol is added, the mixture is filtered, and the solvents are removed by evaporation under reduced pressure. The residue is taken up in 20 ml ethyl acetate, and the resulting solution is washed with saturated NaCl (2 x 10 ml) and is then dried by filtration over anhydrous magnesium sulfate. The solvent is removed by evaporation under reduced pressure and the product 2-(benzyloxycarbonyl)amino-3-oxocyclobutanecarboxylate [45] (as a mixture of diastereomers) is purified by chromatography on silica gel.

Example 14

Preparation of 3-*exo*-(Benzyloxycarbonyl)amino-2-*exo*-Hydroxynorbornyl-7-*anti*-carboxylate [53] and 3-*exo*-(Benzyloxycarbonyl)amino-2-Oxonorbornyl-7-*anti*-carboxylate [54]

The reaction sequence is shown in Fig. 25. Methyl 2-*exo*-hydroxynorbornyl-7-*anti*-carboxylate [51] is prepared by a published procedure (25).

Preparation of *anti*-7-Carboxymethylnorbornyl-2,3-*exo*-Cyclic Carbamate [52]

To a stirred solution of 0.60 g (3.53 mmol) of methyl 2-*exo*-hydroxynorbornyl-7-*anti*-carboxylate and 0.67 ml (8.25 mmol) of pyridine in 60 ml benzene at room temperature is added in one portion 0.68 g (2.3 mmol) triphosgene, and the resulting solution stirred for 1 1/2 hr. The solution is then quickly washed with saturated NaCl (3 x 6 ml), the organic layer is dried by filtration over anhydrous magnesium sulfate, and the solvent is removed by evaporation under reduced pressure. The resulting product is dissolved in 16 ml of dry DMF, and 0.77 g (11.9 mmol) of sodium azide is added. The solution is stirred at room temperature for 2 hr, 55 ml of water is added, and the solution is extracted with diethyl ether (5 x 30 ml). The organic extracts are combined and washed with saturated NaCl (3 x 32 ml), are dried by filtration over anhydrous magnesium sulfate, and the solvent is removed by evaporation under reduced pressure. The product is purified by chromatography on silica gel to yield *anti*-7-carboxymethylcyclobutanyl-2-*exo*- azidoformate, which is dissolved in 5 ml dry methylene chloride. This solution was sealed in a glass reaction tube and is heated to 135°C for 1 hr. The solvent is removed by evaporation under reduced pressure

and the product is purified by chromatography on silica gel to yield *anti*-7-carboxymethylnorbornyl-2,3-*exo*-cyclic carbamate [52].

Preparation of 3-*exo*-(Benzyloxycarbonyl)amino-2-*exo*-Hydroxynorbornyl-7-*anti*-Carboxylate [53]

0.143 g (0.68 mmol) of *anti*-7-carboxymethylnorbornyl-2,3-*exo*-cyclic carbamate and 0.384 g (6.88 mmol) of potassium hydroxide are dissolved in 20 ml of 1:1 (v:v) dioxane:water. The solution is refluxed for 23 hours. The resulting solution is extracted with diethyl ether (2 x 20 ml) and 5 ml of water added. The solution was cooled to 0°C and is vigorously stirred. Then 0.25 g (1.0 mmol) of carbobenzyloxy chloroformate is added, and the solution is stirred at room temperature for 1 hr. The mixture is acidified to pH 1 with 1M HCl and is extracted with methylene chloride (3 x 30 ml). The combined organic layers are washed with saturated NaCl (30 ml) and are dried by filtration over anhydrous magnesium sulfate. The solvent is removed by evaporation under reduced pressure and the product is purified by chromatography on silica gel to yield 3-*exo*-(benzyloxycarbonyl)amino-2-*exo*-hydroxynorbornyl-7-*anti*-carboxylate [53].

Preparation of 3-*exo*-(Benzyloxycarbonyl)amino-2-Oxonorbornyl-7-*anti*-Carboxylate [54]

To a stirred solution of 0.123 g (0.39 mmol) of 3-*exo*-(benzyloxycarbonyl)amino-2-*exo*-hydroxynorbornyl-7-*anti*-carboxylate in 20 ml acetone is added 0.235 ml (0.591 mmol) of Jones' reagent (prepared by dissolving 2.5 g chromium(VI) oxide in 2.15 ml concentrated sulfuric acid and diluting to 10 ml with water) at room temperature over a period of 15 s. After an additional

30 s, 2 ml of 2-propanol is added, the mixture is filtered, and the solvents are removed by evaporation under reduced pressure. The residue is taken up in 20 ml ethyl acetate, is washed with saturated NaCl (2 x 10 ml), and is dried by filtration over anhydrous magnesium sulfate. The solvent is removed by evaporation under reduced pressure and the product is purified by chromatography on silica gel to yield 3-*exo*-(benzyloxycarbonyl)amino-2-oxonorbornyl-7-*anti*-carboxylate [54].

Example 15

Preparation of 3-*endo*-(Benzyloxycarbonyl)amino-2-*endo*-Hydroxynorbornyl-7-*anti*-carboxylate [63] and 3-*endo*-(Benzyloxycarbonyl)amino-2-Oxonorbornyl-7-*anti*-carboxylate [64]

The syntheses of these compounds are identical to those discussed above for the *exo*-isomers [53] and [54], except that methyl 2-*endo*-hydroxynorbornyl-7-*anti*-carboxylate [61] is utilized in place of methyl 2-*endo*-hydroxynorbornyl-7-*anti*-carboxylate [51], as is shown in the reaction sequence in Fig. 26. Methyl 2-*endo*-hydroxy-norbornyl-7-*anti*-carboxylate [61] is prepared by a published procedure (26).

Example 16

Preparation of Amidine Hapten Immunogen Coupling of (2-[(6-maleimido)butanoyl]amino]-3-phenyl-propylimino-D-alanine to Bovine Serum Albumin And to Keyhole Limpet Hemocyanin

Two protein conjugates were prepared for the immunization of mice and for coating the solid phase in an enzyme-linked immunosorbent assay (ELISA) screening procedure for detection of antigen-specific antibody. Thiolated bovine serum albumin (BSA) was prepared by

mixing 30 mg of BSA with a 100-fold molar excess of iminothiolane (Traut's reagent) (27), 5.1 mg of Traut's reagent in 1 M triethanolamine, at pH 8.0, for 90 min. at 4°C. BSA-SH was purified by size-exclusion chromatography on a PD-10 desalting column (Sephadex G-25M) equilibrated and eluted with 50 mM phosphate, pH 7.1. A 100-fold molar excess of (2-[(6-maleimido)butanoyl]amino]-3-phenyl- propylimino-D-alanine (hapten, prepared as described in Example 6 above) (2.8 mg in 0.5 ml) was resuspended in 50 mM phosphate, pH 7.1, and mixed for 3 hours at room temperature with 5 mg of BSA-SH. The hapten-BSA conjugate was purified from uncoupled hapten by gel filtration using 10 mM phosphate, pH 7.4, 0.15 M NaCl (PBS) as the equilbration and elution buffer. A free-thiol analysis (28) of the conjugate showed that the hapten to protein ratio was 23:1 (using a M.W. of 64,000 for BSA). Protein concentrations were determined by the bicinchoninic acid method (29), using BSA as the standard.

The amidine hapten keyhole limpet hemocyanin (KLH) conjugate was prepared in a similar fashion with 3 mg of KLH-SH and 1.7 mg of the amidine hapten-GMBS reagent, except that dialysis of the hapten-KLH conjugate against 10 mM phosphate, pH 7.4, 0.15 M NaCl (PBS) was performed instead of gel filtration to remove unconjugated hapten. The thiol analysis indicated that three haptens were coupled per KLH (using a M.W. of 64,000, for easier comparison to BSA).

Immunization of Mice, Preparation of Hybridomas, and Screening of Antibodies for Binding Activity

Twelve-week-old BALB/c mice were injected intraperitoneally (IP) with 10 μg of the amidine hapten-KLH conjugate emulsified in complete Freund's adjuvant.

The mice were boosted with the hapten-KLH conjugate emulsified in incomplete Freund's adjuvant after three weeks with 10 μg and after another three weeks with 30 ug. The inverse serum titer was 51,200 by ELISA. A final intrasplenic injection of 10 μg of the conjugate in PBS was administered three days before the mice were sacrificed and the splenocytes prepared for fusion. A fusion was performed by standard methods (30) using SP2/0 myelomas as the fusion partner. Of the 45 hybridomas which produced anitbodies against the hapten-BSA conjugate by ELISA, 24 produced antibodies which bound the cys-maleimide-linker hapten in a competitive inhibition ELISA. Of those 24, 17 also reacted with the acetylated-hapten.

Chemiluminescent Assay for Catalytic Activity

The production of D-alanine was indirectly measured by the D-amino acid oxidase catalyzed conversion of D-alanine to its alpha-keto acid, which is accompanied by a stoichiometric production of hydrogen peroxide. The H_2O_2 is in turn utilized in the chemiluminescent reaction with luminol and the resulting luminescence measured on a Berthold Bilumat LB9500C luminometer.

Eleven ml of the supernatant fluids from 24 hybridomas were concentrated, then washed and resuspended in 0.75 ml of 20 mM PBS, pH 8.0. One ml of the substrate (1 mM Ac-D-phe-D-ala in 20 mM PBS, pH 8.0) was added to 0.5 ml of the antibody solutions and incubated overnight at 37°C. All solutions were filter sterilized through a 0.2 micron filter unit. A reaction cocktail was prepared one hour before the chemiluminescent assay was performed and was composed of:

- 990 µl of 50 mM phosphate buffered saline at pH 12, containing 10 mM EDTA (PBD-EDTA)
- 15 µl of a 4 mg/ml microperoxidase stock solution
- 75 µl of a 400 mg/ml solution of diazabicyclooctane (DABCO)
- 375 µl of a 250 mM luminol solution in PBS-EDTA.

To eliminate the contribution of any endogenous H_2O_2 from the antibody sample, 100 µl of the antibody/substrate sample was mixed with 97 µl of the reaction cocktail and incubated at room temperature for 15 - 30 min. Then 3 µl of D-amino acid oxidase (a 4.2 mg/ml stock in 3.6 M ammonium sulfate, pH 6.5) was added to the reaction mixture, mixed and the luminescence monitored over 6 min. Eight hybridomas yielded signals greater than two times the signal of a negative control antibody. Four of these hybridomas were stable antibody producers after cloning. Ascites fluids are prepared for confirmation assays on the HPLC.

Example 17

Preparation of Phosphonate Ester Hapten Immunogen

BSA-SH and KLH-SH were prepared as described the previous example. Five mg of each of the proteins were mixed with 5 mg of O-[(6-maleimido)hexanyl]amino](2-phenyl-ethyl) phosphinyl lactic acid disodium salt (hapter, prepared as described in Example 4 above) for 3 hours at room temp. The conjugates were dialyzed against PBS to remove the uncoupled hapten.

Immunization of Mice, Preparation of Hybridomas, and Screening of Antibodies for Binding Activity.

Eight-week-old BALB/c mice were immunized intraperitoneally (IP) with 50 µg of the hapten-KLH conjugate emulsified in complete Freund's adjuvant. The

mice were boosted IP with 10 μg of the antigen in incomplete Freund's adjuvant after three and eight weeks. The inverse serum titer was 25,600 by ELISA. Four weeks after the last injection, 10 μg of the antigen in PBS was injected intrasplenically. The mice were sacrificed three days later and a fusion with the splenocytes and SP2/0 myelomas was performed by standard methods. Forty-two hybridomas produced antibodies against the hapten-BSA conjugate by ELISA and could be inhibited by the free-hapten in a competitive inhibition assay. All but one bound to both the Ac-hapten and the cys-maleimide-linker-hapten.

Catalytic Antibody Assay

Antibodies from 41 supernatant fluids were assayed directly for activity against Ac-L-phe-D-ala and Ac-D-phe-D-ala by the chemiluminescent assay described earlier with the following modifications. Two aliquots of unconcentrated supernatant fluids (0.5 ml each) were mixed with 1 ml of 1 mM substrate solutions and incubated at 37°C for 2-8 days. The reaction cocktail used a 2 mg/ml stock of horseradish peroxidase instead of the microperoxidase. Eight hybridomas yielded a luminescent signal greater than 2 times the negative control supernatant against the Ac-D-phe-D-ala substrate. None of the 41 hybridomas yielded a positive luminescent signal for the Ac-L-phe-D-ala substrate. Four of the eight hybridomas remained stable antibody producers after cloning. One of the four also yielded a positive luminescent signal for the Ac-L-phe-D-ala substrate. Ten-fold concentrates of the antibody supernatant fluids from the four cloned hybridomas were rescreened for catalytic activity against the Ac-D-phe-D-ala and the cys-maleimide-linker-D-phe-D-ala substrates by the

following modified chemiluminescent assay. The concentrated antibody, 0.23 ml, was incubated with 0.48 ml of the 1 mM substrate stock solutions (in 20 mM Tris-HCl, pH 8.0) overnight at 37°C. An aliquot (34 µl) of the antibody/substrate mixture was then mixed with 66 µl of 20 mM Tris-HCl and 3 µl of D-amino acid oxidase. After three min., 97 µl of the reaction cocktail (71 parts of 50 mM Tris-HCl, pH 9.0; 25 parts of 250 mM luminol in Tris-buffer, and 1 part of micro peroxidase at 4 mg/ml in 10 mM Tris-HCl, pH 8.0) was injected and the luminescence measured. Two of the hybridomas yielded positive luminescent signals for both substrates greater than one standard deviation of the signal obtained with the antibody alone. Ascites fluids are then prepared, the antibodies are purified by methods known in the art and are used to catalyze peptide bond hydrolysis.

Example 18

Preparation of Dialkyl Phosphinate Hapten Immunogen

BSA-SH and KLH-SH were prepared as described earlier. [6-Maleimido)hexanoyl]amino-2-phenylethyl(2-carboxy-1-propyl)hydroxyphosphonous acid (6mg), the hapten as prepared in Example 5 above, was dissolved in water and mixed with 5 mg of each of the two proteins for three hours at room temperature. The conjugates were dialyzed against PBS. The free thiol analysis showed that the ratio of hapten to BSA and KLH (using a M.W. of 64,000) was 8:1 and 3:1, respectively.

Immunization of Mice, Preparation of Hybridomas, and Screening of Antibodies for Binding Activity

Nine-week-old BALB/c mice were immunized intraperitoneally (I.P.) with 50 µg of the dialkyl

phosphinate hapten-KLH conjugate emulsified in complete Freund's adjuvant. The mice were boosted IP after three, seven, ten, and 17 weeks with 10 μg of the conjugate emulsified in incomplete Freund's adjuvant. The inverse serum titer was 102,400 by ELISA. The final injection with 10 μg of the conjugate in PBS was given intrasplenically six weeks later. The mice were sacrificed three days after the last injection. A fusion was performed by standard methods using the BALB/c splenocytes and SP2/0 myelomas as the fusion partner. The hybridomas were screened for hapten-BSA specific antibody production by ELISA. Ten hybridomas produced antibodies which were competitively inhibited by Ac-hapten from binding to the hapten-protein conjugate. These hybridomas are then cloned to insure monoclonality.

Example 19

Preparation of Phosphonamidate Immunogens

BSA-SH and KLH-SH were prepared as described earlier except that the uncoupled Traut's reagent was removed by gel filtration with 50 mM phosphate at pH 8.4. The hapten, [(6-maleimido)hexanoyl]amino](2-phenylethyl)hydroxyphosphinyl D-alanine, prepared as described above in Example 3, (7-10 mg) was divided into two portions and mixed with 5 mg each of BSA-SH and KLH-SH at room temperature for three hours. The conjugates were dialyzed against PBS at pH 8.0 and then stored frozen at -20°C. The thiol determination results indicated that 26 hapten were conjugated to the BSA molecules and 12 haptens to KLH (assuming a M.W. of 64,000). These conjugates were injected into BALB/c mice.

Example 20

Methodology For The Production, Screening And Isolation Of Monoclonal Catalytic Antibodies That Cleave The "Flap" Region Of Human Renin.

The inhibition of renin, an aspartic proteinase whose action initiates the renin-angiotensin cascade has been the object of intense investigation in recent years. The potential for treatment of hypertension through the inhibition of renin has resulted in the synthesis of a variety of potent renin inhibitors based on the peptide sequence of the natural substrate angiotensinogen. An alternative approach is the use of a proteolytic antibody to renin.

It has been reported that the flap region of human renin is a hairpin with a loop region of four amino acid residues leading to the carbonyl group of Tyr 83 interacting with the amino groups of Thr 85 and Gly 86 (31). The cyclic decapeptide CLRYSTGTVC, [80-89] human renin, was found to adopt this same hairpin structure.

In this example, monoclonal antibodies are elicited with a difluroketone containing immunogen according to the invention. The monoclonal antibodies will catalyze the cleavage of the peptide sequence

81			85	86				90
Leu-	Arg-	Tyr-	Ser-	Thr-	Gly-	Thr-	Val-	Ser-Gly

in the human renin molecule between residues 85 and 86. Cleavage causes disruption of the catalytic machinery of the enzyme since residues 85 and 86 constitute the "flap" region which holds the substrate in the catalytic site (32). It has previously been demonstrated that rabbit polyclonal antiserum elicited to a synthetic peptide fragment (sequence 81-90) could inhibit human renin activity by 40% as measured by its reaction with synthetic human tetradecapeptide substrate (33).

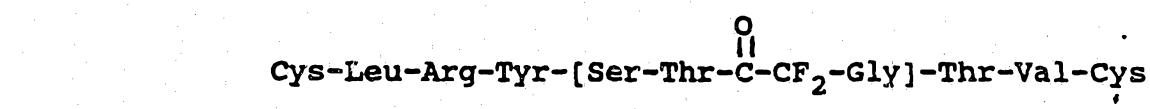
In order to raise a catalytic antibody to this flap region of human renin, the cyclic peptide hapten CLRYST(TS)GTVC, where (TS) represents an analog of the target amide (peptide) bond is synthesized. In this example, the synthesis of the cyclic peptide hapten CLRYST(TS)GTVC follows the solid-phase approach (34) wherein ST(TS)G, corresponds to a difluoroketone transition state analog tripeptide in accordance with the invention and is incorporated into the assembled peptide through its anhydride (34). The completed peptide is fully deprotected and cleaved from the solid support using trifluoroacetic acid. Cyclization of the peptide is achieved by allowing the peptide to stand at low concentration for one hour in an aqueous solution (pH 8) in order to generate a disulphide bridge between the two terminal cysteine residues. The N-terminal amino group of the peptide allows it to be attached to a carrier protein for immunization of mice.

A. **Preparation of the Immunogen**

1. **Peptide Synthesis**

The peptide hapten is synthesized by the solid phase technique using the polyamide-Kieselguhr composite resin (34). The side chain protecting groups are the following: O-tert-butyl (tyrosine, glutamic acid, serine, threonine); N-4-methoxy-2,3,6-trimethyl benzenesulphonyl (arginine); and S-trityl (cysteine). The temporary protection of the N function is by fluorenylmethoxycarbonyl which is removed in 10 minutes with piperidine/DMF : 20/80. The coupling reactions are carried out using FMOC- amino acid anhydrides (34). The protected peptidyl-resin is fully deprotected by treatment with trifluoroacetic acid/thioanisole/m-cresol/thiophenol/ ethanedithiol solution: 90/2/2/2/4

for 3 hrs. After filtration the filtrate is concentrated under vacuum to a small volume. Ether is added to give a precipitate of the peptide. The ethereal supernatant is removed and the peptidic precipitate is washed twice with ether to yield the peptide hapten



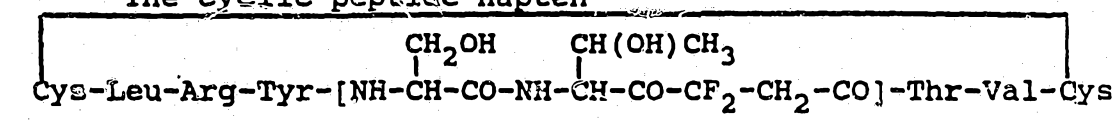
wherein the bracketed moiety is a difluoroketone transition state analog tripeptide isostere of the invention.

2. Cyclization of Peptide Hapten

The peptide hapten is cyclized to obtain the " β-hairpin" conformation by forming a disulphide bond between the two terminal cysteine residues. The disulfide bond is completed in one hour by air oxidation of an aqueous solution (pH 8) at a concentration of 0.3 mg of peptide per milliliter. The oxidized product is then removed by lyophilization and purified by HPLC.

3. Conjugation of the Hapten to the Carrier Molecule

The cyclic peptide hapten



is conjugated to keyhole limpet hemocyanin (KLH) using glutaraldehyde (35). Coupling efficiency is 50-80% as estimated by binding of a trace amount of I125 peptide added to the reaction mixture.

B. Preparation and Screening of Monoclonal Antibodies

KLH conjugated peptide (50 μg) in complete Freund's adjuvant is injected into BALB/c mice. Hybridoma fusions are carried out by standard methods using SP2/0 myeloma as the fusion partner. Polyclonal antiserum response and hybridoma secreting cells resulting from the fusion are screened for binding activity by ELISA.

Wells of plastic microtiter plates (Falcon 3915 Probind, Becton Dickinson Labware CA, USA) are coated with 50 μL of peptide or renin (5 μg/mL) in Tris-HCl buffer (0.1 M, pH 9.6).

Plates are first incubated for 30 minutes at 37°C and then overnight at room temperature. After washing three times with Tween- containing phosphate-buffered saline (PBS-Tween 0.1%, pH 7.4), 50 μL of serial dilutions of antisera in PBS-BSA 1% pH 7.4 are added in peptide- coated duplicate wells and incubated for 2 hours at 37°C. Plates are washed three times again with PBS-Tween 0.1% and wells are then treated with 50 μL of alkaline phosphatase-labelled goat anti-mouse IgG diluted 1:500 (Sigma, MO, USA). Incubation is carried out for 1 hour at 37°C.

Additional extensive washing with PBS-Tween 0.1% is followed by incubation with 150 μL of alkaline phosphatase substrate (2 tablets/10 ml of Sigma 104-105) dissolved in 0.1 M glycine-NaOH buffer (pH 10.4) containing $MgCl_2$ and $ZnCl_2$, 1/L. The enzymatic reaction is allowed to proceed for 2 hours at 37°C and stopped by addition of 50 uL of Na_2CO_3 (1.5 M).

Absorbance is read at 405 nm in a Titerteck multiskan ELISA reader (Flow laboratories). Titer expression is determined by multiplying the optical density by the maximal dilution giving an absorbance three times as high as the negative control (consisting of pooled normal mouse sera diluted 1:100).

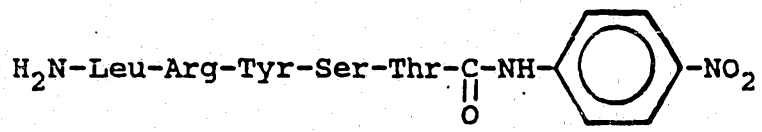
Hybridomas giving a positive reaction in this screening assay against the peptide, renin or both are chosen for further study. IgG is purified from ascites fluid by HPLC with a Bakerbond ABx HPLC column.

C. **Catalysis of Peptide Cleavage by Catalytic Antibody Specific for the "Flap" Region of Human Renin.**

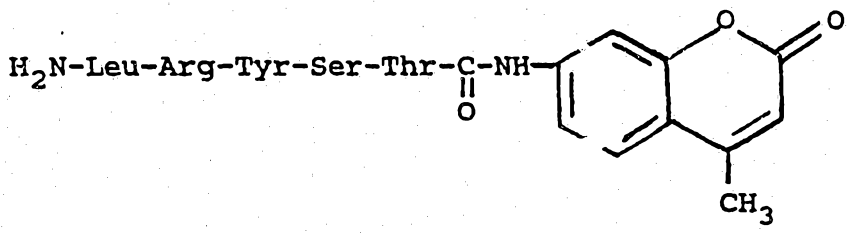
The decapeptide substrate

H_2N-Leu-Arg-Tyr-Ser-Thr-Gly-Thr-Val-Ser-Gly-CO_2H

(2.7 μM) is incubated with the catalytic antibodies produced by the procedure outlined above and the reaction monitored by reverse phase HPLC analysis of the mixture. The reaction is carried out at pH values optimal for high K_{cat} by the catalytic antibody (optimum pH is determined employing the chromogenic p-nitroanilide substrate



or the fluorescent coumarin substrate



and taking into account the binding energy of the catalytic antibody for the residues on the C-terminal side of the cleavage site with change in pH).

Antibodies that show the best K_{cat} values for cleavage of the decapeptide substrate are tested for their ability to inhibit human renin.

D. Binding of Anti-Transition-State Analog Antibodies to Human Renin and Inhibition of its Enzymatic Activity by Cleavage of Residues 85-86 in the "Flap" Region.

Inhibition of plasma renin activity - the ability of the catalytic antibodies to inhibit renin activity - is tested on a pool of human plasma having a high renin activity (40 ng of angiontensin I/h/mL). Plasma (25 μl) is pre-incubated with 100 μl of the catalytic antibody in PBS (pH 7.5) containing 1% EDTA (final volume 0.2 mL) for various periods of time. Next, an excess of plasma renin substrate (200 pmol) is added in order to ensure zero-order behavior, and the mixture is incubated for 30 min. at 37°C in PBS (pH 5.7). The final dilution of the catalytic antibody is 1:5 and 1:50. The angiotensin I generated is measured by radioimmunoassay (36). A blank is included using the same dilutions of the corresponding abzymes. The amount of angiotension I generated is less than that observed with intact renin, thus indicating cleavage of residues 85-86 in the "flap" region and inhibition.

Example 21

In vivo Preparation of Immunogenic Transition-State Analog Containing Peptide from HIV gp120 Coat Protein

A. Preparation of the Immunogen

The octapeptide sequence -Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr- from HIV gp120 coat protein is important for virus interaction with the OKT4 antigen of T4 helper/inducer cells. Synthetic peptides identical or very similar in sequence to this octapeptide strongly inhibit attachment of HIV to the antigen receptor (37). Computer assisted searches have demonstrated homology to a peptide found in the envelope region of the Epstein-Barr virus. In addition, strong homologies exist

between the HIV octapeptide and peptides which occur in human lymphoadenopathy virus (LAV) and in human T-cell leukemia virus (HTLV-IIIb) isolates. An additional homology exists between the HIV peptide and a sequence comprising residues 19-26 of bovine pancreatic ribonuclease A (RNase A). This sequence contains the exposed subtilisin cleavage sites of RNase A between residues 20 and 21 and 21 and 22 (38).

Peptide haptens according to the invention are:

(1) -Ala-[Ser-U-Thr]-Thr-Thr-Asn-Tyr-Cys

(2) -Ala-Ser-[Thr-U-Thr]-Thr-Asn-Tyr-Cys

(3) -Ala-Ser-Thr-[Thr-U-Thr]-Asn-Tyr-Cys

(4) -Ala-Ser-Thr-Thr-[Thr-U-Asn]-Tyr-Cys

The dipeptide analogs isosteres (indicated in brackets) wherein U represents various amide bond analog arrays of atoms of the haptens of the invention (e.g., U is A in haptens of formula I and U is A-Z in haptens of formula IV) atoms as described earlier are incorporated into the peptides as described in Example 19. Each peptide hapten is coupled with keyhole limpet hemocyanin (KLH) carrier protein through the terminal cysteine residue utilizing the cross-linker m-maleimidobenzoyl-N-hydroxysuccinimide ester (39).

B. **Preparation of Monoclonal Antibodies**

BALB/C mice are immunized with the KLH-peptide analog conjugates emulsified in complete Freund's adjuvant. A blood sample is obtained from each mouse and the serum separated by centrifugation and stored at 4°C. Sera obtained in this way are screened for binding activity to the original transition-state analog immunogen by standard ELISA procedures. Antibody producing mice immunized as described above and assayed for reactivity with the transition state analog peptide

immunogens are sacrificed and their spleens removed and hybidoma cells are prepared using SP2/0 myeloma cells as described in Example 19B above.

C. Screening Hybridoma Cells Producing Catalytic Monoclonal Antibodies

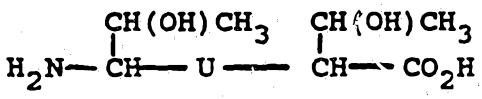
Screening for binding of antibodies to respective transition state analog-containing peptides is performed essentially as described in Example 6 above. Hybridomas secreting monoclonal antibodies and giving a positive binding reaction are chosen for further study. IgG is purified from ascites fluid by HPLC with Bakerbond AB_xHPLC.

Example 22

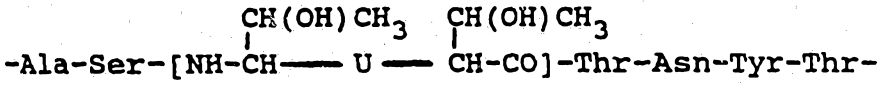
In vitro Elicitation of Catalytic Monoclonal Antibodies Against A Viral Epitope That Selectively Inhibits Human Immunodeficiency Virus (HIV)

A. Preparation of the Immunogen

The dipeptide transition state isostere,



wherein U is as hereinbefore described, is incorporated into a peptide as described in Example 19 to give the hapten



The resulting hapten is designed to mimic a portion of the HIV gp120 goat protein. The synthetic peptide is used in an in vitro immunization procedure using a

modification of a literature procedure (40). Spleen cells are cultured at 106 cell/ml in a medium consisting of 50% fresh Eagle's MEM with 20% fetal bovine serum 5 x 10^{-5}M ß-mercaptoethanol, 2mM glutamine and 50% conditioned medium from BALB/c mouse thymocytes. To furnish conditioned medium, thymocytes are cultured at 3-5 x 10^6 cells/mL in the same Eagles MEM medium as above. After 48-72 hours the medium is removed, sterilized by filtration and used immediately for in vitro activation. The antigen is added to the spleen cell culture medium at concentrations equivalent to approxamately 1 µg peptide/ml. Spleen cells are cultured in the presence of antigen for four days without a change in the medium and are later hybridized.

Hybridizidations are carried out with the mouse plasmacytoma cell line 45 6TGL.7 (41) obtained from the Cell Distribution Center of the Salk Institute. Spleen cells (either freshly isolated or from in vitro activation cultures) and plasmacytoma cells are washed twice in serin-free Eagles MEM, then fused using PEG 1000 as previously described in the literature (42). Hybrids are selected by treatment of the cultures with HAT (43). Hybrid cells which produce antibody reactive with the peptide transition state analog (as judged by an ELISA assay) are cloned and re-cloned by limiting dilution in conditioned medium from parental plasmacytoma cells.

Screening for binding of antibodies to respective transition state analog-containing peptides or the virus itself is performed essentially as described in Example 19 above. Hybridomas secreting monoclonal antibodies giving a positive binding reaction are chosen for further study. IgG is purified from ascites fluid by HPLC with Bakerbond ABxHPLC. One of ordinary skill in

the art will realize that these antibodies can be tested against the peptide not containing the transition state analog.

The virus replication assay is carried out essentially as described in the literature (44), except that cultures are propagated in microtube wells containing 200 μL. Graded concentrations of purified antibodies obtained from the in-vitro immunization procedure or buffer alone, each in 25 μL, are preincubated for 1 hr. at 37°C in 5% CO2 with 50 TCID50 HTLV-IIIB in 25 μL. Following preincubation, H9 cells (1×10^5 cells in 150 μL RPM1-040 supplemented with 20% heat-inactivated FCS) are added to the wells, yielding final antibody concentrations ranging from 0.1 μgm1-1 to 10 μg ml^{-1}. Microtiter plates are incubated at 37°C in 5% CO_2 for 14 days. Cells are fed by exchanging 100 μL cell-free supernatant fluid on days 3, 7 and 10 with fresh medium, and no further antibody is added during this period. Cell-free supernatant fluid (100 μL) is analyzed for p24 antigen by RIA (Dupont, NEK-040). Since the amount of p24 correlates with the degree of infection and replication of the virus, those wells treated with catalytic antibodies having significant decreases in p24 when compared to virus treated with control antibodies demonstrate the inhibition resulting from cleavage of gp120 by the catalytic antibody.

The C8166 fusion assay is described in the literature (45). Three monoclonal antibodies (clone AHIV1.6; AHIV1.3 and AHIV2.0) are tested in 2 hr. assays. H9 cells (1×10^4) infected chronically with HTLV-IIIB are preincubated with varying concentrations of antibody in 150 μL medium in 96-well plates. All assays are done in triplicate. After 1 hr. incubation at 37°C in 5% CO_2, 3×10^4 C8166 cells (HTLV-I

transformed umbilical cord lymphocytes) in 50 μL are added to the wells. Final well concentrations of antibodies are 41 μgml^{-1} and 5 μgm^{-1}. Preincubation with OKT4A (Ortho Diognostics) at 25 μgml^{-1} served as a control. After the plates are incubated for 2 hrs. at 37°C in 5% CO_2, syncytia (ballooning cytoplasm greater than three lymphocyte cell diameters) are counted. To prevent bias during counting, samples are coded.

The antiviral activity of clones AHIV 1.3, AHIV 1.6 and AHIV 2.0 is examined in HIV-I replication and cell fusion assays. Fig. 27 shows the dose dependent inhibition by clone AHIV 1.3 of HIV-1 p. 24 gag production in infected H9 cells. Fig. 28 shows the dose dependent inhibition by clones AHIV 1.3, AHIV 1.6 and AHIV 2.0 of HIV-induced cell fusion.

The catalytic monoclonal antibodies elicited by in vitro immunization with the transition state dipeptide isostere analog incorporated into the exposed peptide sequence of HIV gp120 can prevent infection by HIV virus by causing rupture of an important region of the viral coprotein involved in binding to the CD4 receptor on lymphocytes. The catalytic monoclonal antibodies break the peptide bond in the chosen sequence (i.e., between the first two threonine residues from the left of the octapeptide shown in Example 20) in a manner analogous to the action of proteolytic enzymes. The mechanism of the antibody catalyzed hydrolysis of the octapeptide or gp120 does not involve a metal ion and consequently may involve either a nucleophile in the active site of the antibody or nucleophilic addition of water activated by amino acid residues in the antibody combining site.

Example 23

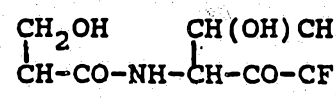
Site Specific Cleavage of the Threonine-Glycine Bond, Residues 9 and 10, in a Tetradecapeptide Containing Two Other Threonine-Glycine Bonds, Residues 4, 5 and 12, 13

In this example, catalytic antibodies are elicited for the purpose of cleaving the threonine-glycine bond at residues 9 and 10 in a tetradecapeptide containing two other threonine-glycine bonds, one at residues 4 and 5 and the other at residues 12 and 13.

Catalytic antibodies are elicited by the methodology described in Example 19 to the tetradecapeptide immunogen:

Ala-Val-Leu-Thr_4-Gly_5-Ala-Val-[Ser-Thr_9-T.S.-Gly_{10}]-Tyr-$Thr_{12}Gly_{13}$-Val.

The tripeptide transition state analog isostere,



H_2N CH-CO-NH-CH-CO-$CF_2CH_2CO_2H$, represented in the immunogen by [Ser-Thr-T.S.-Gly] is incorporated into the assembled peptide through its anhydride, as described in Example 19, to yield the tetradecapeptide immunogen shown above. Antibodies isolated from ascites fluid by AB_xHPLC chromatography and identified as being specific for the tripeptide transition state analog isostere in the tetradecapeptide immunogen are incubated for approximately one hour at 37°C with the tetradecapeptide substate:

Ala-Val-Leu-Thr-Gly-Ala-Val-Ser-Thr-Gly-Tyr-Thr-Gly-Val.

The reaction mixture is then analyzed by HPLC. Acetic acid (3M) is added and the samples are centrifuged for 10 minutes. An aliquot of the resulting supenatant is analyzed by HPLC using a Vydac C- 18 analytical column. Absorbance at 214 nm is monitored and fractions are collected. Resultant peptide fractions are

analyzed by standard amino acid analysis and sequencing. Analysis of the cleavage products from the action of the catalytic anti-transition-state analog antibodies shows that two peptide fragments, a nonapeptide, H_2N-Ala-Val-Leu-Thr-Gly-Ala-Val-Ser- Thr-CO_2H, and a pentapeptide, H_2N-Gly-Tyr-Thr-Gly-Val-CO_2H result. No cleavage is seen at the other Thr-Gly residues in the original tetradecapeptide substrate indicating the extreme specificity of the antibody to only the site of introduction of the tripeptide transition state analog isostere of the invention.

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WE CLAIM:

1. A method for catalyzing the cleavage or formation of a specific amide, ester or glycosidic bond within a molecule which comprises contacting said molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of said amide, ester or glycosidic bond under conditions suitable for said cleavage or formation to take place, said monoclonal antibody having been prepared by a process comprising the steps of:

(a) selecting the specific amide, ester or glycosidic bond to be cleaved or formed;

(b) selecting an antigen comprising an analog of said amide, ester or glycosidic bond to be cleaved or formed, and also comprising moieties surrounding said analog of said amide, ester or glycosidic bond, said moieties substantially corresponding to some or all of the moieties surrounding the amide, ester or glycosidic bond to be cleaved or formed;

(c) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(d) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(e) screening said plurality of monoclonal antibodies to identify a monoclonal anitbody which catalyzes the cleavage or formation of said amide, ester or glycosidic bond.

2. A method for catalyzing the cleavage or formation of a specific amide or ester bond within a specific amino acid sequence contained in a molecule which comprises contacting said molecule with an amount

of a monoclonal antibody effective to catalyze the cleavage or formation of said amide or ester bond under conditions suitable for said cleavage or formation to take place, said monoclonal antibody having been prepared by a process comprising the steps of:

(a) selecting the specific amide or ester bond to be cleaved or formed;

(b) selecting an antigen comprising an analog of said amide or ester bond to be cleaved or formed, and also comprising moieties surrounding said analog of said amide or ester bond, said moieties substantially corresponding to some or all of the moieties surrounding the amide or ester bond to be cleaved or formed;

(c) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(d) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(e) screening said plurality of monoclonal antibodies to identify a monoclonal anitbody which catalyzes the cleavage or formation of said amide or ester bond.

3. A method for catalyzing the cleavage or formation of a specific amide or ester bond within a specific amino acid sequence contained in a molecule which comprises contacting said molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of said amide or ester bond under conditions suitable for said cleavage or formation to take place, said monoclonal antibody having been prepared by a process comprising the steps of:

(a) selecting the specific amide or ester bond to be cleaved or formed;

(b) selecting an antigen comprising a dipeptide analog which (i) mimics one or more high energy intermediates or transition states in the cleavage or formation of said amide or ester bond or (ii) mimics one or more high energy conformations of said amide or ester bond or (iii) mimics both one or more high energy intermediates or transition states in the cleavage or formation of said amide or ester bond and one or more high energy conformations of said amide or ester bond, and said antigen also comprising moieties surrounding the dipeptide analog which substantially correspond to some or all of the moieties surrounding the amide or ester bond to be cleaved or formed;

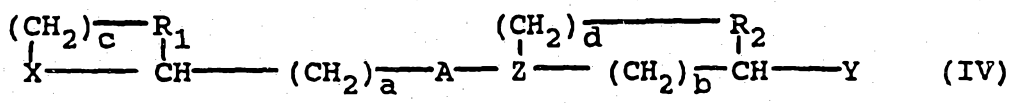
(c) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(d) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(e) screening said plurality of monoclonal antibodies to identify a monoclonal anitbody which catalyzes the cleavage or formation of said amide or ester bond.

4. An immunogen comprising:

(a) a hapten of formula IV



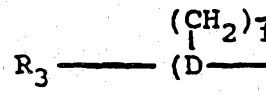
(IV)

or a physiologically acceptable salt thereof, wherein:

a and b are the same or different and each is an integer from 0 to 10;

c and d are the same or different and each is 0 or 2;

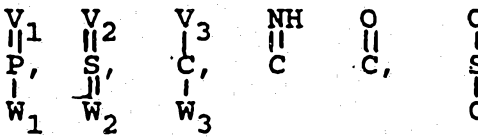
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



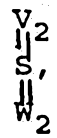
provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

A is  or  ;

Z is O, NH, CH_2, or S when A is , provided that

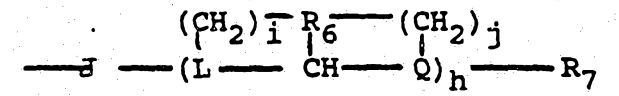
Z is N or CH when d is 2; Z is O, NH or CH_2 when A is ,

provided that Z is N or CH when d is 2; Z is CH_2 or CF_2

when A is  provided that Z is CH or CF when d is 2; and

Z is NH when A is , provided that Z is N when d is 2; Z is CF_2 when A is C=O, provided that Z is CF when d is 2; and Z is O or CH_2 when A is Si or B, provided that Z is CH when d is 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1 is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S provided that when c is 2, G is N, CH or CF;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

V_3 is OH, NH_2, or $NHNH_2$;

W_1 is OH, NH_2, NH(C_1-C_{10})alkyl, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C1-C10) alkyl or $NHNH_2$;

W_3 is H or CH_2NH_2;

and wherein

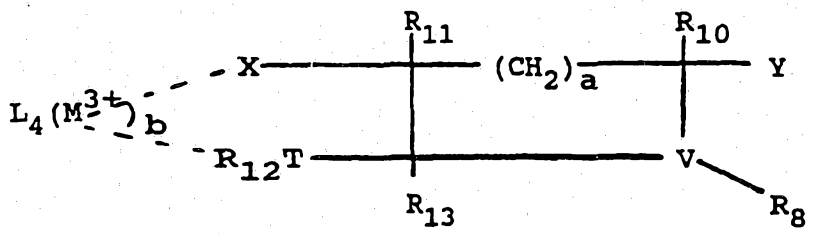
one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$, provided that if Z is O or NH when V_1 is O and W_1 is OH, then at least one of R_1, R_2, R_4 and R_6 is bound to one of said remaining substitutents R_1, R_2, R_4 and R_6.

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

5. An immunogen comprising:

(a) a hapten of formula V



(V)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

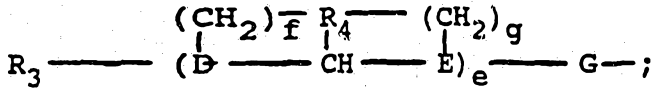
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

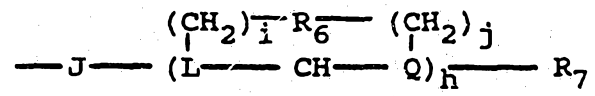
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylphenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the

aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-$_4$)alkoxy- carbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or F; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

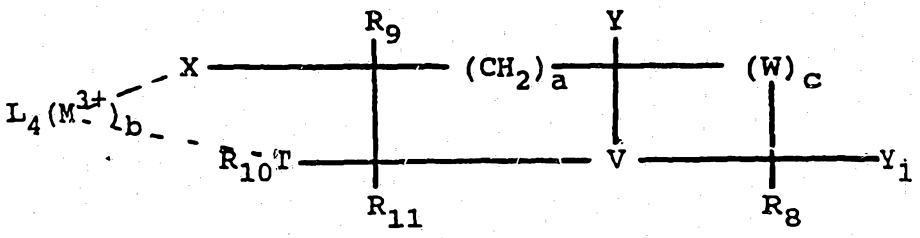
one or more of R_4, R_6, R_9, R_{10} and R_{11} are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9, R_{10} and R_{11} provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u$-S-S-$(CH_2)_v$-, $-(CH_2)_v$-, -S-$(CH_2)_v$-S-, $-(CH_2)_u$-S-$(CH_2)_v$-, $-(CH_2)_u$-CH=CH-$(CH_2)_v$-, $-(CH_2)_u$ - NH-CO-$(CH_2)_v$-, $-(CH_2)_u$-NH-$(CH_2)_v$- and $-(CH_2)_u$-phenyl-$(CH_2)_v$-; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

6. An immunogen comprising:

(a) a hapten of formula VI



(VI)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 and R_9 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

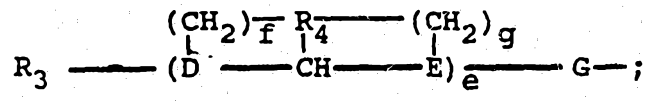
T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N,

it is directly adjacent to another CH or N and the two are joined by a double bond;

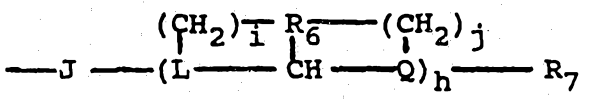
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O

or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

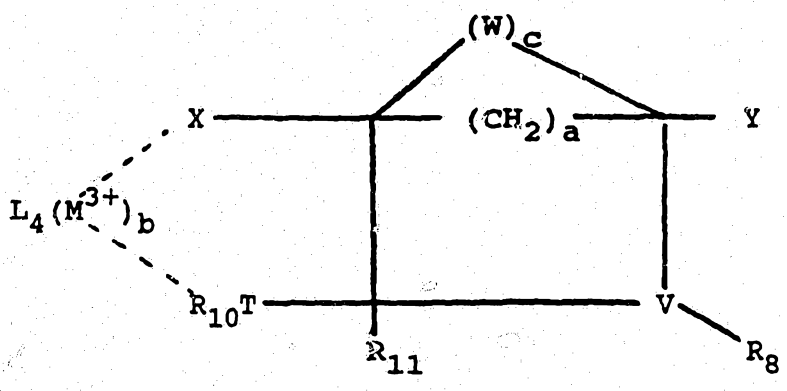
one or more of R_4, R_6, R_8 and R_9 are unbound or bound to one or more of said remaining substituents R_4, R_6, R_8 and R_9 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-phenyl-(CH_2)_v-$;

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

7. An immunogen comprising:

(a) a hapten of formula VII



(VII)

or a physiologically acceptable salt thereof wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9 is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

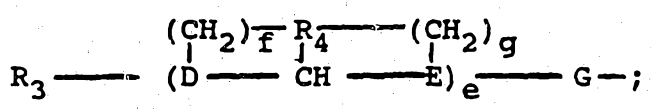
M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

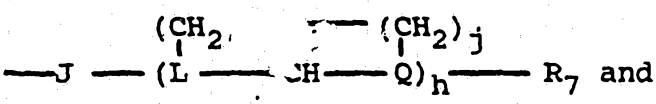
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



and Y is additionally a side chain of a naturally occurring amino acid or an analog of said side chain;

h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxy-carbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O, C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are

joined by a double bond provided that L or Q is C when i or j is 2;

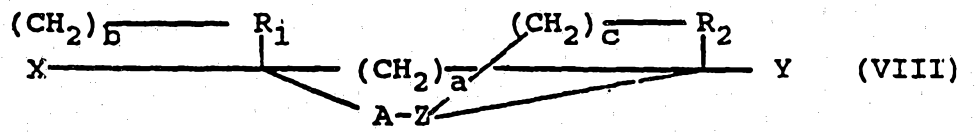
and wherein

one or more of R_4, R_6, R_9 and Y, when Y is a side chain of a naturally occurring amino acid or analog of said side chain, are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9 and Y provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-phenyl-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

8. A hapten of formula VIII



(VIII)

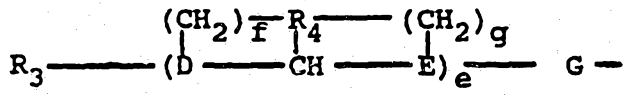
or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b and c are the same or different and each is 0 or 2;

X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the

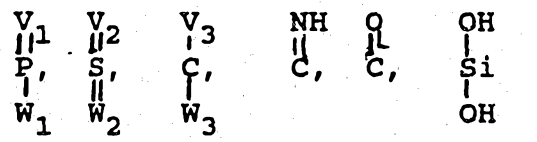
aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or

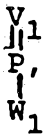


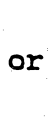
provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

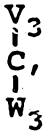
e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

A is  ;

Z is O, NH, CH_2, or S when A is , provided that

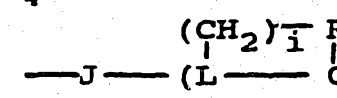
Z is N or CH when c is 2; Z is O, NH or CH_2 when A is ,

provided that Z is N or CH when c is 2; Z is CH_2 or CF_2 when A is , provided that Z is CH or CF when c is 2; and

Z is NH when A is , provided that Z is N when c is 2; Z is CF_2 when A is C=O, provided that Z is CF when c is 2; and Z is O or CH_2 when A is Si or B, provided that Z is CH when c is 2;

Y is COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups

are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1 is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen, $CONH_2$ or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f

and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O, C=S, provided that when b is 2, G is N, CH or CF;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})-alkyl or $NHNH_2$;

V_3 is OH, NH_2, or $NHNH_2$;

W_1 is OH, NH_2, NH(C_1-C_{10}) alkyl, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

W_3 is H or CH_2NH_2;

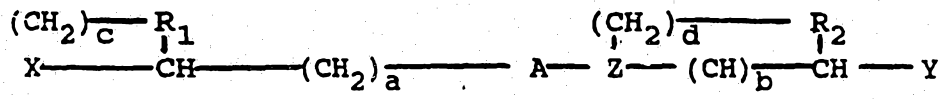
and wherein

one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of

$-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

9. A hapten of formula IX



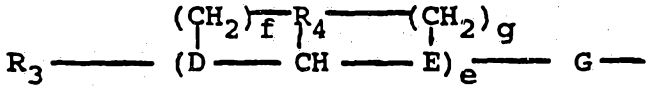
(IX)

or a physiologically acceptable salt thereof, wherein:

a and b are the same or different and each is an integer from 0 to 10;

c and d are the same or different and each is 0 or 2;

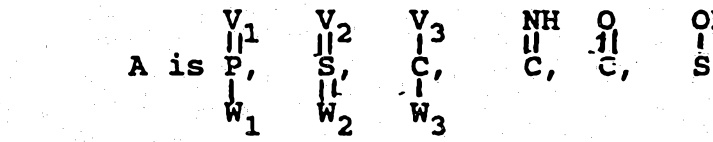
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or

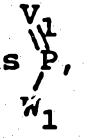


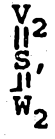
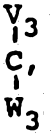
provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

e is an integer from 1 to 10;

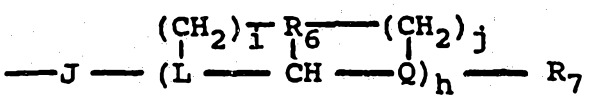
f and g are 0 or 2 provided f and g are not both 2;

A is ;

Z is O, NH, CH_2, or S when A is , provided that

Z is N or CH when d is 2; Z is O, NH or CH_2 when A is , provided that Z is N or CH when d is 2; Z is CH_2 or CF_2 when A is , provided that Z is CH or CF when d is 2; and Z is NH when A is , provided that Z is N when d is 2; Z is CF_2 when A is C=O, provided that Z is CF when d is 2; and Z is O or CH_2 when A is Si or B, provided that Z is CH when d is 2;

Y is COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1 is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen, or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S, provided that when c is 2, G is N, CH or CF;

J, NH, O, S, CH_2, CF_2, is C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

V_3 is OH, NH_2, or $NHNH_2$;

W_1 is OH, NH_2, NH(C_1-C_{10})alkyl, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

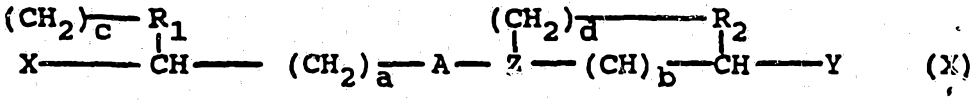
W_3 is H or CH_2NH_2;

and wherein

one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$, provided that at least one of R_1, R_2, R_4 and R_6 is bound to at least one of said remaining substituents R_1, R_2, R_4 and R_6.

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

10. A hapten of formula X



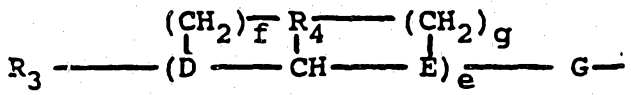
(X)

or a physiologically acceptable salt thereof, wherein:

a and b are the same or different and each is an integer from 0 to 10;

c and d are the same or different and each is 0 or 2;

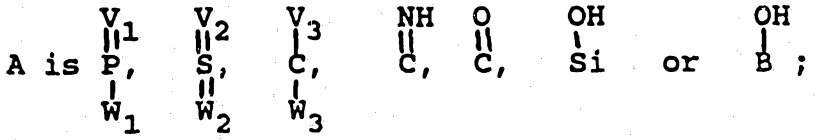
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



provided that when c is 2, X is O, S, NH or NH protected by a protecting group as defined above;

e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

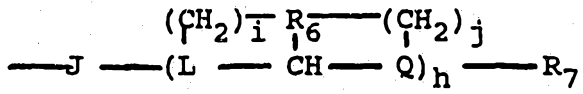


Z is O, NH, CH_2, or S when A is $\overset{V_1}{\underset{W_1}{P}}$, provided that

Z is N or CH when d is 2; Z is O, NH or CH_2 when A is $\overset{V_2}{\underset{W_2}{S}}$, provided that Z is N or CH when d is 2; Z is CH_2 or CF_2 when A is $\overset{V_3}{\underset{W_3}{C}}$, provided that Z is CH or CF when d is 2;

Z is NH when A is $\overset{NH}{\underset{}{C}}$, provided that Z is N when d is 2; Z is CF_2 when A is C=O, provided that Z is CF when d is 2; and Z is O or CH_2 when A is Si or B, provided that Z is CH when d is 2;

Y is COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_1 and R_2 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain provided that when c is 2, R_1 is CH_2 and when d is 2, R_2 is CH_2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2; D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2, provided that at least one of said D substituents is different from NH or at least one of said E substituents is different from C=O;

G is NH, O, S, CH_2, CF_2, C=O or C=S, provided that when c is 2, G is N, CH or CF;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

V_1 is O or S;

V_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

V_3 is OH, NH_2, NH(C_1-C_{10})alkyl or $NHNH_2$;

W_1 is OH, NH_2, SH, H, $NHNH_2$ or CH_2NH_2;

W_2 is O, a lone pair of electrons, NH, N(C_1-C_{10})alkyl or $NHNH_2$;

W_3 is H or CH_2NH_2;

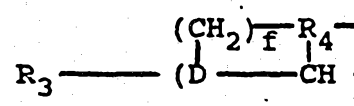
and wherein

one or more of R_1, R_2, R_4 and R_6 is unbound or bound to one or more of said remaining substituents R_1, R_2, R_4, and R_6, provided R_1 is unbound when c is 2, R_2 is unbound when d is 2, R_4 is unbound when f or g is 2 and R_6 is unbound when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$;

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

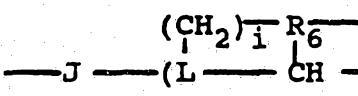
provided that when

(i) X is



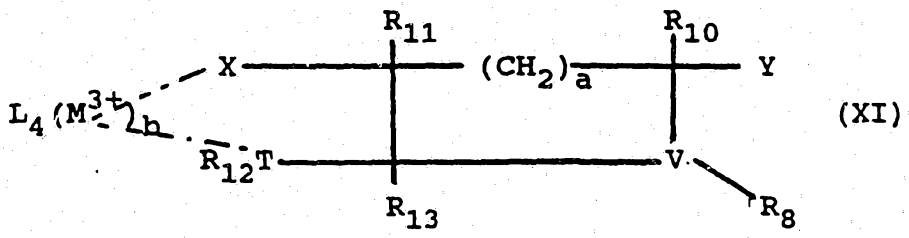
, then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

11. A hapten of formula XI



(XI)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

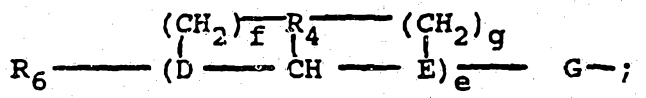
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

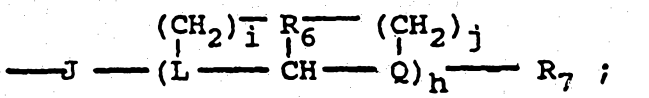
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are

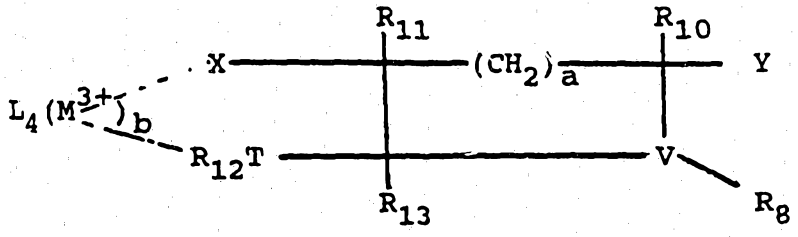
joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

one or more of R_4, R_6, R_9, R_{10} and R_{11} are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9, R_{10} and R_{11} provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-$phenyl$-(CH_2)_v-$, provided that at least one of said substituents R_4, R_6, R_9, R_{10} and R_{11} is bound to at least one of said remaining substituents R_4, R_6, R_9, R_{10} and R_{11}; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

12. A hapten of formula XII



(XII)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

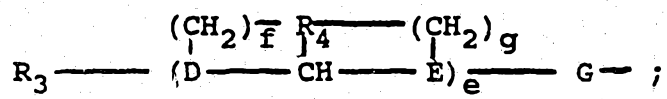
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

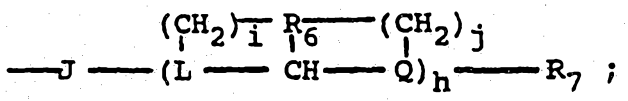
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is hydrogen or (C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or F; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

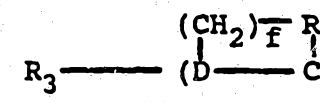
and wherein

one or more of R_4, R_6, R_9, R_{10} and R_{11} are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9, R10 and R_{11}, provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-$phenyl$-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

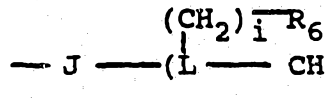
provided that when

(i) X is



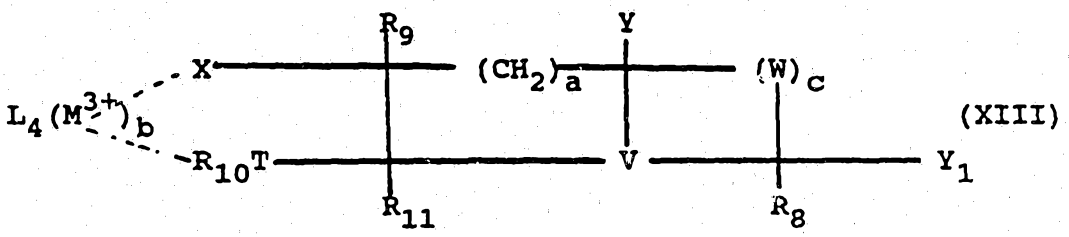
, then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

13. A hapten of formula XIII



or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 and R_9 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

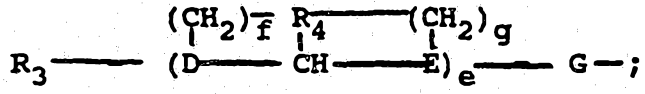
T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal

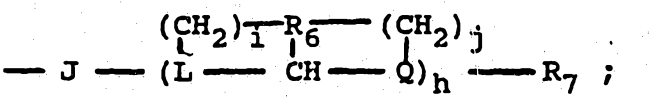
amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an

analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are O, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

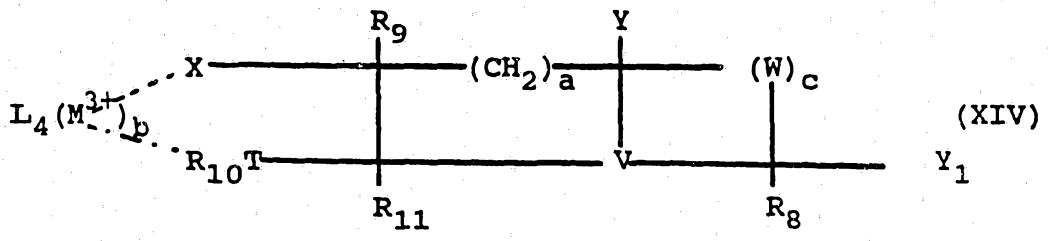
and wherein

one or more of R_4, R_6, R_8 and R_9 are unbound or bound to one or more of said remaining substituents R_4, R_6, R_8 and R_9 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said

remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-phenyl-(CH_2)_v-$, provided that at least one of said substituents R_4, R_6, R_8 and R_9 is bound to at least one of said remaining substituents R_4, R_6, R_8 and R_9; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

14. A hapten of formula XIV



(XIV)

or a physiologicly acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 and R_9 are the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

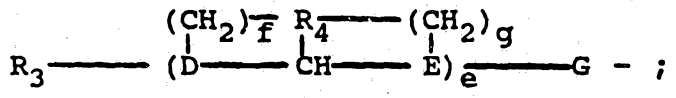
M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

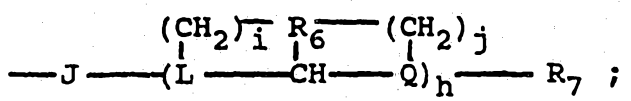
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, or OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

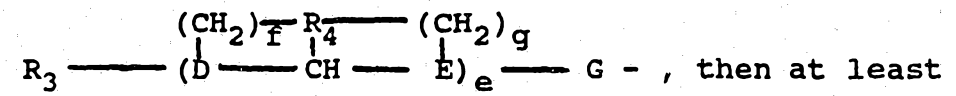
J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O

or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

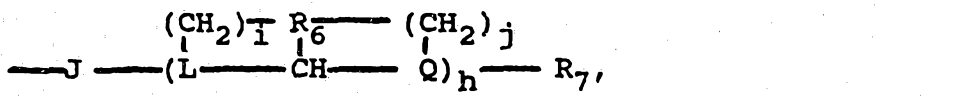
one or more of R_4, R_6, R_8 and R_9 are unbound or bound to one or more of said remaining substituents R_4, R_6, R_8 and R_9 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$, provided that at least one of said substituents R_4, R_6, R_8 and R_9 is bound to at least one of said remaining substituents R_4, R6, R8 and R_9; and provided that when

(i) X is



then at least one of said E substituents is different from C=O or at least one of said G or D substituents is different from NH; or

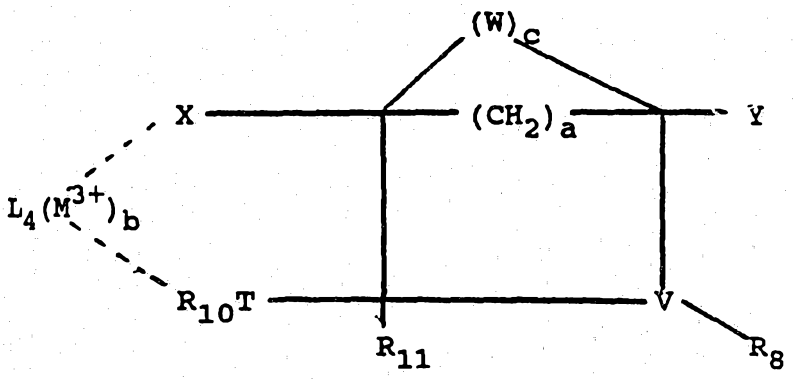
(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

15. A hapten of formula (XV)



(XV)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9 is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

X is OH, SH, NH_2, or NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, $(C_1-$

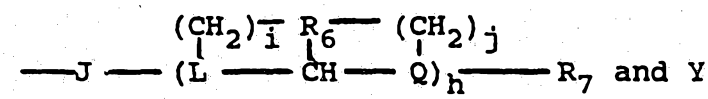
C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



and Y is additionally a side chain of a naturally occurring amino acid or an analog of said side chain;

h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

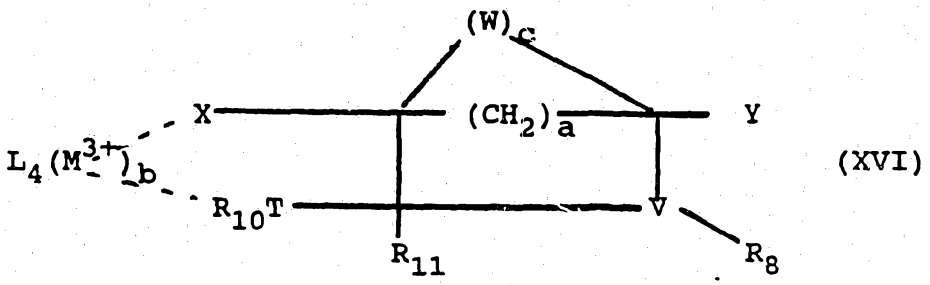
and wherein

one or more of R_4, R_6, R_9 and Y, when Y is a side chain of a naturally occurring amino acid or analog of

said side chain, are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9 and Y provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-$phenyl$-(CH_2)_v-$, provided at least one of said substituents R_4, R_6, R_9 and Y is bound to at least one of said remaining substituents R_9 and Y; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

16. A hapten of formula XVI



(XVI)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9 is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{10} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{10} is a second bond, then there is no substituent R_{11};

R_{11} is hydrogen;

L is a ligand;

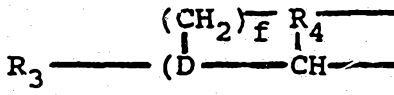
M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N, CH or CF;

W is O, S, NH, CH_2, or CF_2, and when c > 1, W in each occurrence is any of said aforementioned substituents or CH or N, provided that if W is CH or N, it is directly adjacent to another CH or N and the two are joined by a double bond;

X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



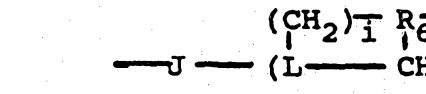
;

e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by

halogen, (C_1- C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



and Y is additionally a side chain of a naturally occurring amino acid or an analog of said side chain;

h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly

adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or F; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

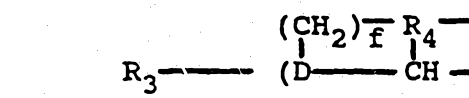
and wherein

one or more of R_4, R_6, R_9 and Y, when Y is a side chain of a naturally occurring amino acid or analog of said side chain, are unbound or bound to one or more of said remaining substituents R_4, R_6, R_9 and Y provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u$-phenyl-$(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

provided that when

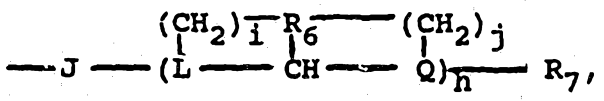
(i) X is



, then at least one of said E substituents is different from C=O or at

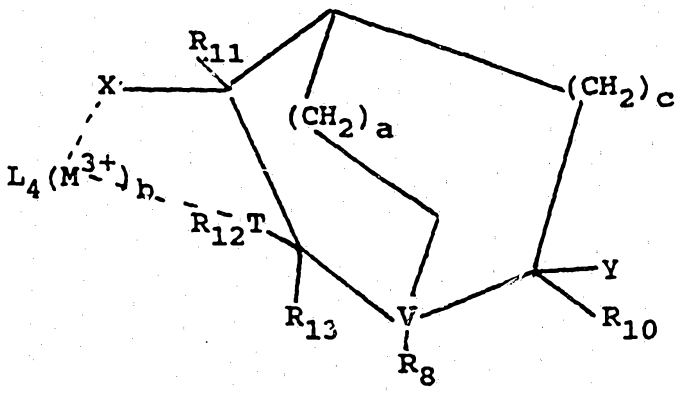
least one of said G or D substituents is different from NH; or

(ii) Y is



then at least one of said L substituents is different from NH and at least one of said J or Q substituents is different from C=O.

17. A hapten of formula XVII



(XVII)

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 1 to 10;

R_8 is hydrogen, fluorine or CHR_9Y_1;

R_9, R_{10} and R_{11} are the same or not all the same and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

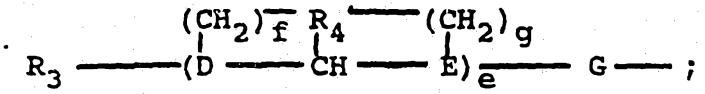
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is C or N provided that if V is N, then there is no substituent R_8;

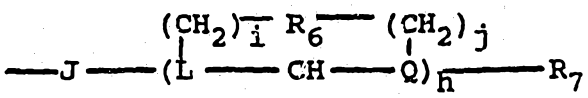
X is OH, SH, NH_2, protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y and Y_1 are the same or different and each is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is N, CH or CF; and when h > 1 and when L and Q are directly adjacent to each other, L and Q are CH or N and are

joined by a double bond provided that L or Q is C when i or j is 2;
and wherein

one or more of R_9, R_{10}, R_{11}, R_4 and R_6 are unbound or bound to one or more of said remaining substituents R_9, R_{10}, R_{11}, R_4 and R_6 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$ and $-(CH_2)_u-$phenyl$-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

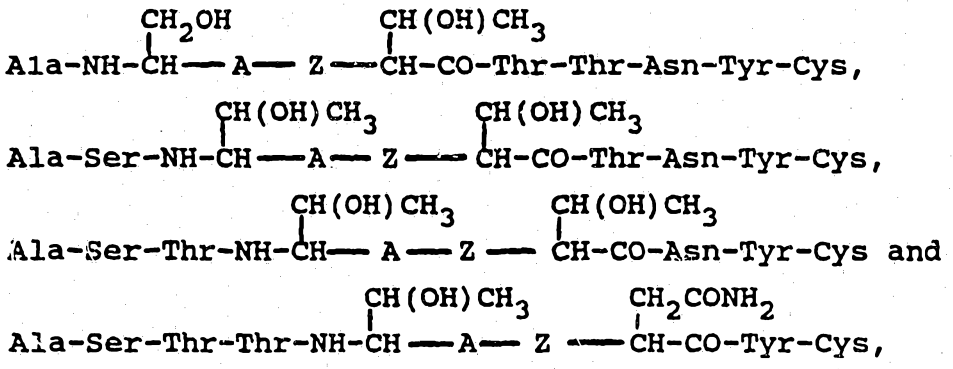
18. A hapten as claimed in claim 17 wherein R_8 is hydrogen, R_{12} is a second bond between the T atom and the carbon atom to which T is attached, Y is oxygen, a is 1, and b is 0.

19. A hapten as claimed in claim 17 wherein R_8, R_{12} and R_{13} are hydrogen; T is oxygen, a is 1 and b is 0.

20. An immunogen comprising a hapten as in claims 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 and a carrier molecule, said compound being coupled to said carrier molecule by a suitable coupling moiety.

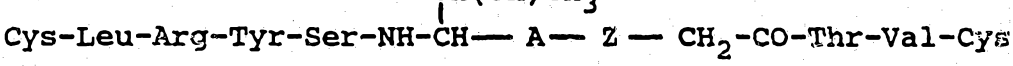
21. An immunogen as recited in claim 4, wherein the hapten is 5-(serinyl)amino 3,3-difluoro 4-oxo 6-hydroxy heptanoic acid.

22. An immunogen according to claim 4 wherein said hapten is selected from the group consisting of



wherein A and Z are as defined in claim 4.

23. An immunogen according to claim 4 wherein said hapten is



wherein A and Z are as defined in claim 4.

24. An immunogen according to claim 23 wherein said hapten has β-turn configuration mimicking the configuration of native protein wherein the sulfur atoms in the two terminal cysteine residues are joined to form a disulphide bridge.

25. A catalytic antibody elicited by an immunogen as in claims 4, 5, 6 or 7.

26. A catalytic antibody elicited by an immunogen comprising a hapten as in claims 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 and a carrier molecule said compound

being coupled to said carrier molecule by a suitable coupling moiety.

27. A catalytic antibody which can catalyze a chemical reaction of interest and which is elicited through in vitro or in vivo techniques by an antigen comprising a hapten as in any one of claims 4-17, said catalytic antibody having been prepared by a process comprising the steps of:

(a) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(b) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(c) screening said plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes said chemical reaction of interest.

28. A method for producing catalytic antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by an antigen comprising a hapten as in any one of claims 4-17, wherein said method comprises the steps of:

(a) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(b) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(c) screening said plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes said chemical reaction of interest.

29. A method for catalyzing the cleavage or formation of an amide, peptide or ester bond in a molecule comprising contacting said molecule with an effective amount of a catalytic antibody elicited by an antigen comprising a hapten as in any one of claims 4-17.

30. A method for treating acquired immune deficiency syndrome by inhibiting human immunodificiency virus which comprises treating a patent with an effective amount of a catalytic antibody elicited with a hapten of claim 22.

31. A method for treating hypertension by inhibiting human renin activity which comprises treating a patient with an effective amount of a catalytic antibody elicited with a hapten of claim 23.

32. A method for catalyzing the cleavage of myohaemerythrin between the glycine residues at positions 85 and 86 of the myohaemerythrin molecule comprising contacting said molecule with an effective amount of a catalytic antibody elicited by an antigen comprising a hapten of any one of claims 11-17.

33. A method for catalyzing the formation of a glycine-glycine bond between myohaemerythrin fragment 1-85 and myohaemerythrin fragment 86- 118 comprising contacting said molecule with an effective amount of a catalytic antibody elicited by an antigen comprising a hapten of any one of claims 11-17.

34. An immunogen as recited in claim 4, wherein the hapten is N-[N-(1-carboxy-2-phenyl)ethylaminosulfonylacetyl]glycine.

35. An immunogen as recited in claim 4, wherein the hapten is 3-(N-carbobenzyloxymethyl)sulfonyl-2-methylpropanoic acid.

36. An immunogen as recited in claim 4, wherein the hapten is 5-amino-3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid.

37. The hapten which is 5-[N-benzyloxycarbonylmethylamido]-3,3-difluoro-2-methyl-4-oxo-6-phenylhexanoic acid.

38. An immunogen as recited in claim 4, wherein the hapten is amino-(2-phenylethyl)hydroxyphosphinyl D-alanine.

39. An immunogen as recited in claim 4, wherein the hapten is amino-(2-phenylethyl)hydroxyphosphinyl D-alanine.

40. An immunogen as recited in claim 4, wherein the hapten is O-[1-amino-(2-phenylethyl)phosphinyl]lactic acid disodium salt.

41. An immunogen as recited in claim 4, wherein the hapten is 1-amino-2-phenylethyl(2-carboxy-1-propy)hydroxyphosphonous acid.

42. An immunogen as recited in claim 4, wherein the hapten is 2-amino-3-phenylpropylimino-D-alanine.

43. An immunogen as recited in claim 5, wherein the hapten is (S)-3-(benzyloxycarbonyl)amino-2-oxo-1-azetidineacetic acid.

44. An immunogen as recited in claim 5, wherein the hapten is (3'S,2R)[3-amino-2-oxo-1-azetidinyl]-3-methylbutanoic acid.

45. An immunogen as recited in claim 5, wherein the hapten is *cis*-3-(benzyloxycarbonyl)amino-4-carboxy-2-azetidinone.

46. An immunogen as recited in claim 5, wherein the hapten is 3-(benzyloxycarbonyl)amino-2-oxo-1-cyclobutaneacetic acid.

47. An immunogen as recited in claim 5, wherein the hapten is 3-(benzyloxycarbonyl)amino-2-hydroxy-1-cyclobutaneacetic acid.

48. An immunogen as recited in claim 5, wherein the hapten is 2-(benzyloxycarbonyl)amino-3-hydroxycyclobutanecarboxylate.

49. An immunogen as recited in claim 5, wherein the hapten is 2-(benzyloxycarbonyl)amino-3-oxocyclobutanecarboxylate.

50. The hapten which is 3-*exo*-(benzyloxycarbonyl)amino-2-*exo*-hydroxynorbornyl-7-*anti*-carboxylate.

51. The hapten which is 3-*exo*-(benzyloxycarbonyl)amino-2-oxonorbornyl-7-*anti*-carboxylate.

52. The hapten which is 3-*endo*-(benzyloxycarbonyl)amino-2-*endo*-hydroxynorbornyl-7-*anti*-carboxylate.

53. The hapten which is 3-*endo*-(benzyloxycarbonyl)amino-2-oxonorbornyl-7-*anti*-carboxylate.

54. The method of claim 2, wherein said amide bond is a peptide bond.

55. The method of claim 3, wherein said amide bond is a peptide bond.

56. The method of claim 3, wherein said dipeptide analog comprises a hapten as recited in any of claims 4-17.

57. An immunogen substantially as hereinbefore described with reference to any one of the Examples.

58. A hapten substantially as hereinbefore described with reference to any one of the Examples.

59. A catalytic antibody substantially as hereinbefore described with reference to any one of the Examples.

60. A catalytic antibody when prepared by the method of any one of claims 1 to 3.

Dated 17 August, 1993
Igen, Inc.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

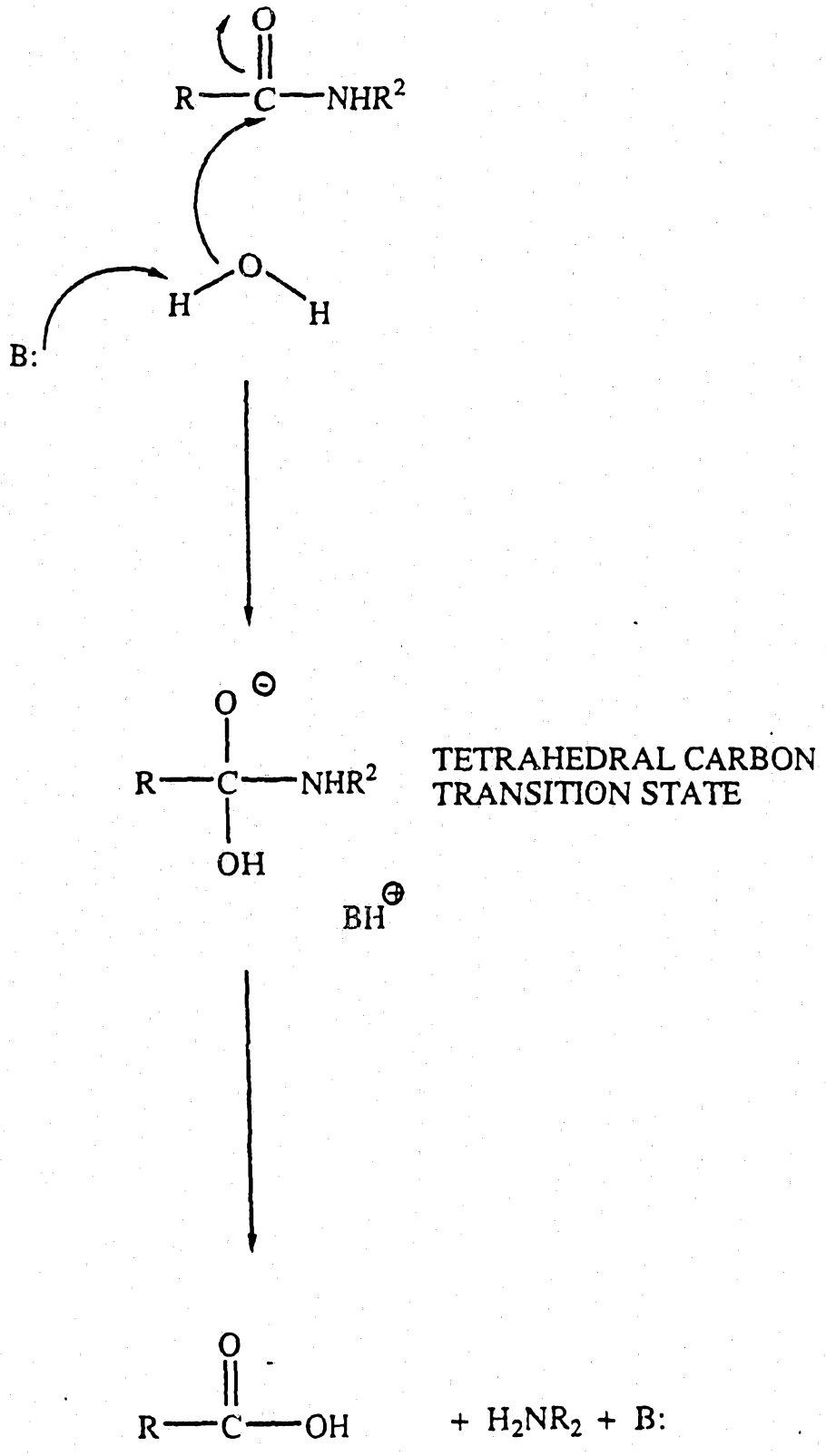

R—C—NHR2
O
H
H
B:
R—C—NHR2
O
OH
TETRAHEDRAL CARBON
TRANSITION STATE
BH⊕
R—C—OH
+ H2NR2 + B:

Fig. 1

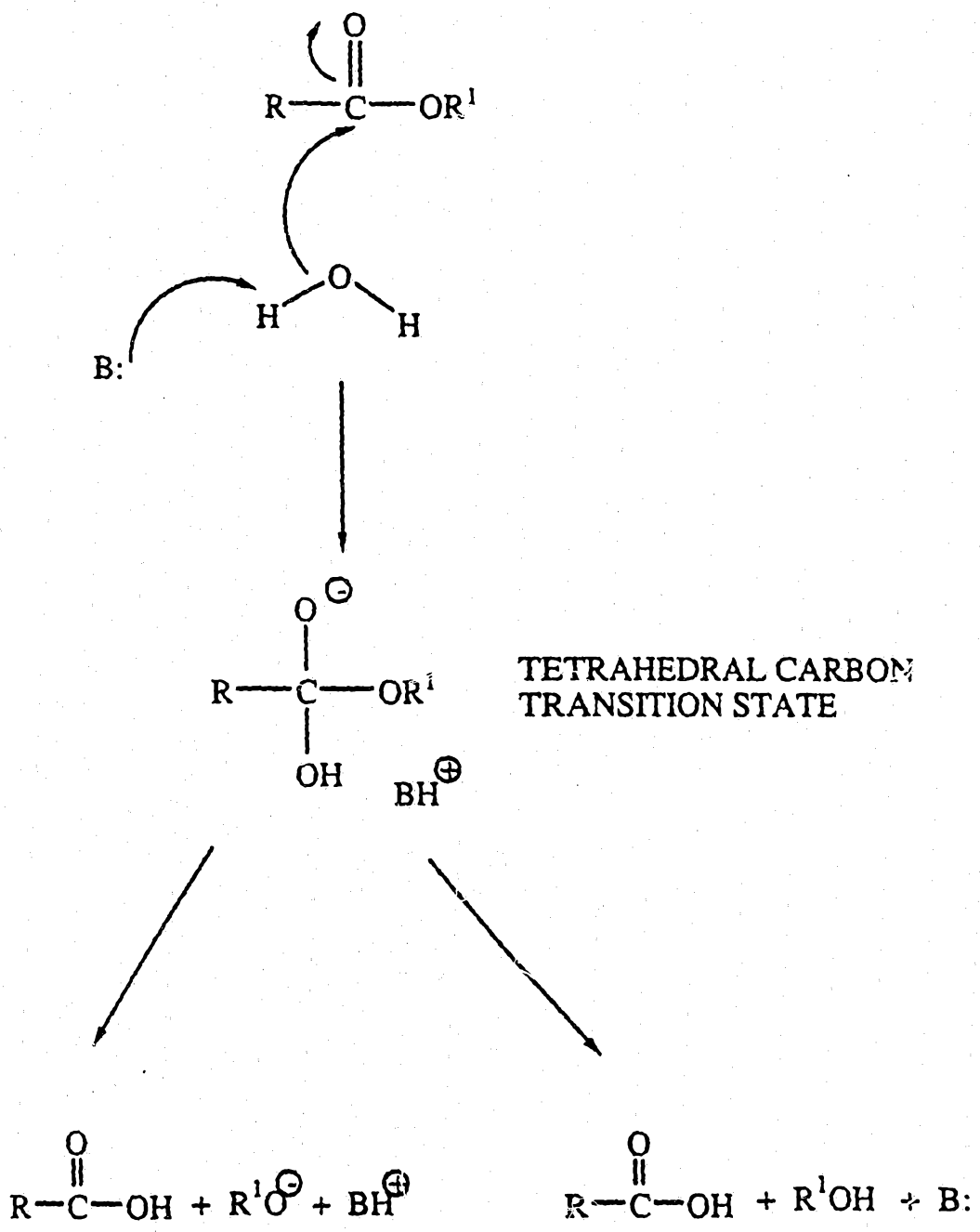
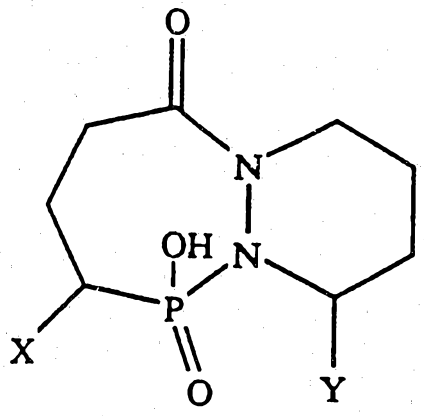
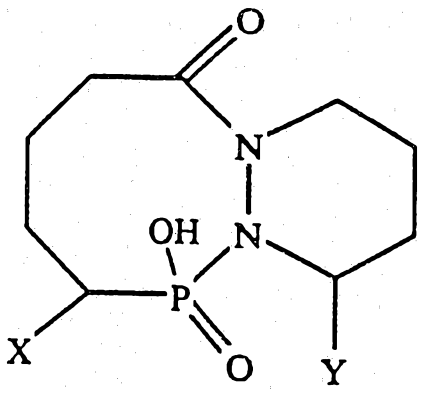

R—C—OR¹
O
B:
H
H
R—C—OR¹
O
OH
BH⊕
TETRAHEDRAL CARBON
TRANSITION STATE
R—C—OH + R¹O⊖ + BH⊕
R—C—OH + R¹OH + B:

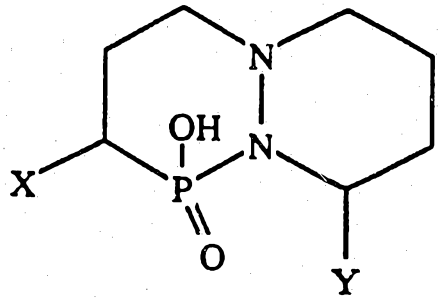
Fig. 2



(A)

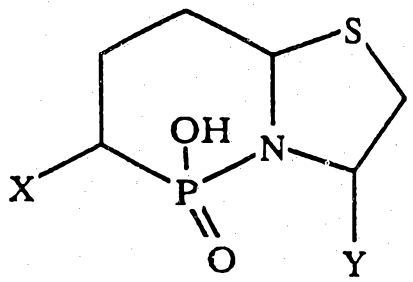


(B)

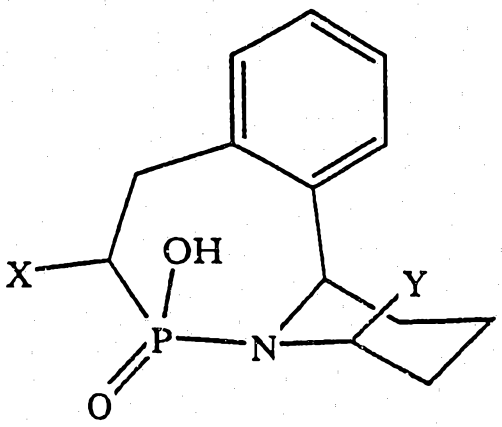


(C)

Fig. 3



(A)



(B)

Fig. 4

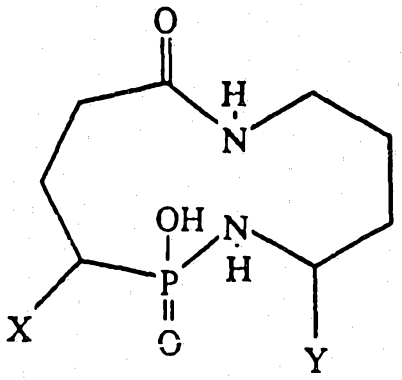


Fig. 5

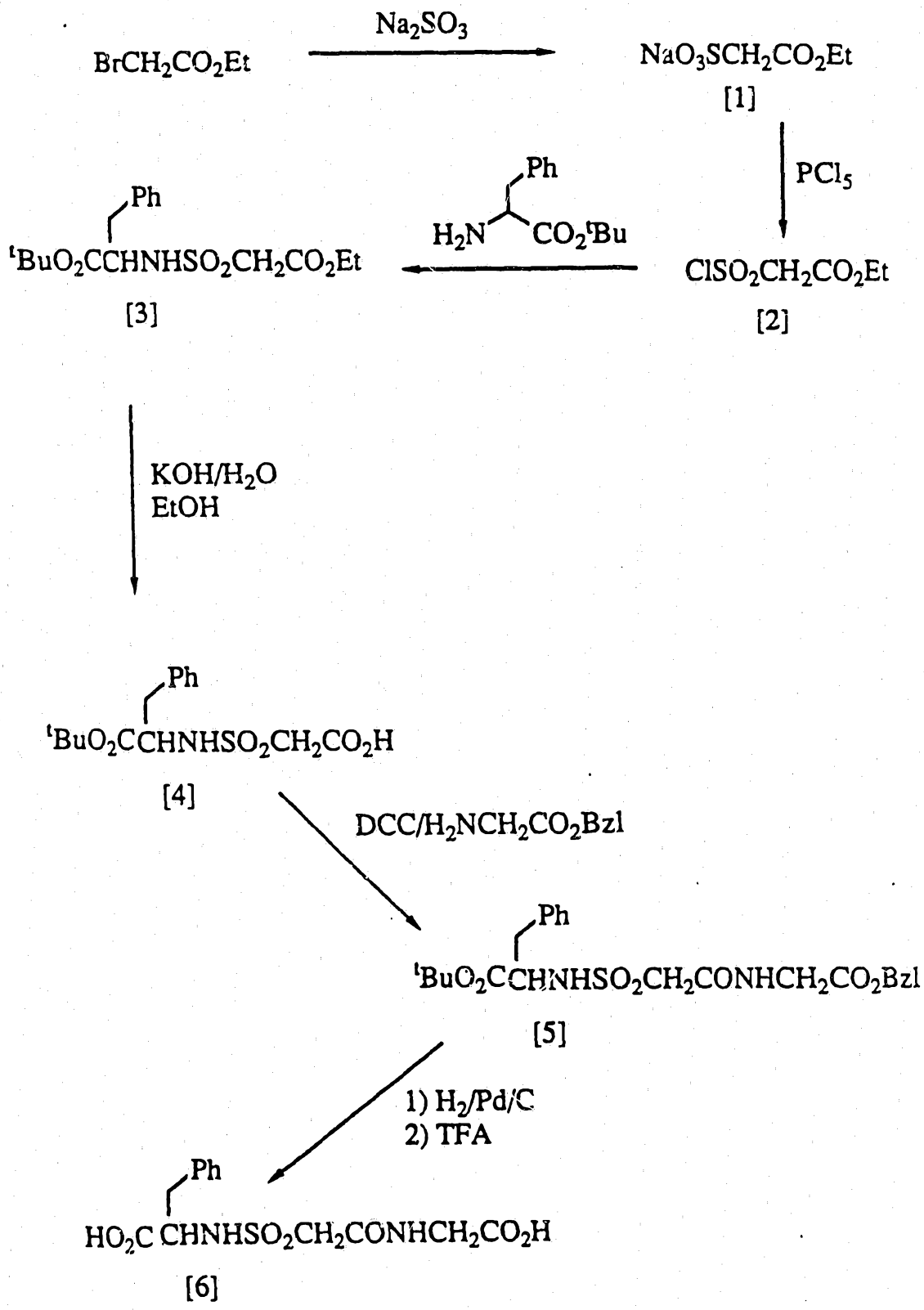

Na2SO3
BrCH2CO2Et
NaO3SCH2CO2Et
[1]
PCl5
Ph
H2N
CO2tBu
tBuO2CCHNHSO2CH2CO2Et
[3]
ClSO2CH2CO2Et
[2]
KOH/H2O
EtOH
tBuO2CCHNHSO2CH2CO2H
[4]
DCC/H2NCH2CO2Bzl
tBuO2CCHNHSO2CH2CONHCH2CO2Bzl
[5]
1) H2/Pd/C
2) TFA
HO2C CHNHSO2CH2CONHCH2CO2H
[6]

Fig. 6

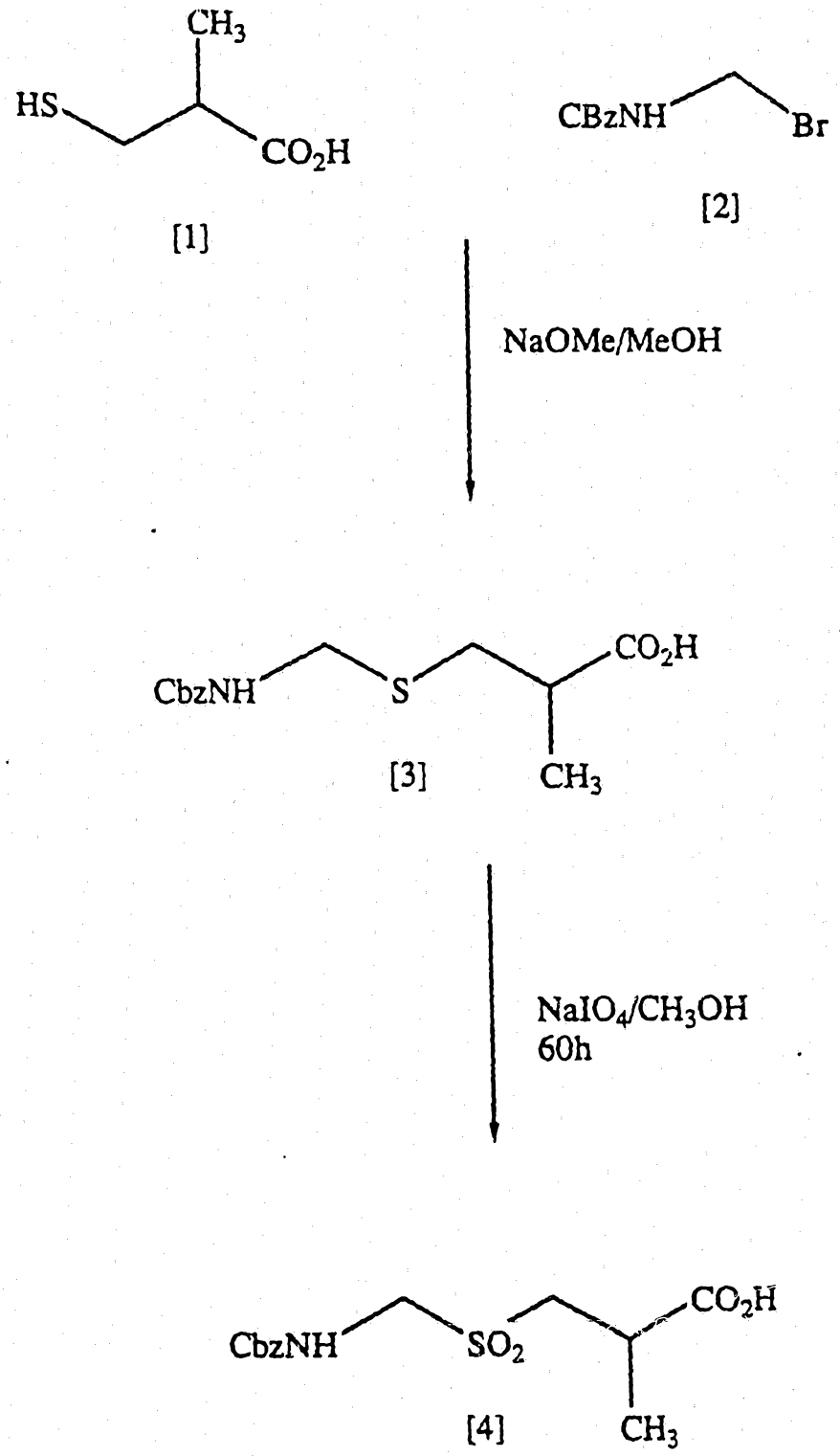

CH3
HS
CO2H
[1]
CBzNH
Br
[2]
NaOMe/MeOH
CbzNH
S
CO2H
[3]
CH3
NaIO4/CH3OH
60h
CbzNH
SO2
CO2H
[4]
CH3

Fig. 7

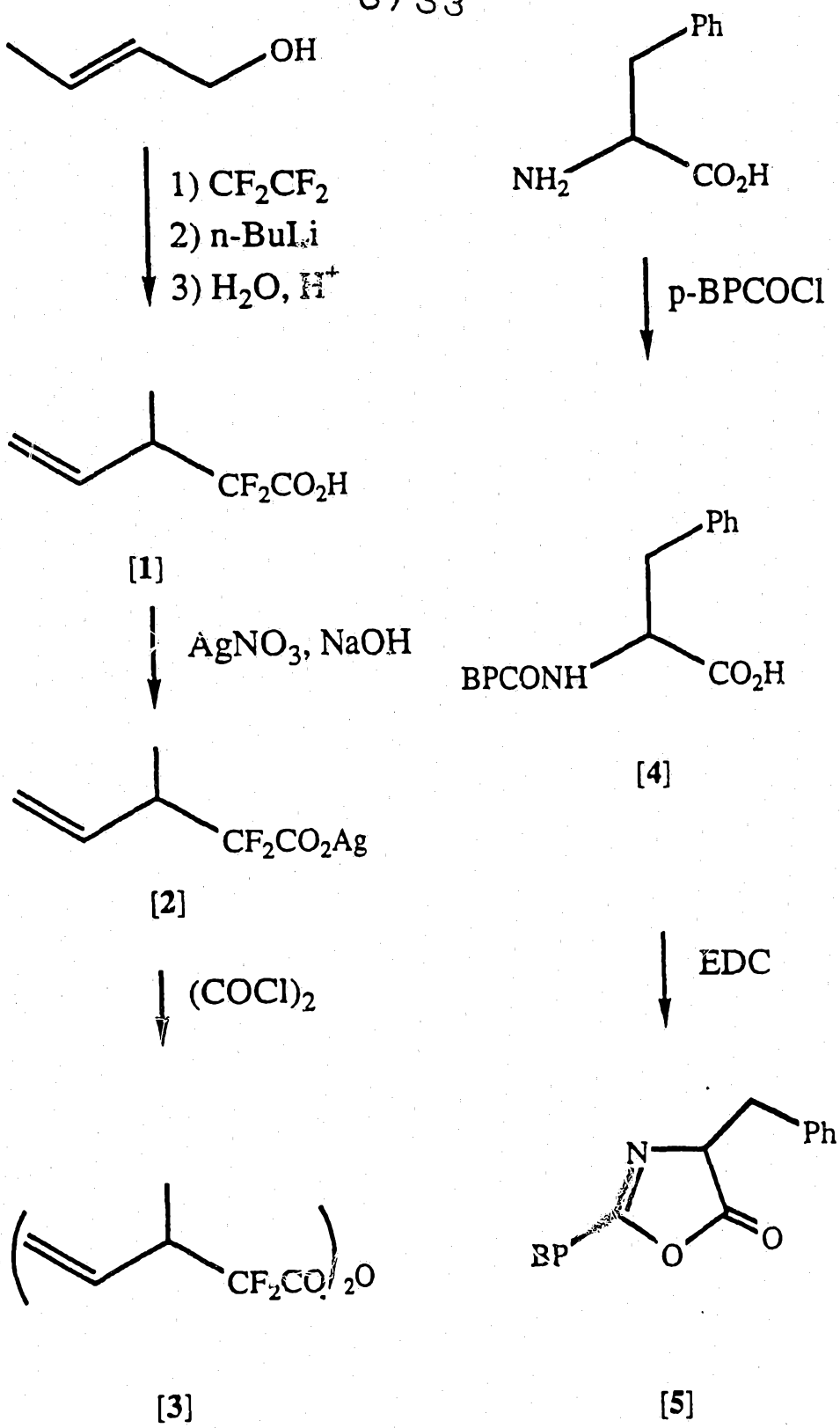

OH
1) CF_2CF_2
2) n-BuLi
3) H_2O, H^+
CF_2CO_2H
[1]
$AgNO_3$, NaOH
CF_2CO_2Ag
[2]
$(COCl)_2$
$CF_2CO)_2O$
[3]
Ph
NH_2
CO_2H
p-BPCOCl
Ph
BPCONH
CO_2H
[4]
EDC
Ph
N
BP
O
O
[5]

Fig. 8

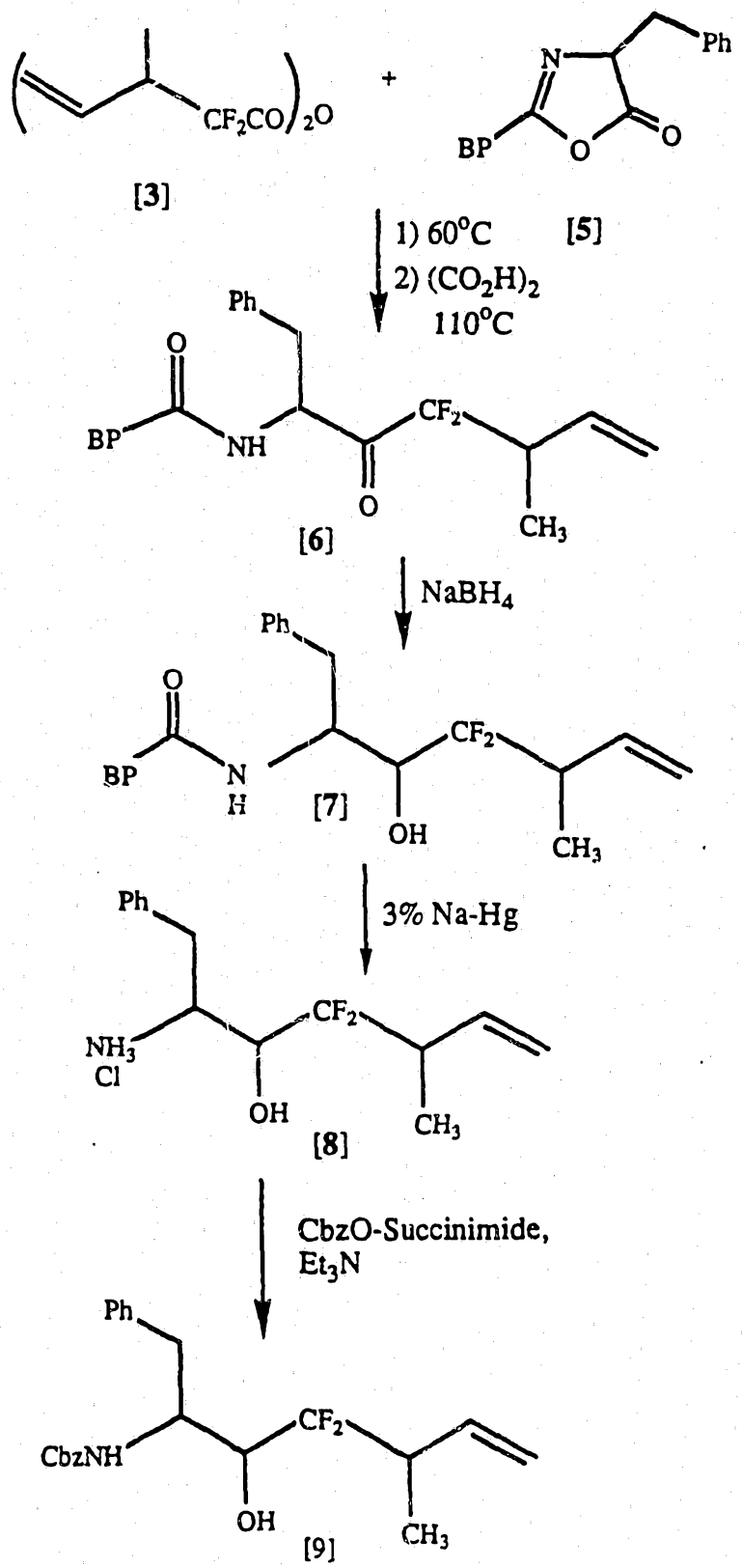


Fig. 8 (cont'd)

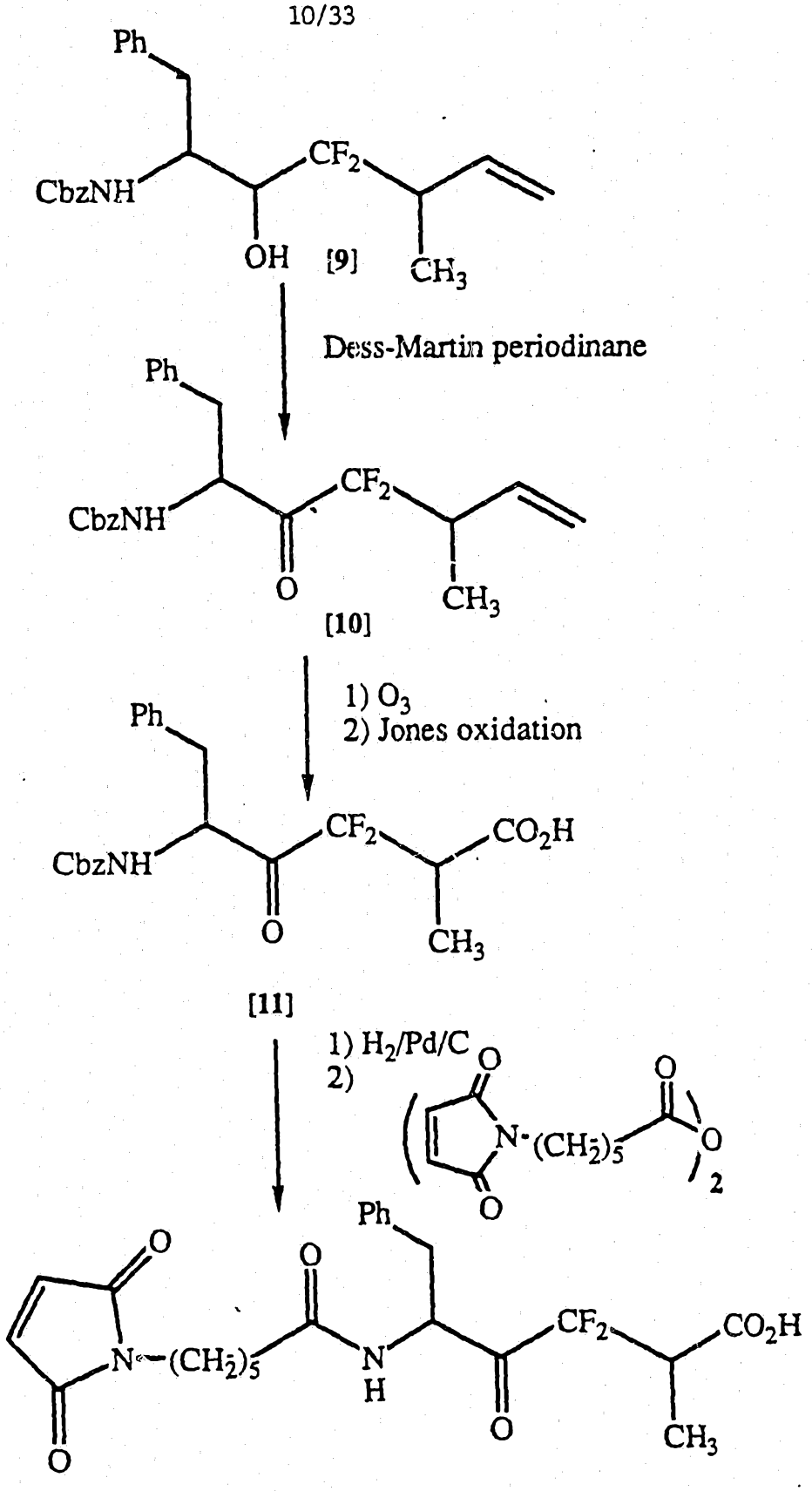

Ph
CbzNH
CF2
OH
[9]
CH3
Dess-Martin periodinane
Ph
CbzNH
CF2
O
CH3
[10]
1) O3
2) Jones oxidation
Ph
CbzNH
CF2
CO2H
O
CH3
[11]
1) H2/Pd/C
2)
O
N
(CH2)5
O
O
2
Ph
O
O
N
(CH2)5
N
H
CF2
CO2H
O
O
CH3
[12]

Fig. 8 (cont'd)

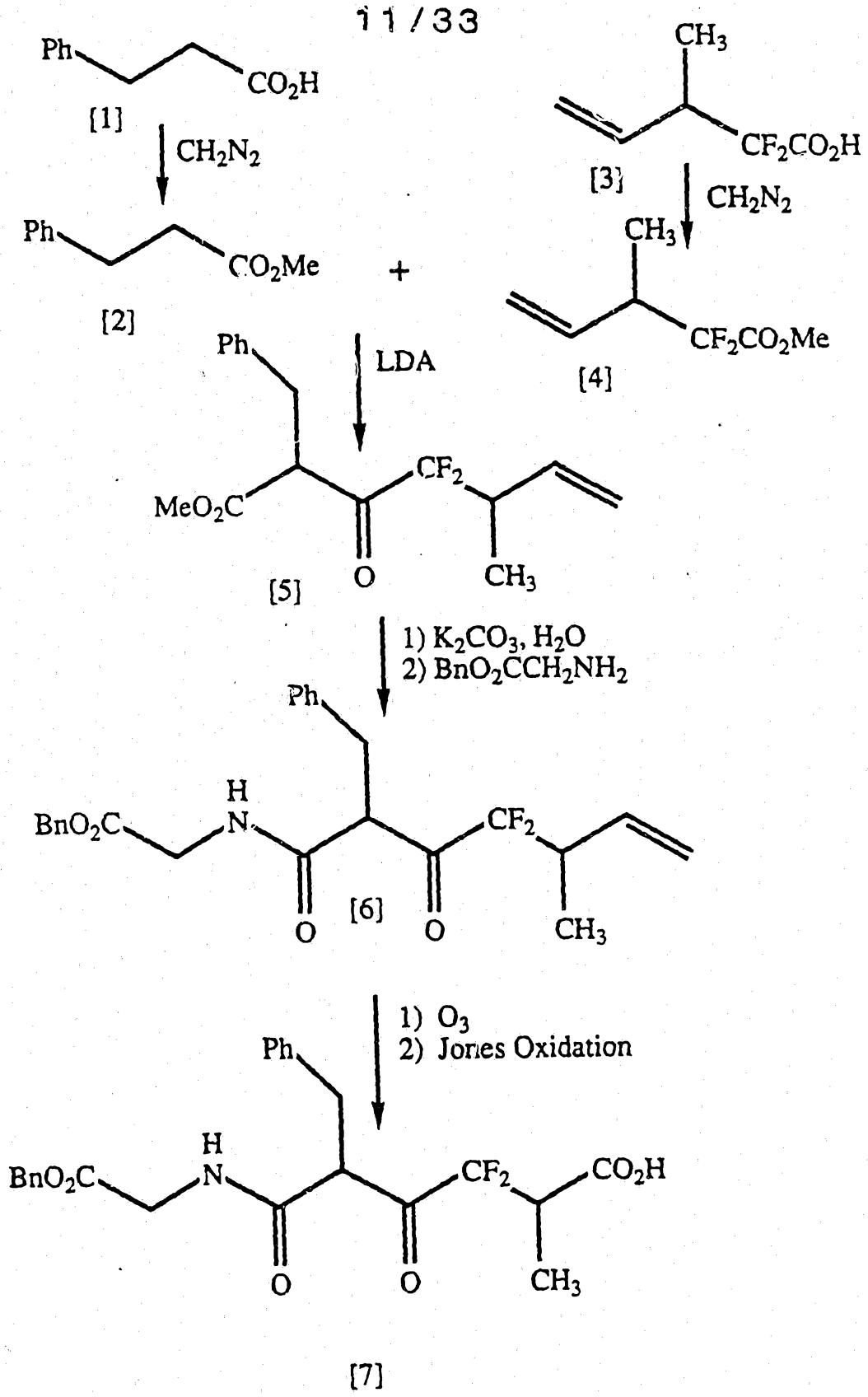

Ph
CO2H
[1]
CH2N2
Ph
CO2Me
[2]
+
CH3
CF2CO2H
[3]
CH2N2
CH3
CF2CO2Me
[4]
LDA
Ph
MeO2C
CF2
O
CH3
[5]
1) K2CO3, H2O
2) BnO2CCH2NH2
Ph
BnO2C
H
N
CF2
O
O
CH3
[6]
1) O3
2) Jones Oxidation
Ph
BnO2C
H
N
CF2
CO2H
O
O
CH3
[7]

Fig. 9

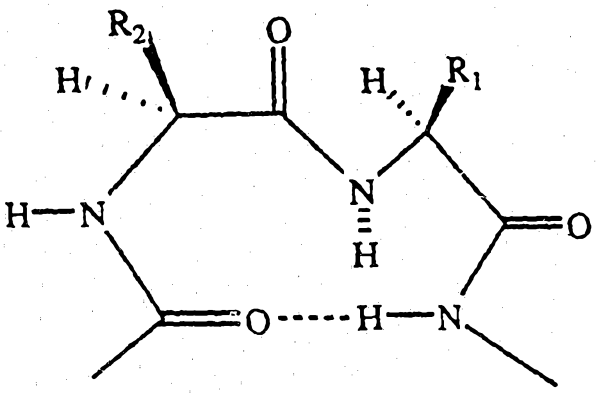


Fig. 10

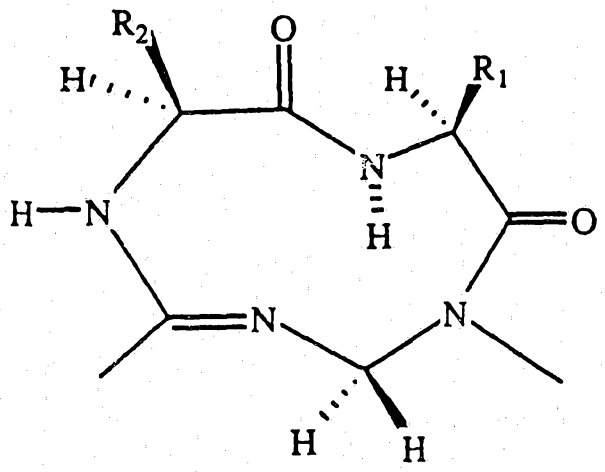


Fig. 11

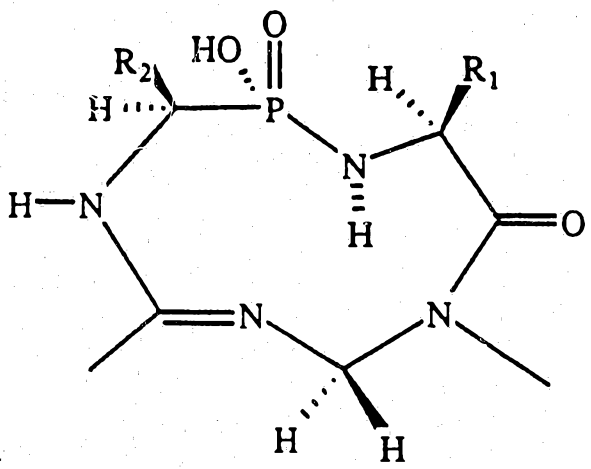
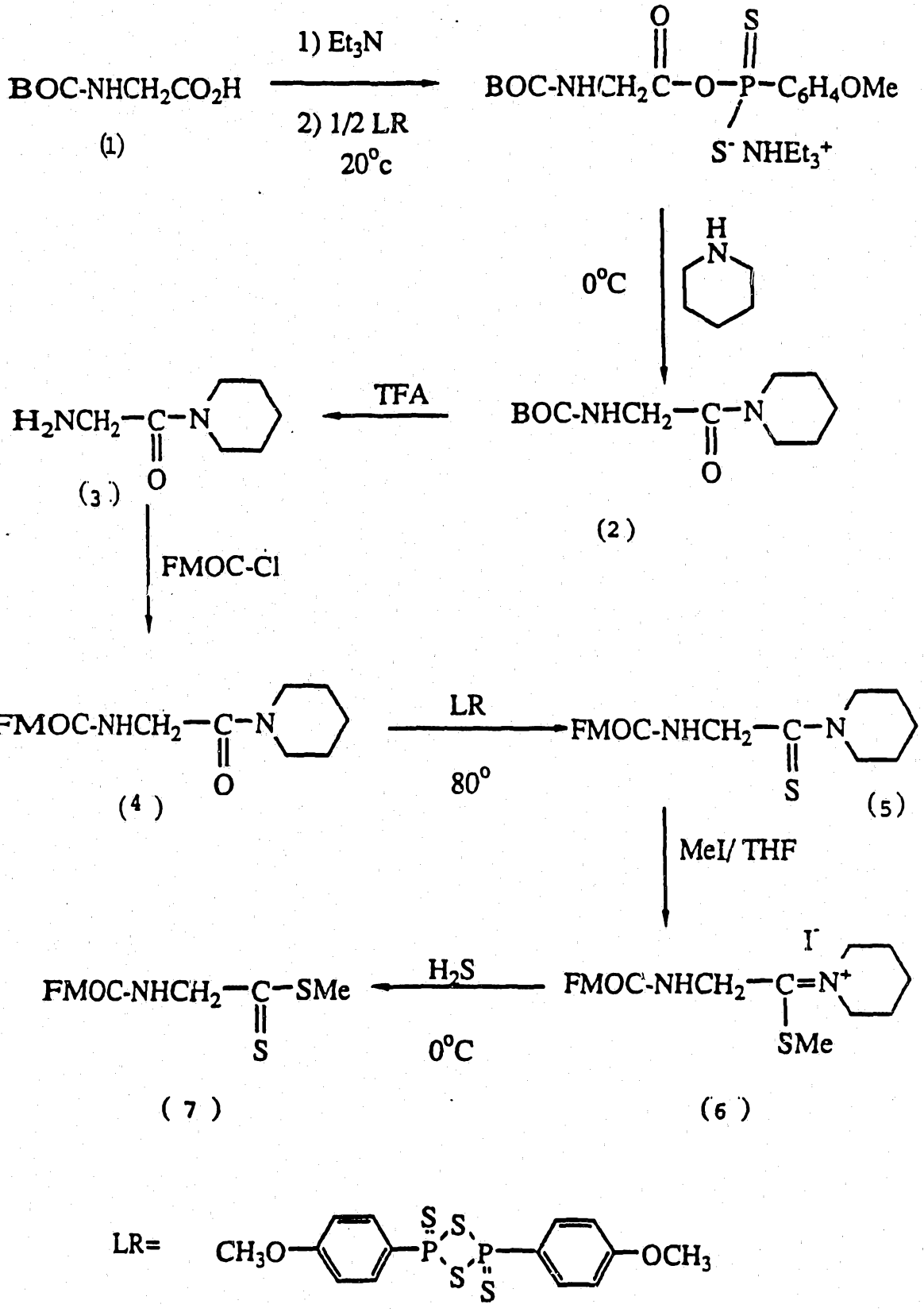


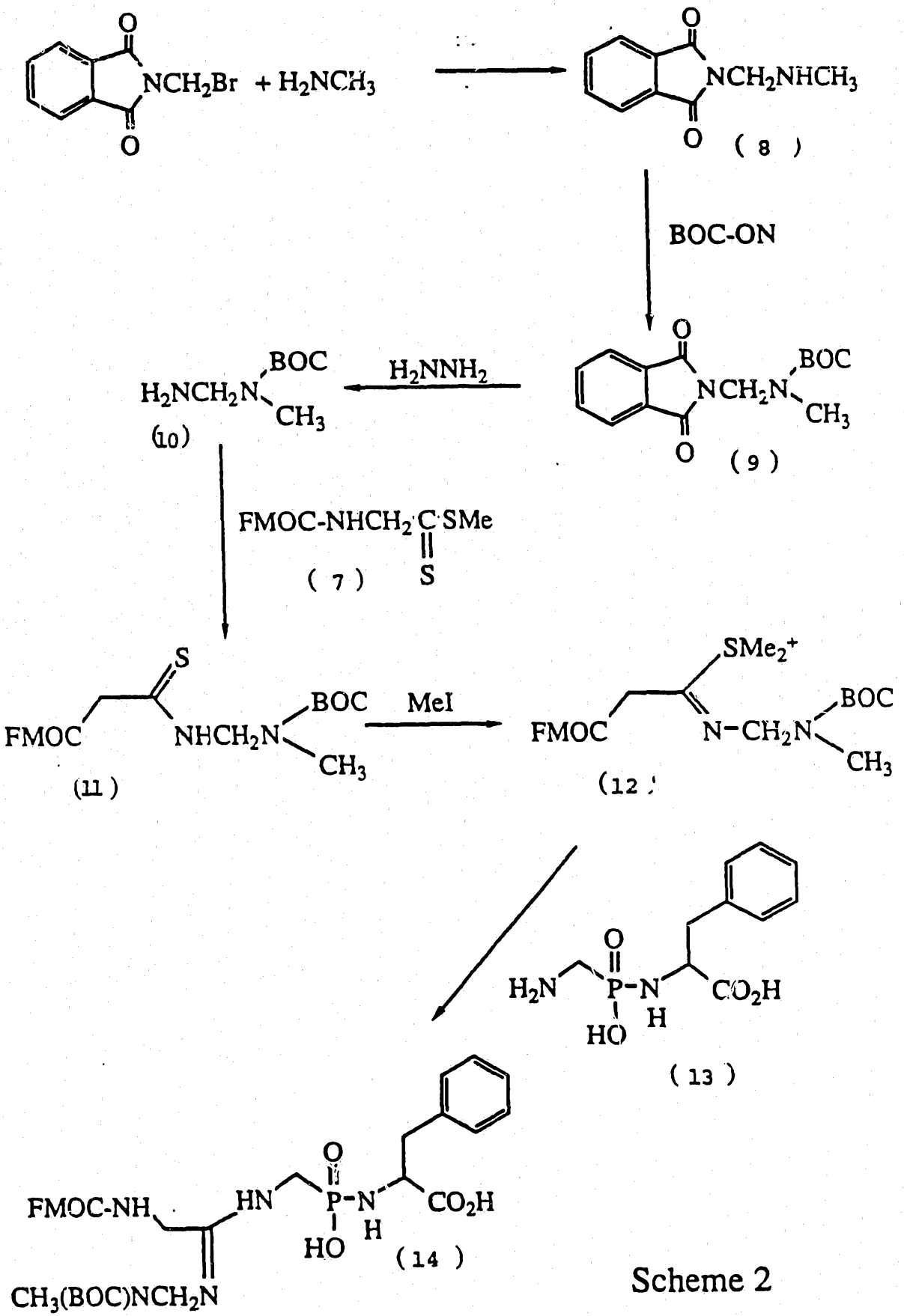
Fig. 12



LR=

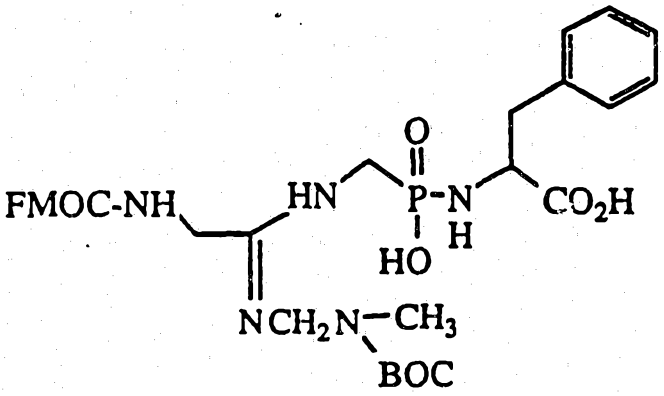
(Lawesson's reagent)

Fig. 13 Scheme 1

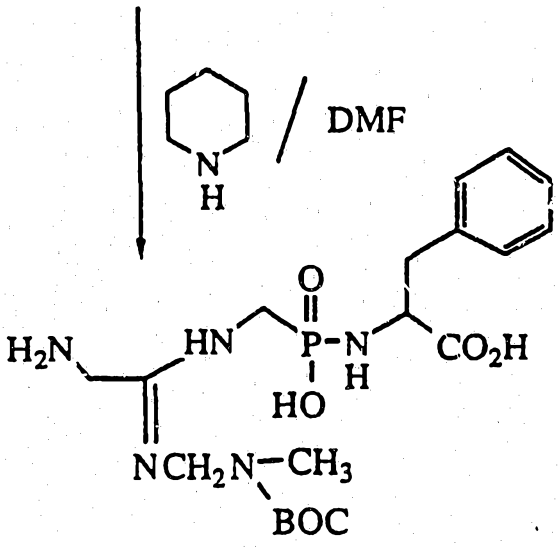


Scheme 2

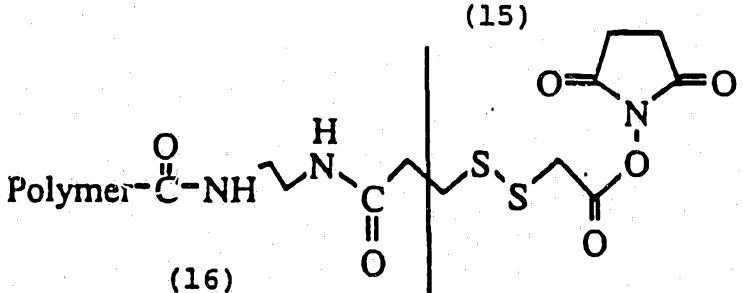
Fig. 14



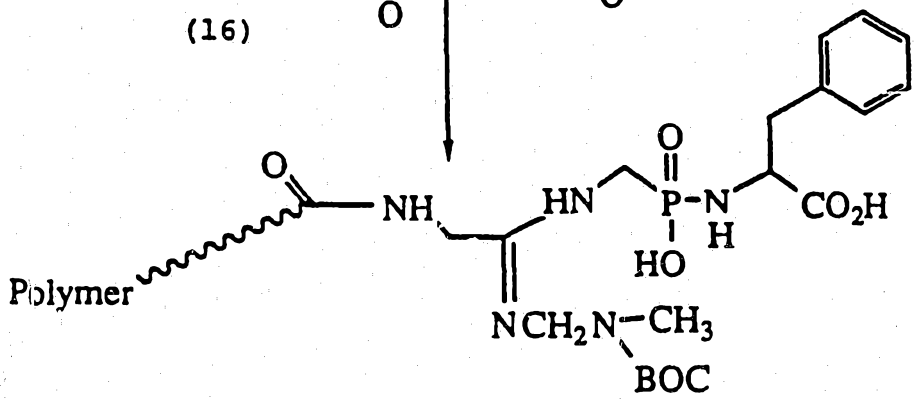
(14)



(15)

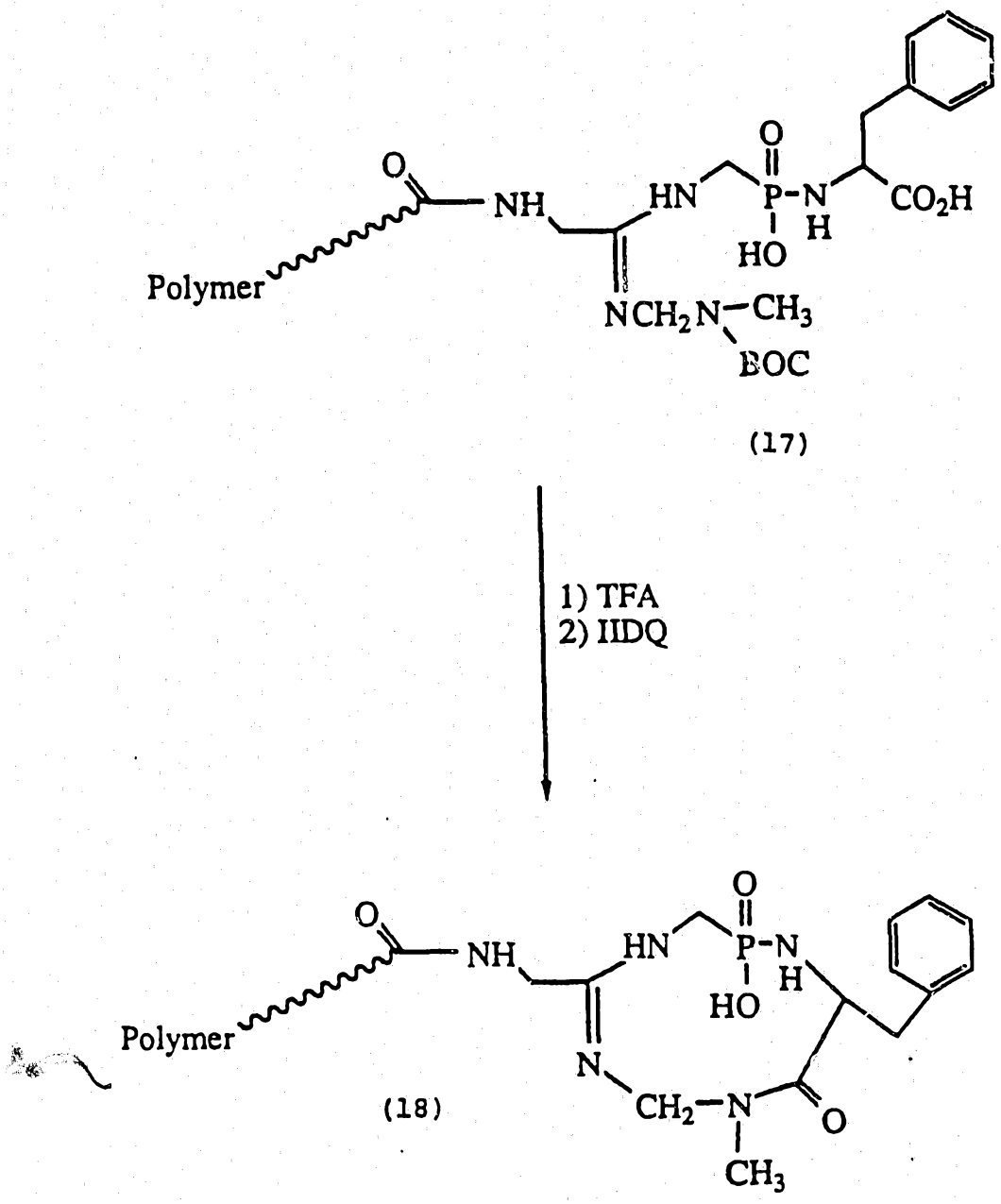


(16)



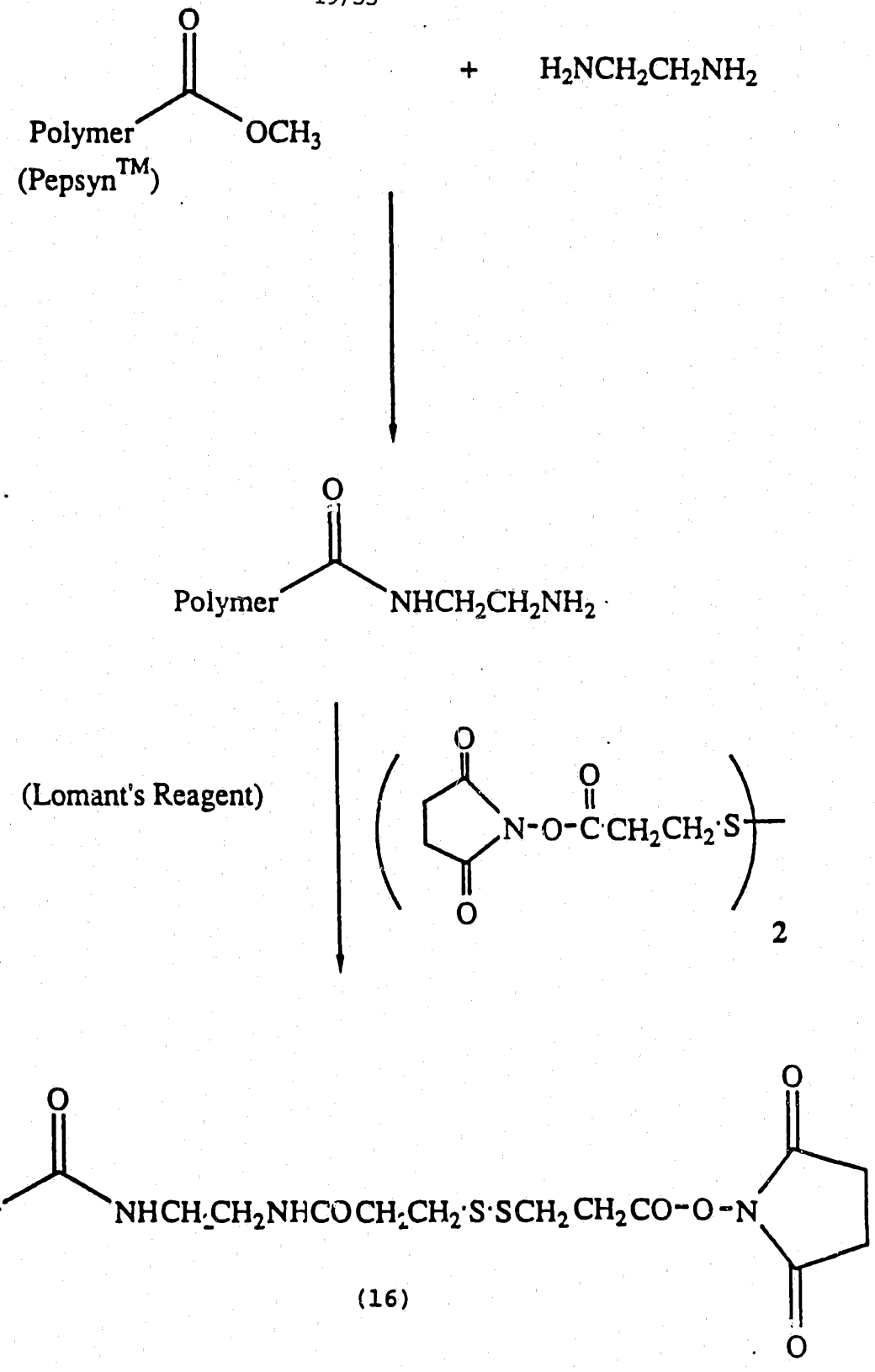
(17)

Fig. 14 (cont'd)



Scheme 2 (continued)

Fig. 14 (cont'd)



Scheme 3

Fig. 15

Fig 16

SUBSTITUTE SHEET

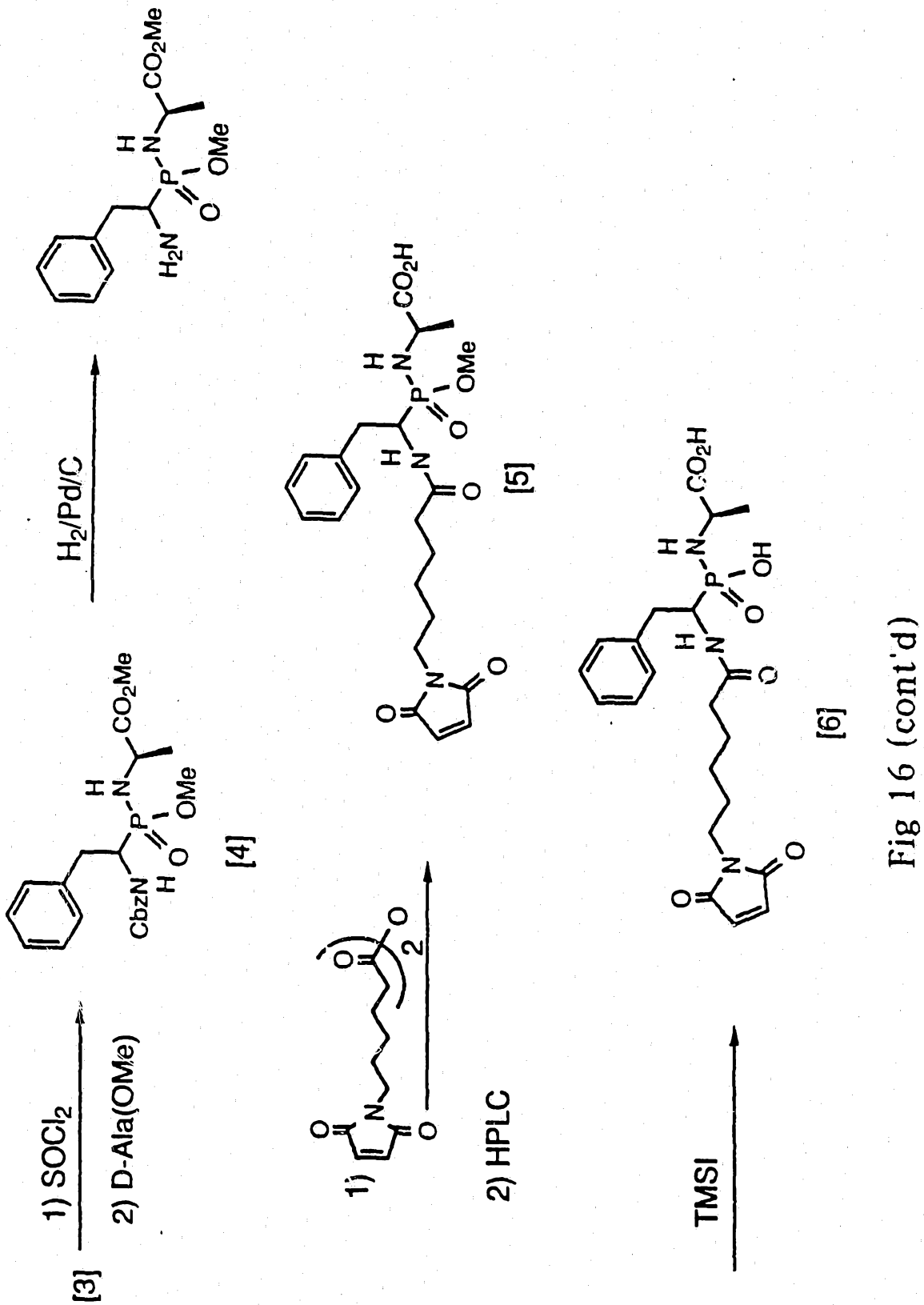


Fig 16 (cont'd)

SUBSTITUTE SHEET

Fig 17

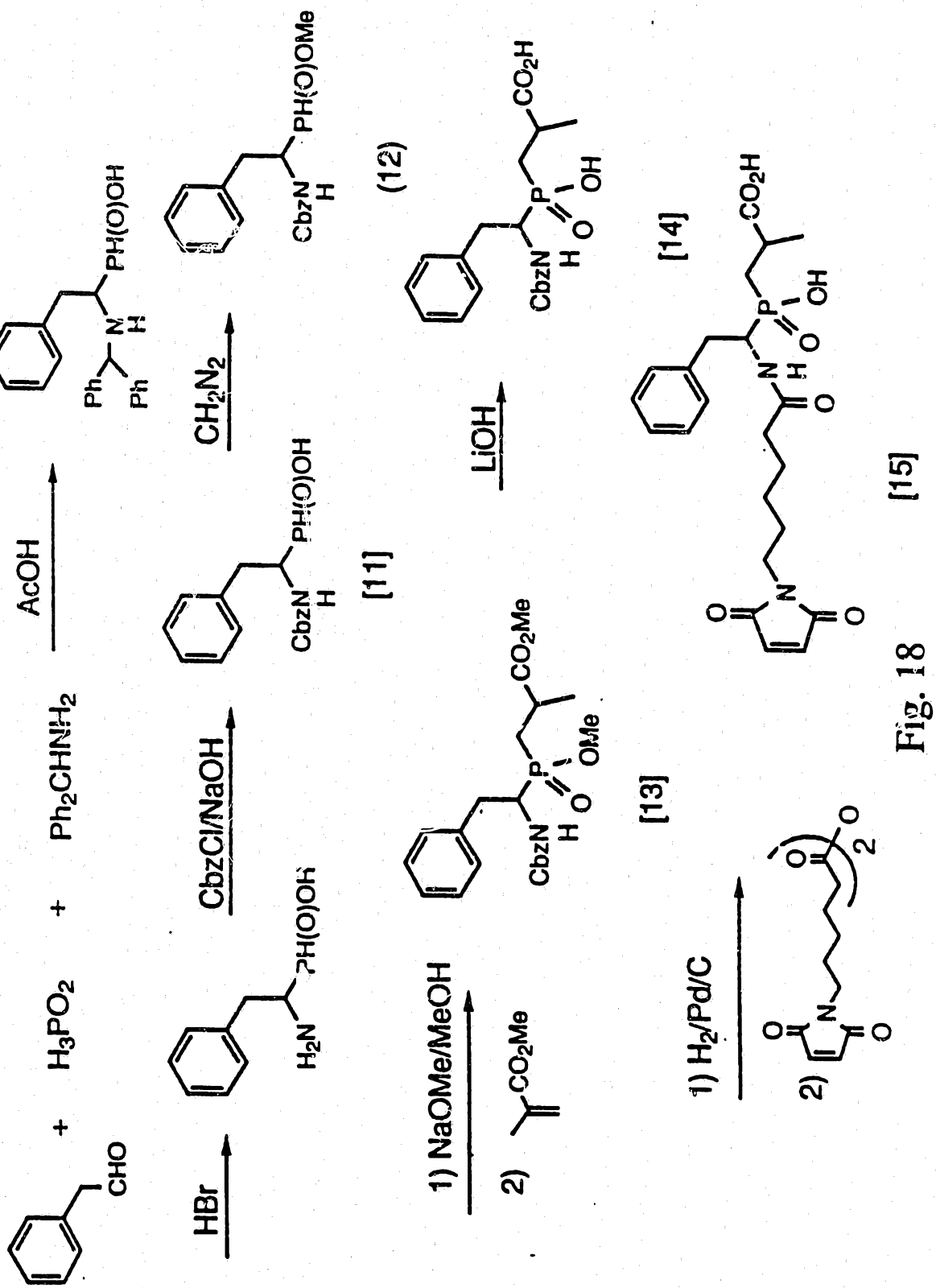

CHO
+ H_3PO_2 + Ph_2CHNH_2
AcOH
HBr
CbzCl/NaOH
CH_2N_2
LiOH
1) NaOMe/MeOH
2)
1) H_2/Pd/C
2)
[11]
(12)
[13]
[14]
[15]

Fig. 18

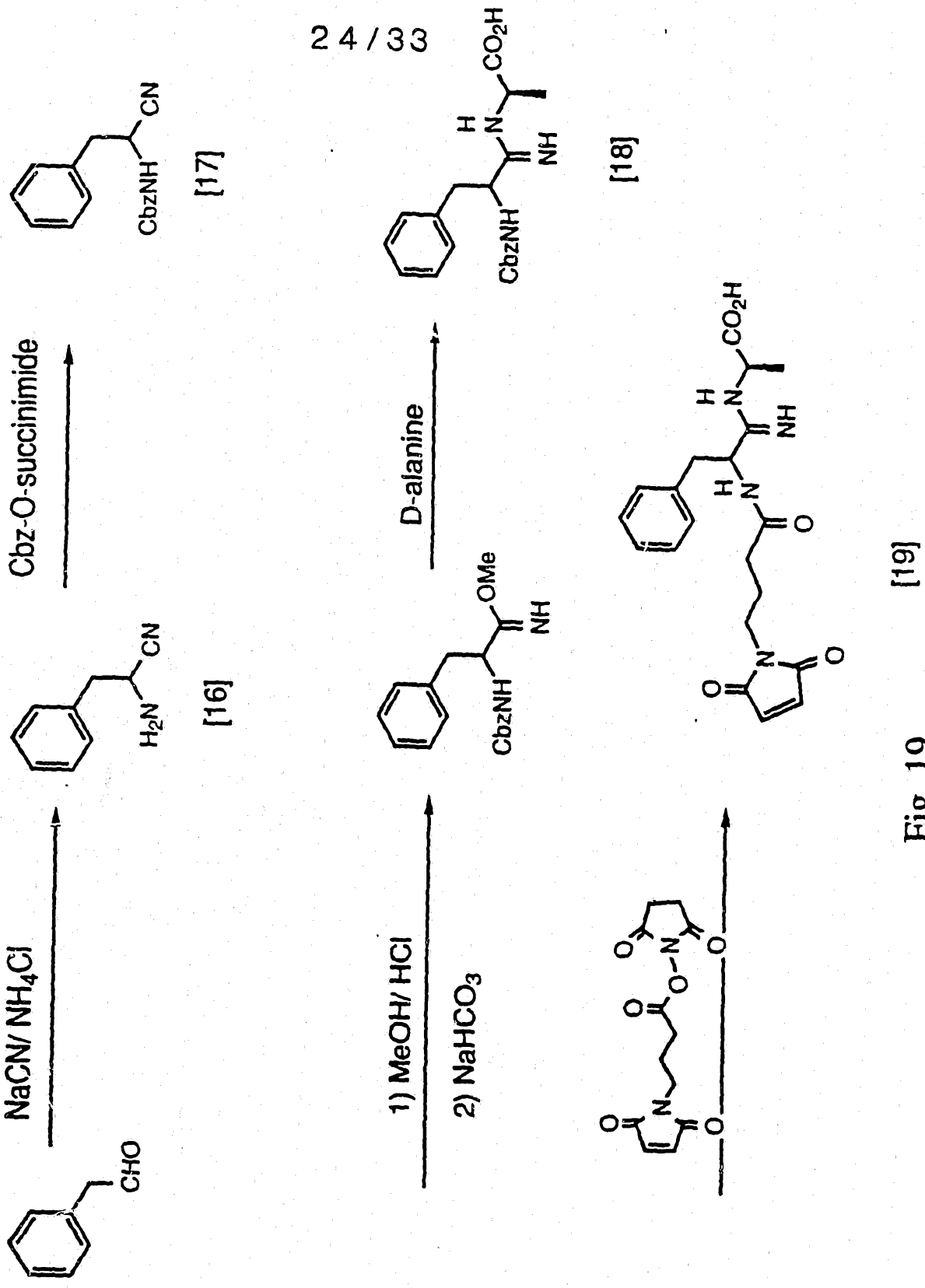

NaCN/ NH4Cl
Cbz-O-succinimide
1) MeOH/ HCl
2) NaHCO3
D-alanine
CHO
CN
CbzNH
H2N
OMe
NH
CO2H
H
N
O
[16]
[17]
[18]
[19]

Fig. 19

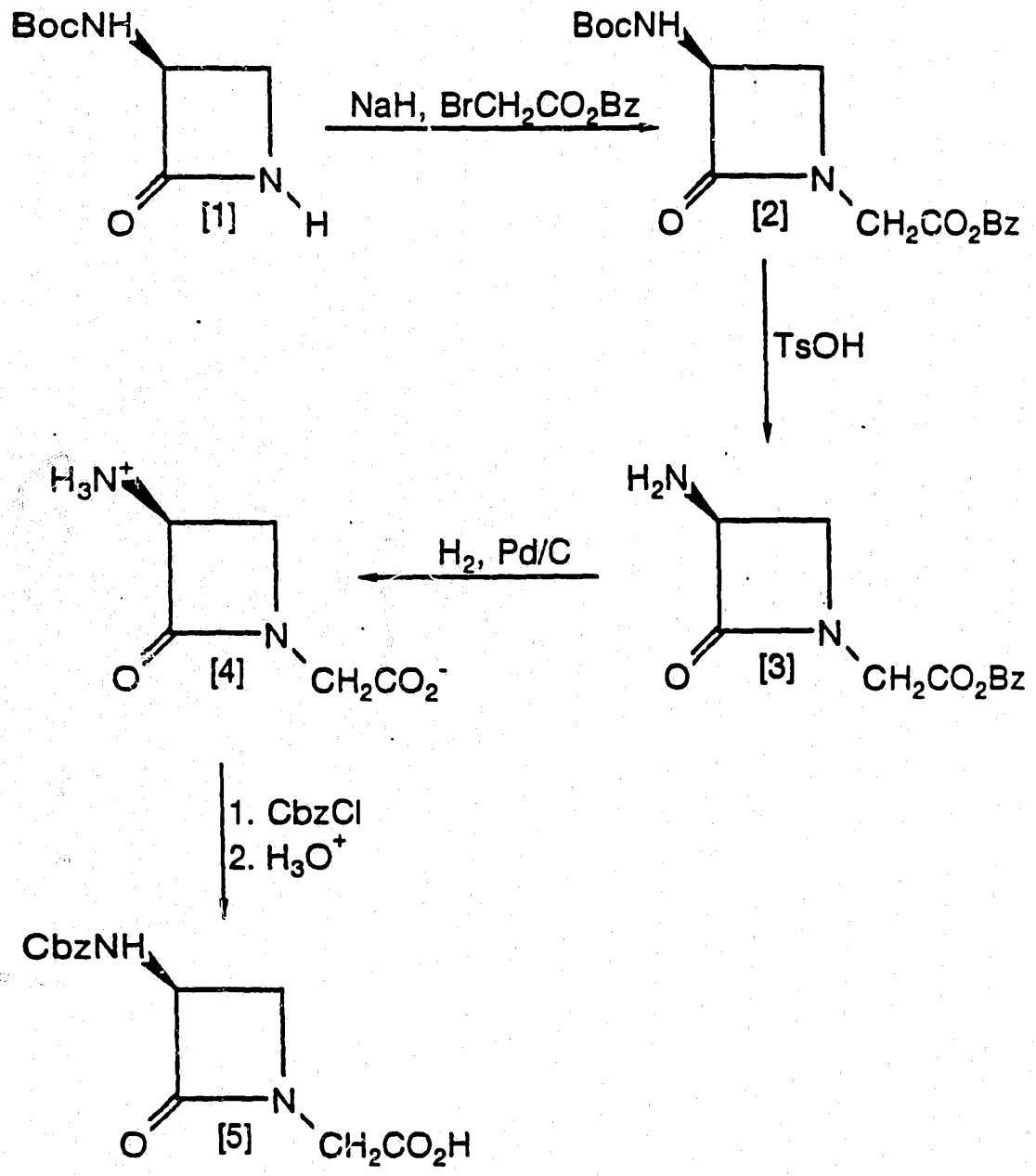

BocNH
NaH, BrCH2CO2Bz
[1]
[2]
CH2CO2Bz
TsOH
H3N+
H2N
H2, Pd/C
[4]
CH2CO2-
[3]
1. CbzCl
2. H3O+
CbzNH
[5]
CH2CO2H

Figure 20

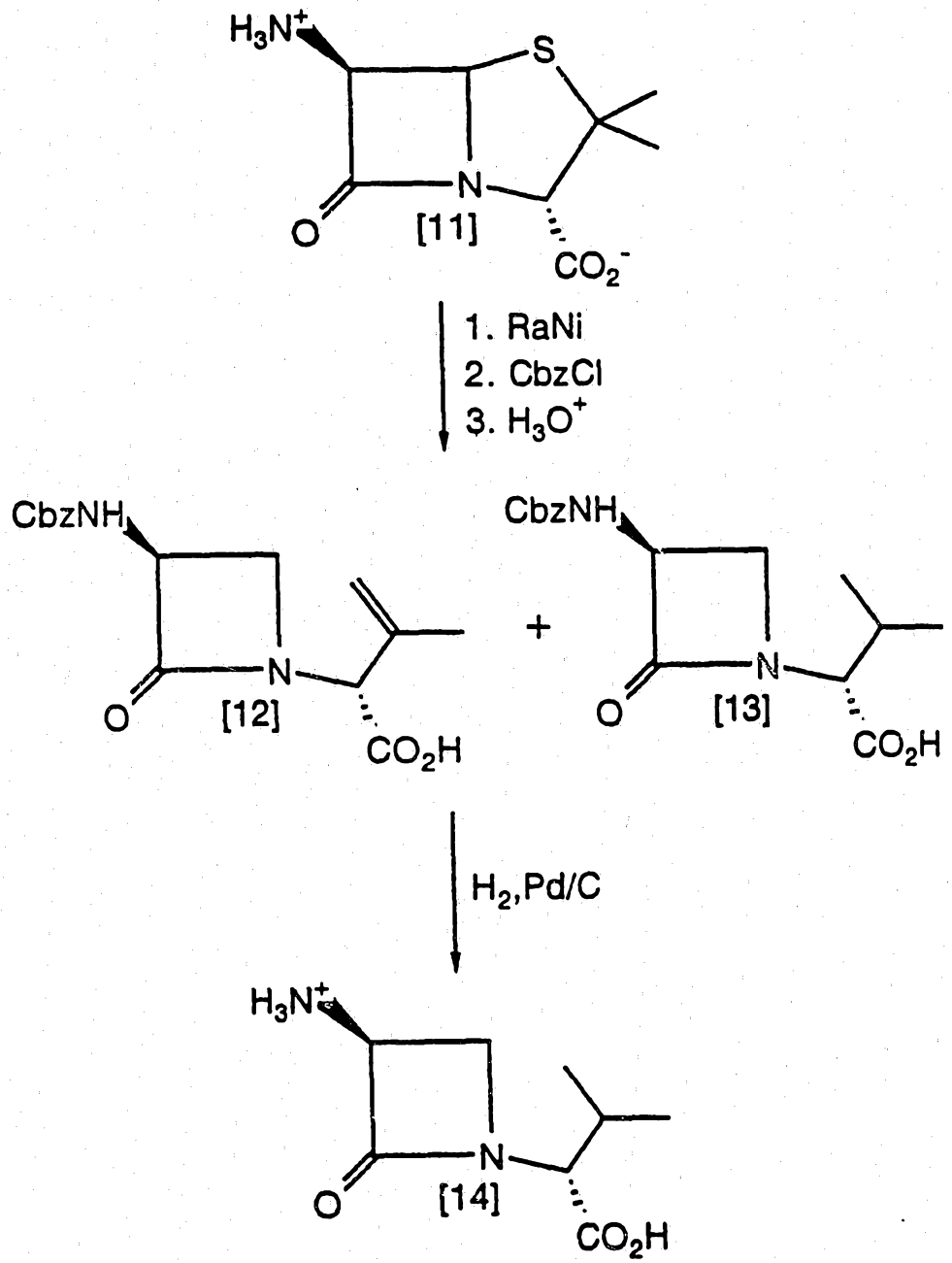

H3N+
S
N
O
[11]
CO2-
1. RaNi
2. CbzCl
3. H3O+
CbzNH
N
O
[12]
CO2H
+
CbzNH
N
O
[13]
CO2H
H2,Pd/C
H3N+
N
O
[14]
CO2H

Figure 21

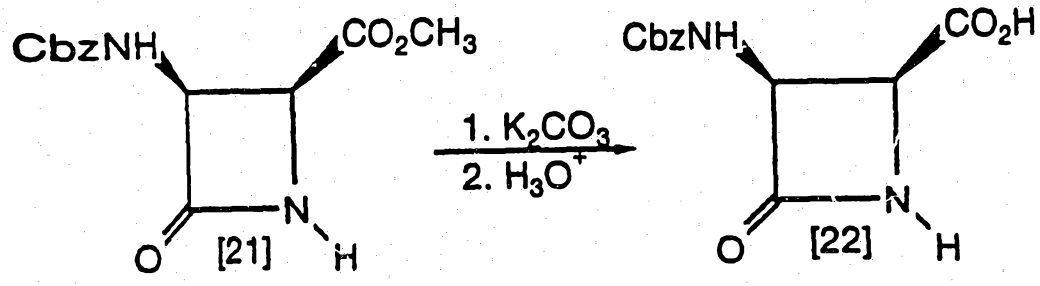


Figure 22

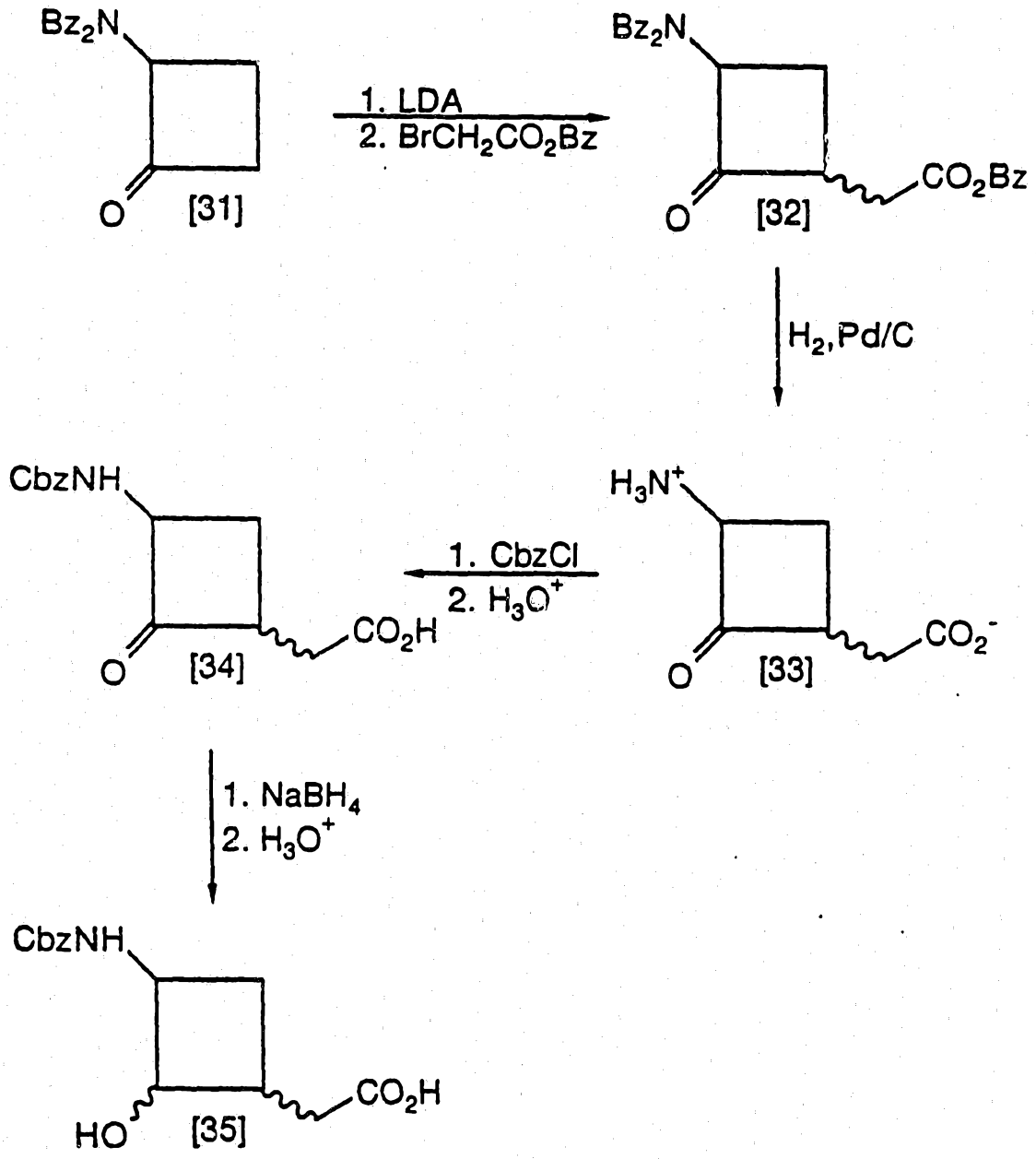

Bz2N
O
[31]
1. LDA
2. BrCH2CO2Bz
Bz2N
O
[32]
CO2Bz
H2,Pd/C
CbzNH
O
[34]
CO2H
1. CbzCl
2. H3O+
H3N+
O
[33]
CO2-
1. NaBH4
2. H3O+
CbzNH
HO
[35]
CO2H

Figure 23

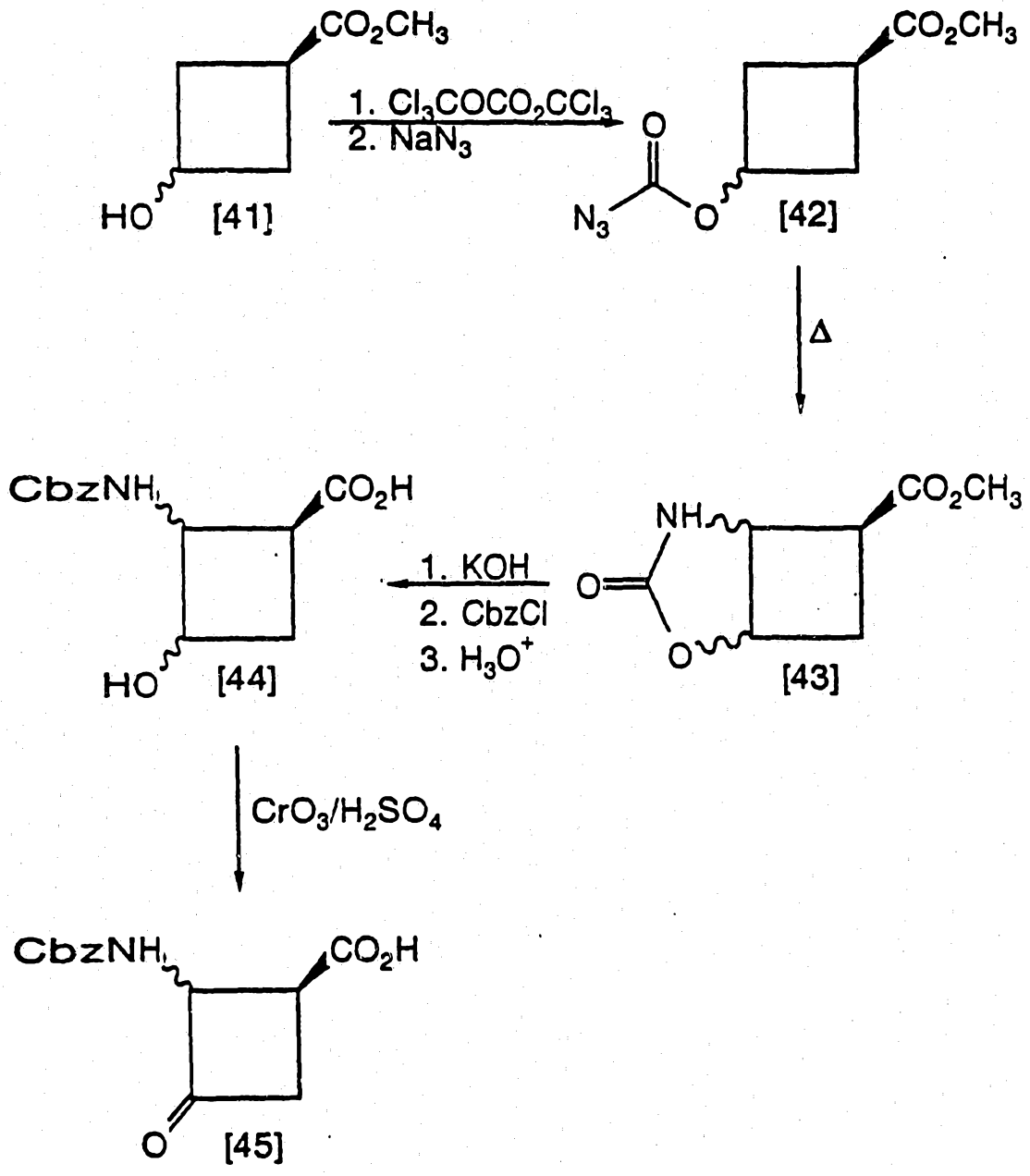

CO2CH3
1. Cl3COCO2CCl3
2. NaN3
HO
[41]
N3
O
[42]
Δ
CbzNH
CO2H
HO
[44]
1. KOH
2. CbzCl
3. H3O+
NH
[43]
CrO3/H2SO4
CbzNH
CO2H
O
[45]

Figure 24

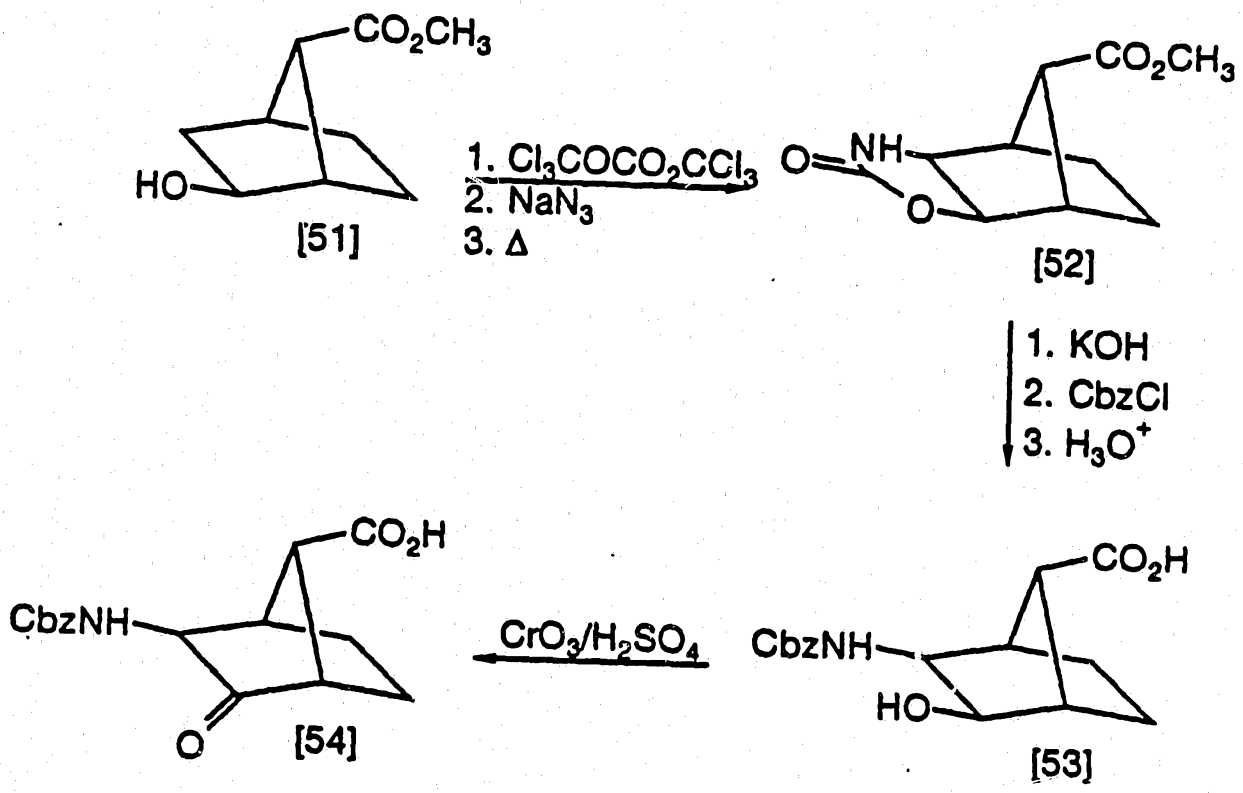

CO2CH3
HO
[51]
1. Cl3COCO2CCl3
2. NaN3
3. Δ
O
NH
O
CO2CH3
[52]
1. KOH
2. CbzCl
3. H3O+
CO2H
CbzNH
O
[54]
CrO3/H2SO4
CbzNH
HO
CO2H
[53]

Figure 25

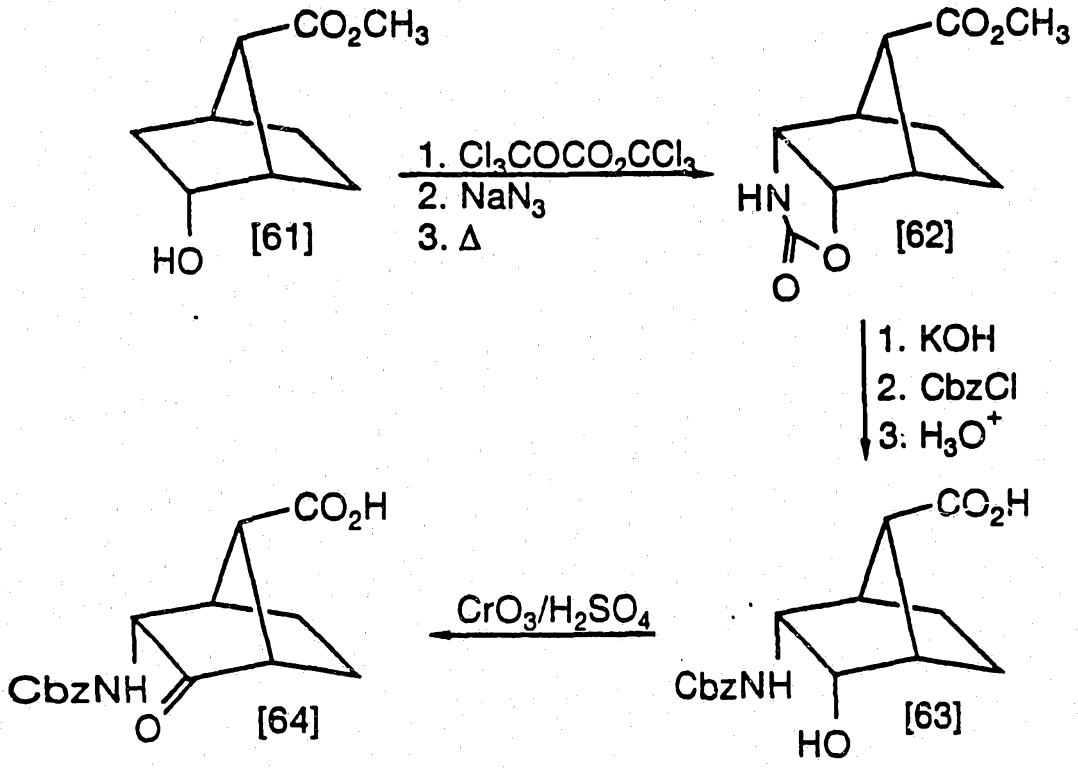

CO2CH3
HO
[61]
1. Cl3COCO2CCl3
2. NaN3
3. Δ
HN
O
O
[62]
CO2CH3
1. KOH
2. CbzCl
3. H3O+
CO2H
CbzNH
O
[64]
CrO3/H2SO4
CO2H
CbzNH
HO
[63]

Figure 26

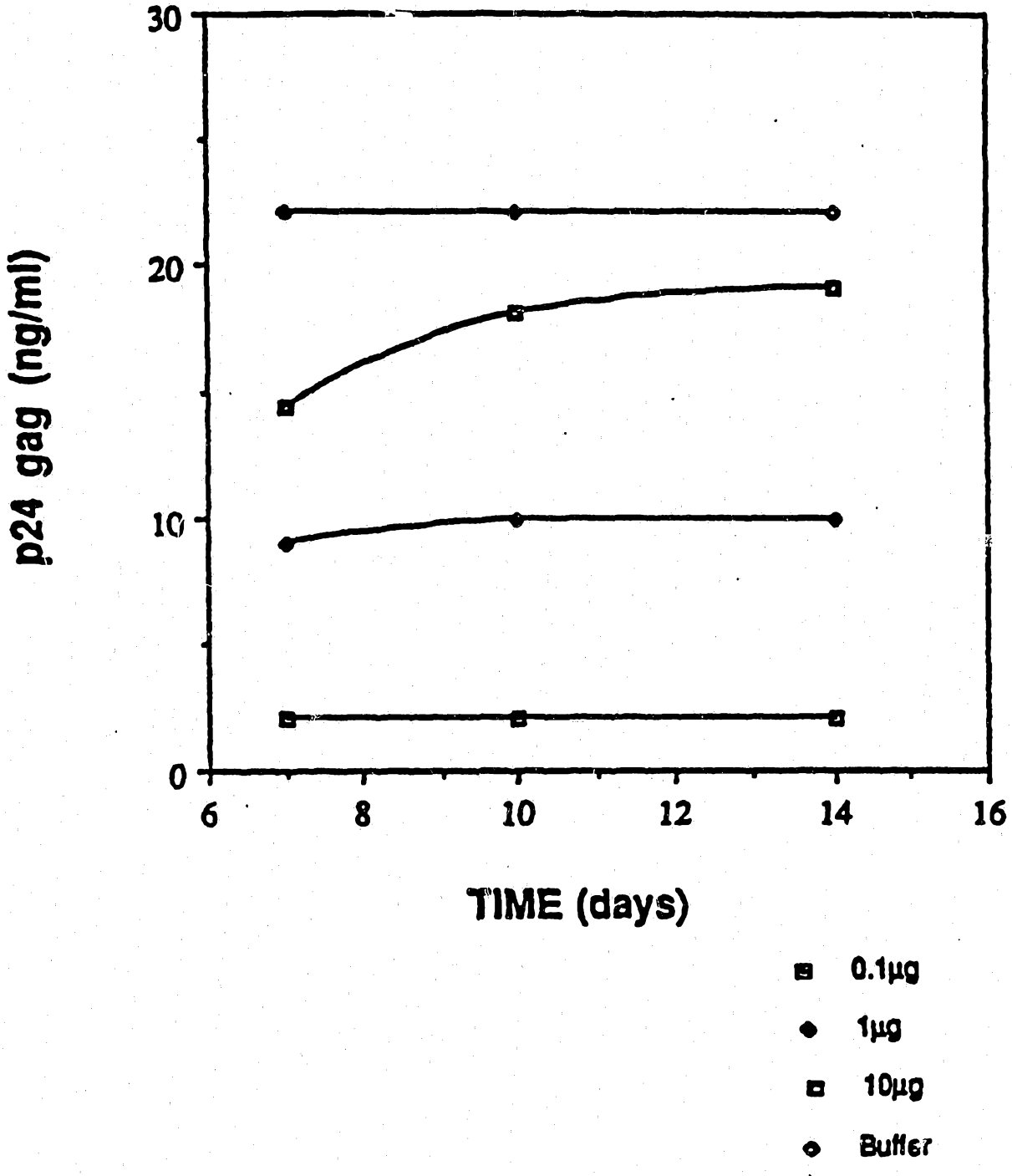

30
20
10
0
p24 gag (ng/ml)
6
8
10
12
14
16
TIME (days)
0.1µg
1µg
10µg
Buffer

Figure 27

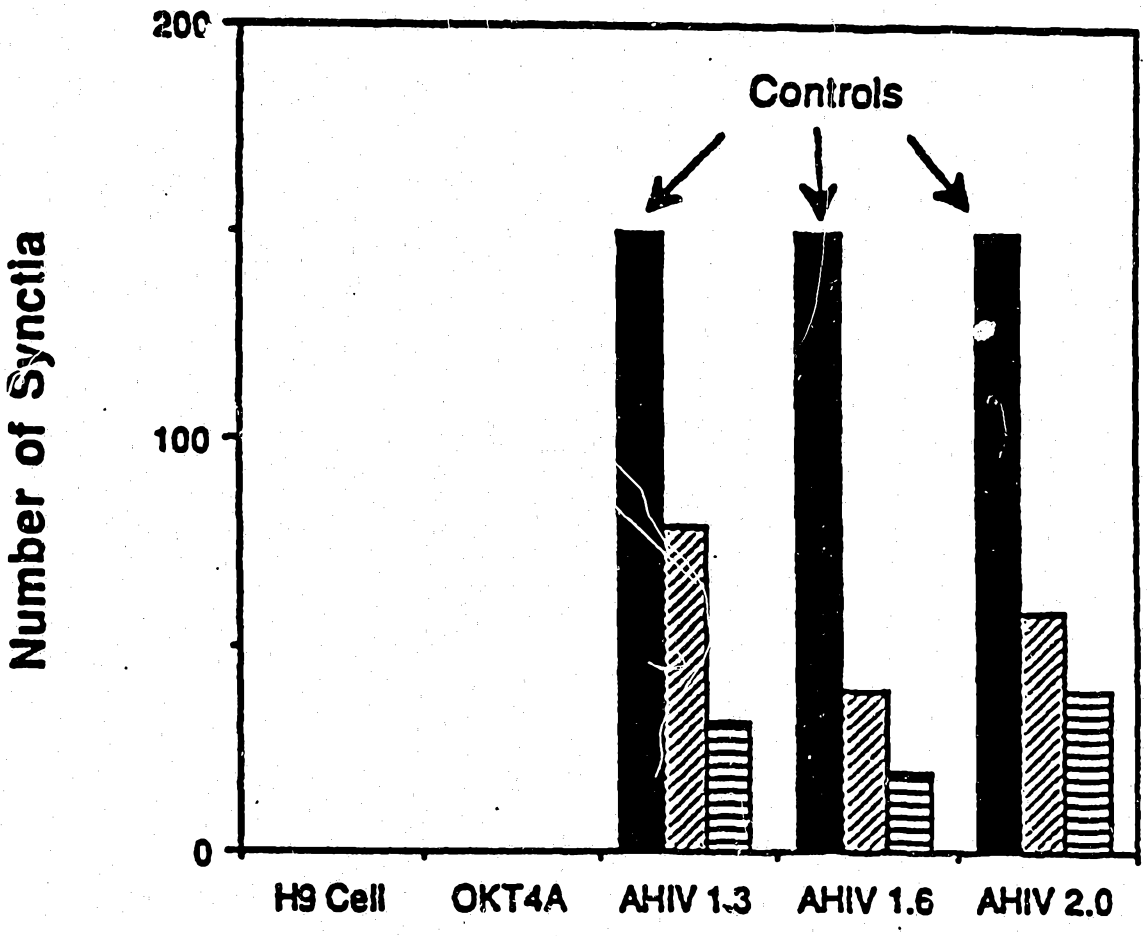

200
100
0
Number of Synctia
Controls
H9 Cell
OKT4A
AHIV 1.3
AHIV 1.6
AHIV 2.0

Figure 28

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/01951

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12N 9/14,9/16,9/48,9/88; C07K 15/00; C07C 79/46

U.S.Cl: 435/195,196,212,232; 530/387; 560/21

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/183,195,196,212,232; 530/387; 560/21

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Computer Structure Search - CA

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	BIOCHEM. BIOPHYS. ACTA, Volume 629, issued 1980, F. Kohen, "Antibody-Enhanced Hydrolysis OF Steroid Esters", see pages 328-337.	1-3,54,55
Y	SCIENCE, Volume 240, issued 22 April 1988, P.G. Schultz, "The Interplay Between Chemistry And Biology In The Design Of Enzymatic Catalysts", see pages 426-432.	1-3,54,55
Y	SCIENCE, Volume 234, issued 19 December 1986, S.J. Pollack, "Selective Chemical Catalysis By An Antibody", see pages 1570-1573.	1-3,54,55
Y	SCIENCE, Volume 234, issued 19 December 1986, A. Tramontano, "Catalytic Antibodies", see pages 1566-1570.	1-3,54,55
Y	WO, A, 85/02414 (IGEN, INC.) 6 June 1985, see entire document.	1-3,54,55

* Special categories of cited documents: [10]

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

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

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

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

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

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

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

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
08 August 1989	06 SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Charles L. Patterson, Jr.

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. ☐ Claim numbers , because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☒ Claim numbers 4-49, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

The instant claims are unduly alternative to such an extent that they are incomprehensible and thus no meaningful international search can be done. For example, to the extent understood, claim 4(a) encompasses no fewer than 3,000,000,000 structures. There is also confusion in understanding claim 4(a) and others in that "X" as defined would have an incorrect valence for O, S, or N.

3. ☒ Claim numbers 56, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

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

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	US, A, 4,659,567 (TXAMONTANO, ET.AL.), 21 April 1987, see entire document.	1-3,54,55
Y	US, A, 4,792,446 (KIM, ET. AL.), 20 December 1987, see entire document.	1-3,54,55