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(54) Titre : COMPOSES DE CAPTURE, RECUEILS DE CEUX-CI ET PROCEDES D'ANALYSE DU PROTEOME ET  
COMPOSITIONS COMPLEXES  
(54) Title: CAPTURE COMPOUNDS, COLLECTIONS THEREOF AND METHODS FOR ANALYZING THE PROTEOME  
AND COMPLEX COMPOSITIONS

(57) Abrégé/Abstract:

Capture compounds and collections thereof and methods using the compounds for the analysis of biomolecules are provided. In particular, collections, compounds and methods are provided for analyzing complex protein mixtures, such as the proteome. The compounds are multifunctional reagents that provide for the separation and isolation of complex protein mixtures. Automated systems for performing the methods also are provided.



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**ABSTRACT**

Capture compounds and collections thereof and methods using the compounds for the analysis of biomolecules are provided. In particular, collections, compounds and methods are provided for analyzing complex protein mixtures, such as the proteome. The compounds are multifunctional reagents that provide for the separation and isolation of complex protein mixtures. Automated systems for performing the methods also are provided.

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CAPTURE COMPOUNDS, COLLECTIONS THEREOF AND METHODS FOR  
ANALYZING THE PROTEOME AND COMPLEX COMPOSITIONS  
RELATED APPLICATIONS

FIELD

- 5        Provided herein are compounds and methods using the compounds to specifically and selectively analyze biomolecules. In particular, the compounds and methods are useful for analyzing the proteome.

BACKGROUND

- 10        Understanding the basis of disease and the development of therapeutic and preventative treatments has evolved over the last century from empirical observation and experimentation to genome wide mutation scanning. The revolution in genomics has provided researchers with the tools to look for a genomic basis for disease. The Human Genome effort has generated a raw sequence of the 3 billion base pairs of the human
- 15        genome and revealed about 35,000 genes. Genetic variations amongst different individuals and in and in between populations are being studied

in order to determine the association with the predisposition to disease or the correlation to drug efficacy and/or side effects. The promise of personalized medicine based on a panel of genetic markers has tantalized the healthcare community and provides an important goal for those  
5 focused on providing diagnostic and treatment options for healthcare providers and patients.

With the development of a variety of tools in molecular biology, such as nucleic amplification methods, cloning and expression systems and methods, disease analysis has been based on a genomics, or *bottom*  
10 *up*, approach. This approach presumes that a genetic change or set of changes will have a long reaching effect on protein function by affecting mRNA transcription or protein structure and function.

Technologies have been developed to analyze single nucleotide polymorphisms (SNPs) in an industrial scale (*e.g.*, MassARRAY™ and the  
15 MassARRAY® system, Sequenom, Inc., San Diego, CA) and in pooled samples to study the frequency of SNPs in populations of various gender, ethnicity, age and health condition. The ultimate goal of these efforts is to understand the etiology of disease on the molecular level (*e.g.*, based on genetic variances (pharmacogenomics)), to develop diagnostic assays  
20 and effective drugs with few or no side-effects.

Genomics has fallen short of the original expectation that this strategy could be used to stratify a population relative to a defined phenotype, including differences between normal and disease patient population or populations. Although single genetic markers have been  
25 found to be associated with or cause or predict a specific disease state, genomic information may not be sufficient to stratify individual populations by of the association of an SNP (or SNPs) with a given disease, drug side-effect or other target phenotype. Because of the large number of potential targets and regulatory signals that affect protein

translation, it is not sufficient to establish the differential expression profiles of messenger RNA in comparing phenotypes or populations, such as healthy and disease states, or such as the analyses using expression DNA chips (*e.g.*, GeneChip™ technology, Affymetrix, Inc., Santa Clara, CA; LifeArray™ technology, Incyte Genomics, Inc., Palo Alto, CA). The metabolic activities in a cell are not performed by mRNA but rather by the translated proteins and subsequently posttranslationally modified products, such as the alkylated, glycosylated and phosphorylated products.

10       The study of proteomics encompasses the study of individual proteins and how these proteins function within a biochemical pathway. Proteomics also includes the study of protein interactions, including how they form the architecture that constitutes living cells. In many human diseases such as cancer, Alzheimer's disease, diabetes as well as host  
15       responses to infectious diseases, the elucidation of the complex interactions between regulatory proteins, which can cause diseases, is a critical step to finding effective treatment. Often, SNPs and other nucleic acid mutations occur in genes whose products are such proteins as (1) growth related hormones, (2) membrane receptors for growth hormones,  
20       (3) components of the trans-membrane signal pathway and (4) DNA binding proteins that act on transcription and the inactivation of suppressor genes (*e.g.* p53) causing the onset of disease.

Complex protein mixtures are analyzed by two-dimensional (2D) gel electrophoresis and subsequent image processing to identify changes in  
25       the pattern (structural changes) or intensity of various protein spots. Two-dimensional gel electrophoresis is a laborious, error-prone method with low reproducibility and cannot be effectively automated. This gel technology is unable to effectively analyze membrane proteins. Further, the resolution of 2D gels is insufficient to analyze the profile of all

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proteins present in a mixture.

Available protein chips are limited by their ability to specifically capture hydrophobic and membrane proteins, which are frequently targets of drug development. Once bound to the chip, proteins are highly  
5 unstable and their structures often do not reflect the true conformation found under physiological conditions.

Thus, there is a need to develop technologies for analysis of the proteome that allow scaling up to industrial levels with the features of an industrial process: high accuracy, reproducibility and flexibility in that the  
10 process is high-throughput, automatable and cost-effective. There is a need to develop technologies that permit probing and identification of proteins and other biomolecules in their native conformation using automated protocols and systems therefor. In particular, there is a need to develop strategies and technologies for identification and  
15 characterization of hydrophobic proteins under physiological conditions. Therefore, among the objects herein, it is an object herein to provide such technologies.

#### SUMMARY

Provided herein are methods, capture compounds (also referred to  
20 herein as capture agents) and collections thereof for analysis of the proteome on an industrial level in a high-throughput format. The methods, capture compounds and collections permit sorting of complex mixtures of biomolecules. In addition, they permit identification of protein structures predicative or indicative of specific of phenotypes, such as  
25 disease states, thereby eliminating the need for random SNP analysis, expression profiling and protein analytical methods. The capture compounds, collections and methods sort complex mixtures by providing a variety of different capture agents. In addition, they can be used to identify structural "epitopes" that serve as markers for specific disease

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states, stratify individual populations relative to specific phenotypes, permit a detailed understanding of the proteins underlying molecular function, and provide targets for drug development. The increased understanding of target proteins permit the design of higher efficiency  
5 therapeutics.

Capture compounds, collections of the compounds and methods that use the compounds, singly or in collections thereof, to capture, separate and analyze biomolecules, including, but not limited to, mixtures of biomolecules, including biopolymers and macromolecules, such as  
10 proteins, individual biomolecules, such as proteins, including individual or membrane proteins, are provided. The collections contain a plurality, generally at least two, three, typically at least 10, 50, 100, 1000 or more different capture compounds. The compounds and collections are designed to permit probing of a mixture of biomolecules by virtue of  
15 interaction of the capture compounds in the collection with the components of the a mixture under conditions that preserve their three-dimensional configuration. Each member of the collection is designed 1) to bind, either covalently or other chemical interaction with high binding affinity ( $k_a$ ) such that the binding is irreversible or stable under  
20 conditions of mass spectrometric analysis) to fewer than all, typically about 5 to 20 or more component biomolecules in a mixture, depending upon complexity and diversity of the mixture, under physiological conditions, including hydrophobic conditions, and 2) distinguish among biomolecules based upon topological features. In addition, the capture  
25 compounds generally include a group, such as a single-stranded oligonucleotide or partially single-stranded oligonucleotide, that permits separation of each set of capture compounds.

The capture compounds and collections are used in a variety of methods, but are particularly designed for assessing biomolecules, such

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as biopolymers or components in mixtures from biological samples. The collections are used in top-down unbiased methods that assess structural changes, including post-translational structural changes and, for example, are used to compare patterns, particularly post-translational protein

5 patterns, in diseased versus healthy cells from primary cells generally from the same individual. The cells that serve as the sources of biomolecules can be frozen into a selected metabolic state or synchronized to permit direct comparison and identification of phenotype-specific, such as disease-specific biomolecules, generally proteins.

10 A capture compound includes at a chemical reactivity group X (also referred to herein as a function or a functionality), which effects the covalent or a high binding affinity (high  $k_d$ ) binding, and least one of three other groups (also referred to herein as functions or functionalities). The other groups are selected from among a selectivity function Y that

15 modulates the interaction of a biomolecule with the reactivity function, a sorting function Q for addressing the components of the collection, and a solubility function W that alters solubility of the capture compound, such as by increasing the solubility of the capture compound under selected conditions, such as various physiological conditions, including

20 hydrophobic conditions of cell membranes. Hence, for example, if membrane proteins are targeted, then the capture compounds in the collection are designed with solubility functions that increase or provide for solubility in such environment.

For example, the reactivity group (reactivity function) includes

25 groups that specifically react or interact with functionalities on the surface of a protein such as hydroxyl, amine, amide, sulfide and carboxylic acid groups, or that recognize specific surface areas, such as an antibody, a lectin or a receptor-specific ligand, or interacts with the active site of enzymes. Those skilled in the art can select from a library

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of functionalities to accomplish this interaction. While this interaction can be highly reaction-specific, these compounds can react multiple times within the same protein molecule depending on the number of surface-accessible functional groups. Modification of the reaction conditions  
5 allows the identification of surface accessible functional groups with differing reactivity, thereby permitting identification of one or more highly reactive sites used to separate an individual protein from a mixture. Available technologies do not separate species in the resulting reaction mixture. The collections and compounds provided herein solve that  
10 problem through a second functionality, the selectivity group, which alters binding of the reactivity groups to the biomolecule.

Selectivity functions include a variety of groups, as well as the geometric spacing of the second functionality, a single stranded unprotected or suitably protected oligonucleotide or oligonucleotide  
15 analog. The selective functionality can be separate from the compound and include the solid or semi-solid support. The selective functionality in this embodiment can be porosity, hydrophobicity, charge and other chemical properties of the material. For example, selectivity functions interact noncovalently with target proteins to alter the specificity or  
20 binding of the reactivity function. Such functions include chemical groups and biomolecules that can sterically hinder proteins of specific size, hydrophilic compounds or proteins (*e.g.*, PEG and trityls), hydrophobic compounds or proteins (*e.g.*, polar aromatic, lipids, glycolipids, phosphotriester, oligosaccharides), positive or negatively charged groups,  
25 groups or biomolecules which create defined secondary or tertiary structure.

The capture compounds can also include a sorting function for separation or addressing of each capture compound according to its structure. The sorting function, for example, can be a single-stranded (or

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partially single-stranded) unprotected or suitably protected oligonucleotide or oligonucleotide analog, typically containing between at least about 5 and up to 25, 35, 50, 100 or any desired number of nucleotides (or analogs thereof) containing a sequence-permuted region and optionally  
5 flanking regions. Each such block has a multitude of sequence permutations with or without flanking conserved regions, which is capable of hybridizing with a base-complementary single stranded nucleic acid molecule or a nucleic acid analog. The sorting function can also be a label, such as a symbology, including a bar code, particularly a machine-  
10 readable bar code, a color coded-label, such as small colored bead that can be sorted by virtue of its color, a radio-frequency tag or other electronic label or a chemical label. Any functionality that permits sorting of each set of capture compounds to permit separate analysis of bound biomolecules is contemplated.

15 In certain embodiments, each biomolecule to be captured is derivatized with more than one capture compound provided herein, where each tagged compound provides an additional level of sorting capability. In other embodiments, each of the plurality of compounds that derivatize a single biomolecule is different, allowing for specific and efficient sorting  
20 of the biomolecule mixture (see, *e.g.*, Figure 3). The capture compound also can be multifunctional containing other functionalities that can be used to reduce the complexity of biomolecule mixtures.

Some of the capture compounds include at least a reactivity function and a selectivity function. These capture compounds optionally  
25 include sorting functionalities, which are one or more additional moieties that bind either covalently or noncovalently to a specific molecule to permit addressing of the compounds, such as by separation at discrete loci on a solid support, separation of the compounds on discrete loci. These capture compounds also optionally include one or more solubility

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functions, which are moieties that influence the solubility of the resulting compound, to attenuate or alter the hydrophobicity/hydrophilicity of the compounds (solubility function).

Others of the capture compounds (or capture agents) include at  
5 least two functional portions: a reactivity function and a sorting function. The reactive group that specifically interacts with proteins or other biomolecules (reactivity function); and the other is an entity (sorting functions) that binds either covalently or noncovalently to a specific molecule(s). This entity can be a nucleic acid portion or nucleic acid  
10 analog portion that includes a single-stranded region that can specifically hybridize to a complementary single-stranded oligonucleotide or analog thereof.

The capture compounds are provided as collections, generally as collections of sets of different compounds that differ in all functionalities.  
15 For sorting of complex mixtures of biopolymers the collection includes diverse capture compound members so that, for example, when they are arrayed, each locus of the array contains 0 to 100, generally, 5 to 50 and desirably 1 to 20, typically 5 to 20, different biomolecules at each locus in the array.

20 In practice in one embodiment, a collection of capture compounds is contacted with a biomolecule mixture and the bound molecules are assessed using, for example, mass spectrometry, followed by optional application of tagging, such as fluorescence tagging, after arraying to identify low abundance proteins. In other embodiments, a single capture  
25 compound is contacted with one or plurality of biomolecules, and the bound molecules are assessed.

Also provided herein are methods for the discovery and identification of proteins, which are selected based on a defined phenotype. The methods allow proteins to bind to the target molecules

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under physiological conditions while maintaining the correct secondary and tertiary conformation of the target. The methods can be performed under physiological and other conditions that permit discovery of biologically important proteins, including membrane proteins, that are  
5 selected based upon a defined phenotype.

Before, during or after exposure of one or a plurality of capture compounds to a mixture of biomolecules, including, but not limited to, a mixture of proteins, the oligonucleotide portion, or analog thereof, of these compounds is allowed to hybridize to a complementary strand of  
10 immobilized oligonucleotide(s), or analog(s) thereof, to allow separation, isolation and subsequent analysis of bound biomolecules, such as proteins, by, for example, mass spectrometry, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, colorimetric, fluorescent or chemiluminescent tagging, or to allow for  
15 increased resolution by mass spectrometry, including MALDI-TOF mass spectrometry.

The collections of capture compounds can be used to generate compound arrays to capture target proteins or groups of related proteins that can mimic biological structures such as nuclear and mitochondrial  
20 transmembrane structures, artificial membranes or intact cell walls. Thus, the compounds and compound arrays provided herein are capable of mimicking biological entities and biological surfaces, thereby allowing for capture of biomolecules, including but not limited to proteins, which would otherwise be difficult or impossible to capture, such as those found  
25 in transmembrane regions of a cell.

Samples for analysis include any biomolecules, particularly protein-containing samples, such as protein mixtures, including, but not limited to, natural and synthetic sources. Proteins can be prepared by translation from isolated chromosomes, genes, cDNA and genomic

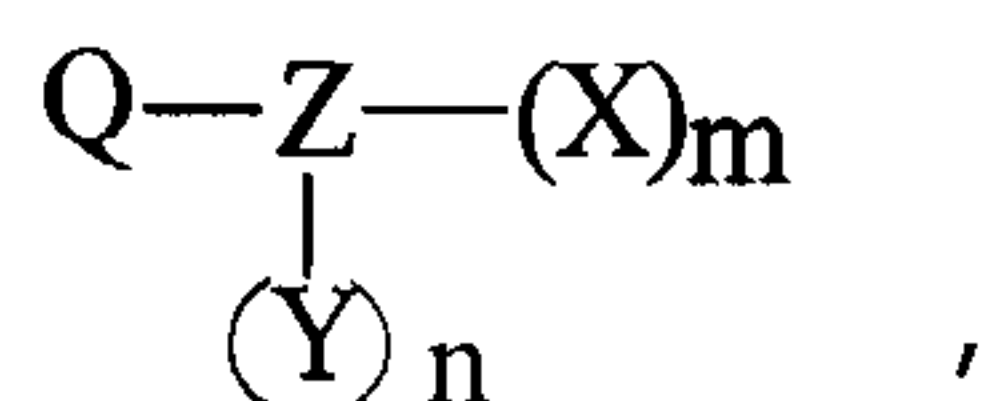
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libraries. Proteins can be isolated from cells, and other sources. In certain embodiments, the capture compounds provided herein are designed to selectively capture different post-translational modifications of the same protein (*i.e.*, phosphorylation patterns (*e.g.*, oncogenes), glycosylation and other post-translational modifications).

Other methods that employ the collections are also provided. In one method, the collections of one or more member capture compounds are used to distinguish between or among different conformations of a protein and, for example, can be used for phenotypic identification, such as for diagnosis. For example, for diseases of protein aggregation, which are diseases involving a conformationally altered protein, such as amyloid diseases, the collections can distinguish between the disease-involved form of the protein from the normal protein and thereby diagnose the disease in a sample.

In one aspect, the invention relates to a collection of capture compounds, comprising a plurality of different capture compounds, wherein each capture compound in the collection has the formula:



wherein:

m is an integer that is 1 to 100;

n is an integer from 1 to 100;

the collection includes at least ten different capture compounds;

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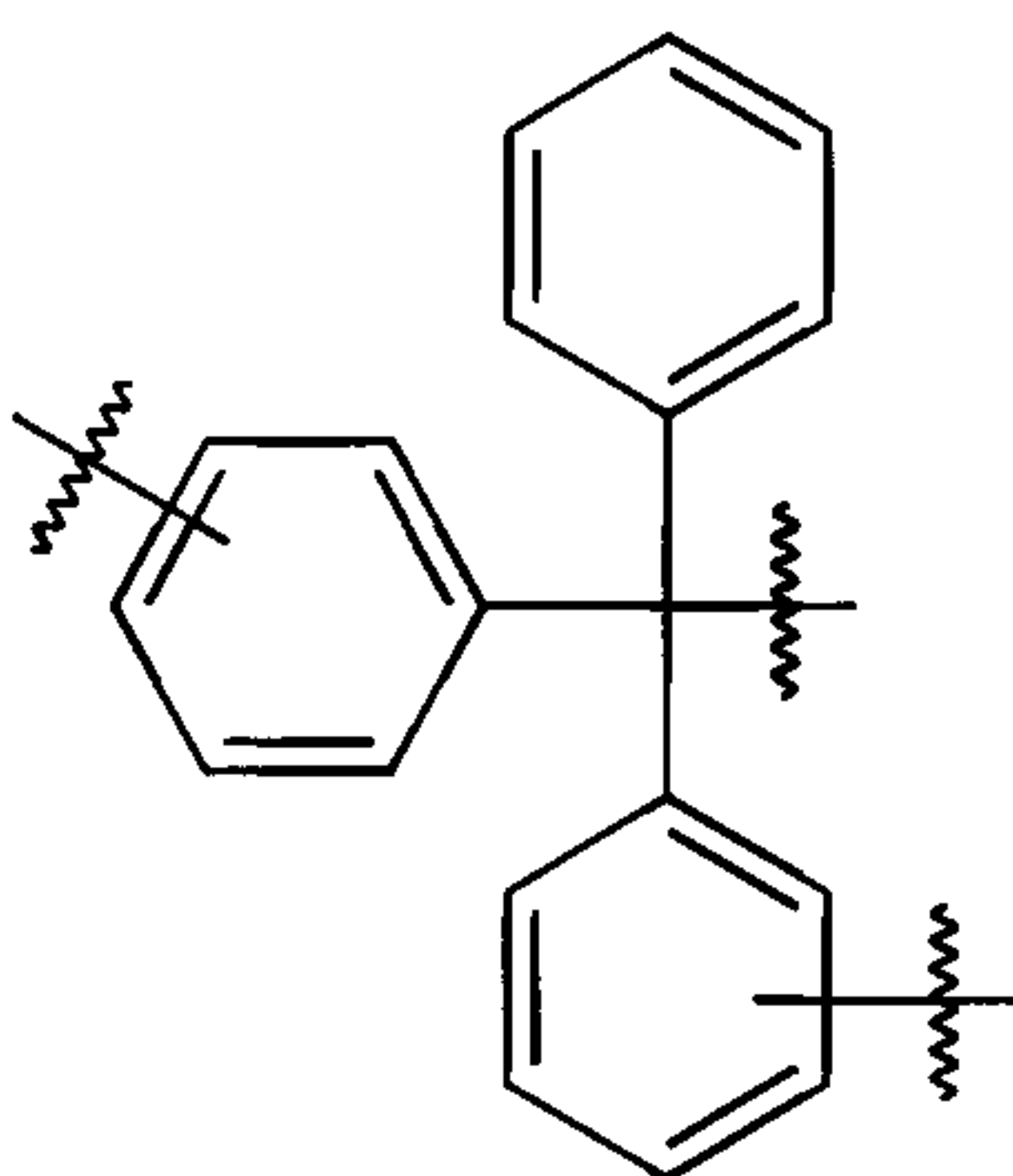
moiety X is selected to covalently bind to biomolecules;

Y modulates one or more of the affinity, steric properties and electronic properties of the capture compound  
5 thereby increasing selectivity of the binding by X such that the capture compound binds to fewer biomolecules when the selectivity moiety Y is present than in its absence;

Q permits separation or immobilization of capture compounds in the collection; and

10 Z is a trityl derivative for presenting functional moieties X, Y and Q;

Z has the formula:



; and

the capture compounds are provided as a mixture in a composition or as an addressable array where each locus of the array contains the same compound or a mixture thereof.

## 20 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the hybridization, separation and mass spectral analysis of a mixture of proteins.

Figure 2 provides a schematic depiction of one embodiment of the apparatus provided herein.

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Figure 3 illustrates a protein tagged with four compounds provided herein, thereby allowing for specific sorting of the protein.

Figure 4 shows the increased and specific  
5 hybridization resulting from use of two or more oligonucleotide tags.

Figure 5 shows tagging of a single protein with two different oligonucleotides in one reaction.

Figure 6 is a flow diagram of recombinant protein  
10 production.

Figure 7 illustrates production of an adapted oligonucleotide dT primed cDNA library.

Figure 8 shows production of an adapted sequence motif specific

cDNA library.

Figure 9 shows production of an adapted gene specific cDNA.

Figure 10 illustrates purification of amplification products from a template library.

5        Figure 11 shows an adapted oligonucleotide dT primed cDNA library as a universal template for the amplification of gene subpopulations.

Figure 12 illustrates decrease of complexity during PCR amplification.

10       Figure 13 shows the attachment of a bifunctional molecule to a solid surface.

Figure 14 shows analysis of purified proteins from compound screening and antibody production.

15       Figure 15 provides synthetic schemes for synthesis of exemplary capture reagents provided herein (see, *e.g.*, Example 4).

Figure 16 provides exemplary reactivity functions for use in the capture reagents provided herein.

Figure 17 provides exemplary selectivity functions for use in the capture reagents provided herein.

20       Figure 18 depicts exemplary points for regulation of metabolic control mechanisms for cell synchronization.

Figures 19 depict cell separation and synchronization methods; Figure 19a depicts methods for separation of cells from blood from a single patient to separate them by phenotype; Figure 19b shows the results of flow cytometry separation of blood cells without labeling; Figure 25 19c shows an example in which synchronized cells in culture are sorted according to DNA content as a way to separate cells by phase of the cell cycle.

Figure 20 shows a schematic of a biomolecule capture assay and

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results using exemplary capture compounds and proteins.

#### DETAILED DESCRIPTION

##### A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong.

In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to an URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

As used herein, an oligonucleotide means a linear sequence of up to about 20, about 50, or about 100, nucleotides joined by phosphodiester bonds. Above this length the term polynucleotide begins to be used.

As used herein, an oligonucleotide analog means a linear sequence of up to about 20, about 50, or about 100, nucleotide analogs, or linear sequence of up to about 20, about 50, or about 100 nucleotides linked by a "backbone" bond other than a phosphodiester bond, for example, a phosphotriester bond, a phosphoramidate bond, a phosphorothioate bond, a methylphosphonate diester bond, a thioester bond, or a peptide bond (peptide nucleic acid).

As used herein, peptide nucleic acid (PNA) refers to nucleic acid analogs in that the ribose-phosphate backbone is replaced by a backbone

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held together by amide bonds.

As used herein, proteome means all the proteins present within a cell.

As used herein, a biomolecule is any compound found in nature, or  
5 derivatives thereof. Biomolecules include, but are not limited to  
oligonucleotides, oligonucleosides, proteins, peptides, amino acids, lipids,  
steroids, peptide nucleic acids (PNAs), oligosaccharides and  
monosaccharides.

As used herein, MALDI-TOF refers to matrix assisted laser  
10 desorption ionization-time of flight mass spectrometry.

As used herein, the term "conditioned" or "conditioning," when  
used in reference to a protein thereof, means that the polypeptide is  
modified to decrease the laser energy required to volatilize the protein, to  
minimize the likelihood of fragmentation of the protein, or to increase the  
15 resolution of a mass spectrum of the protein or of the component amino  
acids. Resolution of a mass spectrum of a protein can be increased by  
conditioning the protein prior to performing mass spectrometry.

Conditioning can be performed at any stage prior to mass spectrometry  
and, in one embodiment, is performed while the protein is immobilized. A  
20 protein can be conditioned, for example, by treating it with a cation  
exchange material or an anion exchange material, which can reduce the  
charge heterogeneity of the protein, thereby for eliminating peak  
broadening due to heterogeneity in the number of cations (or anions)  
bound to the various proteins in a population. In one embodiment,  
25 removal of all cations by ion exchange, except for  $H^+$  and ammonium ions, is  
performed. By contacting a polypeptide with an alkylating agent such as  
alkyliodide, iodoacetamide, iodoethanol, or 2,3-epoxy-1-propanol, the  
formation of disulfide bonds, for example, in a protein can be prevented.  
Likewise, charged amino acid side chains can be converted to uncharged

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derivatives employing trialkylsilyl chlorides.

Since the capture compounds contain protein and nucleic acid portions, conditioning suitable for one or both portions is also contemplated. Hence, a prepurification to enrich the biomolecules to be  
5 analyzed and the removal of all cations, such as by ion exchange, except for H<sup>+</sup> and ammonium, or other conditioning treatment to improve resolution is advantageous for analysis of the nucleic acid portion as well as the protein portion.

Conditioning of proteins is generally unnecessary because proteins  
10 are relatively stable under acidic, high energy conditions so that proteins do not require conditioning for mass spectrometric analyses. There are means of improving resolution, however, in one embodiment for shorter peptides, such as by incorporating modified amino acids that are more basic than the corresponding unmodified residues. Such modification in  
15 general increases the stability of the polypeptide during mass spectrometric analysis. Also, cation exchange chromatography, as well as general washing and purification procedures that remove proteins and other reaction mixture components away from the protein can be used to increase the resolution of the spectrum resulting from mass spectrometric  
20 analysis of the protein.

As used herein, "matrix" refers to the material with which the capture compound biomolecule conjugates are combined for MALDI mass spectrometric analysis. Any matrix material, such as solid acids, including 3-hydroxypicolinic acid, liquid matrices, such as glycerol, known  
25 to those of skill in the art for nucleic acid and/or protein analyses is contemplated. Since the compound biomolecule conjugates contain nucleic acid and protein a mixture (optimal for nucleic acids and proteins) of matrix molecules can be used.

As used herein, macromolecule refers to any molecule having a

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molecular weight from the hundreds up to the millions. Macromolecules include, but are not limited to, peptides, proteins, nucleotides, nucleic acids, carbohydrates, and other such molecules that are generally synthesized by biological organisms, but can be prepared synthetically or  
5 using recombinant molecular biology methods.

As used herein, the term "biopolymer" is refers to a biological molecule, including macromolecules, composed of two or more monomeric subunits, or derivatives thereof, which are linked by a bond or a macromolecule. A biopolymer can be, for example, a polynucleotide, a  
10 polypeptide, a carbohydrate, or a lipid, or derivatives or combinations thereof, for example, a nucleic acid molecule containing a peptide nucleic acid portion or a glycoprotein. The methods and collections herein, though described with reference to biopolymers, can be adapted for use with other synthetic schemes and assays, such as organic syntheses of  
15 pharmaceuticals, or inorganics and any other reaction or assay performed on a solid support or in a well in nanoliter or smaller volumes.

As used herein, biomolecule includes biopolymers and macromolecules and all molecules that can be isolated from living organisms and viruses, including, but are not limited to, cells, tissues,  
20 prions, animals, plants, viruses, bacteria and other organsims.

As used herein, a biological particle refers to a virus, such as a viral vector or viral capsid with or without packaged nucleic acid, phage, including a phage vector or phage capsid, with or without encapsulated nucleotide acid, a single cell, including eukaryotic and prokaryotic cells or  
25 fragments thereof, a liposome or micellar agent or other packaging particle, and other such biological materials. For purposes herein, biological particles include molecules that are not typically considered macromolecules because they are not generally synthesized, but are derived from cells and viruses.

As used herein, a drug refers to any compound that is a candidate for use as a therapeutic or as a lead compound for designing a therapeutic or that is a known pharmaceutical. Such compounds can be small molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules, antibodies, fragments of antibodies or recombinant antibodies. Of particular interest are "drugs" that have specific binding properties so that they can be used as selectivity groups or can be used as for sorting of the capture compounds, either a sorting functionality that binds to a target on a support, or linked to a solid support, where the sorting functionality is the drug target.

As used herein, the term "nucleic acid" refers to single-stranded and/or double-stranded polynucleotides such as deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) as well as analogs or derivatives of either RNA or DNA. Nucleic acid molecules are linear polymers of nucleotides, linked by 3',5' phosphodiester linkages. In DNA, deoxyribonucleic acid, the sugar group is deoxyribose and the bases of the nucleotides are adenine, guanine, thymine and cytosine. RNA, ribonucleic acid, has ribose as the sugar and uracil replaces thymine. Also included in the term "nucleic acid" are analogs of nucleic acids such as peptide nucleic acid (PNA), phosphorothioate DNA, and other such analogs and derivatives or combinations thereof.

As used herein, the term "polynucleotide" refers to an oligomer or polymer containing at least two linked nucleotides or nucleotide derivatives, including a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), and a DNA or RNA derivative containing, for example, a nucleotide analog or a "backbone" bond other than a phosphodiester bond, for example, a phosphotriester bond, a phosphoramidate bond, a methylphosphonate diester bond, a phosphorothioate bond, a thioester bond, or a peptide bond (peptide nucleic acid). The term

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"oligonucleotide" also is used herein essentially synonymously with "polynucleotide," although those in the art recognize that oligonucleotides, for example, PCR primers, generally are less than about fifty to one hundred nucleotides in length.

5 Nucleotide analogs contained in a polynucleotide can be, for example, mass modified nucleotides, which allows for mass differentiation of polynucleotides; nucleotides containing a detectable label such as a fluorescent, radioactive, colorimetric, luminescent or chemiluminescent label, which allows for detection of a polynucleotide; or  
10 nucleotides containing a reactive group such as biotin or a thiol group, which facilitates immobilization of a polynucleotide to a solid support. A polynucleotide also can contain one or more backbone bonds that are selectively cleavable, for example, chemically, enzymatically or photolytically. For example, a polynucleotide can include one or more  
15 deoxyribonucleotides, followed by one or more ribonucleotides, which can be followed by one or more deoxyribonucleotides, such a sequence being cleavable at the ribonucleotide sequence by base hydrolysis. A polynucleotide also can contain one or more bonds that are relatively resistant to cleavage, for example, a chimeric oligonucleotide primer,  
20 which can include nucleotides linked by peptide nucleic acid bonds and at least one nucleotide at the 3' end, which is linked by a phosphodiester bond, or the like, and is capable of being extended by a polymerase. Peptide nucleic acid sequences can be prepared using well known methods (see, for example, Weiler *et al.* (1997) *Nucleic acids Res.*  
25 25:2792-2799).

A polynucleotide can be a portion of a larger nucleic acid molecule, for example, a portion of a gene, which can contain a polymorphic region, or a portion of an extragenic region of a chromosome, for example, a portion of a region of nucleotide repeats such as a short tandem repeat

(STR) locus, a variable number of tandem repeats (VNTR) locus, a microsatellite locus or a minisatellite locus. A polynucleotide also can be single stranded or double stranded, including, for example, a DNA-RNA hybrid, or can be triple stranded or four stranded. Where the  
5 polynucleotide is double stranded DNA, it can be in an A, B, L or Z configuration, and a single polynucleotide can contain combinations of such configurations.

As used herein, a "mass modification," with respect to a biomolecule to be analyzed for mass spectrometry, refers to the inclusion  
10 of changes in constituent atoms or groups that change the molecular weight of the resulting molecule in defined increments detectable by mass spectrometric analysis. Mass modifications do not include radiolabels, such as isotope labels or or fluroescent gropus or other such tags normally used for detection by means other than mass spectrometry.

15 As used herein, the term "polypeptide" means at least two amino acids, or amino acid derivatives, including mass modified amino acids and amino acid analogs, which are linked by a peptide bond and which can be a modified peptide bond. A polypeptide can be translated from a polynucleotide, which can include at least a portion of a coding sequence or a  
20 portion of a nucleotide sequence that is not naturally translated due, for example, to it being located in a reading frame other than a coding frame, or it being an intron sequence, a 3' or 5' untranslated sequence or a regulatory sequence such as a promoter. A polypeptide also can be chemically synthesized and can be modified by chemical or enzymatic  
25 methods following translation or chemical synthesis. The terms "polypeptide," "peptide" and "protein" are used essentially synonymously herein, although the skilled artisan recognizes that peptides generally contain fewer than about fifty to one hundred amino acid residues, and that proteins often are obtained from a natural source and can contain,

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for example, post-translational modifications. A polypeptide can be post-translationally modified by, for example, phosphorylation (phosphoproteins) or glycosylation (glycoproteins, proteoglycans), which can be performed in a cell or in a reaction *in vitro*.

5       As used herein, the term "conjugated" refers to stable attachment, typically by virtue of a chemical interaction, including ionic and/or covalent attachment. Among the conjugation means are streptavidin- or avidin- to biotin interaction; hydrophobic interaction; magnetic interaction (*e.g.*, using functionalized magnetic beads, such as DYNABEADS, which  
10 are streptavidin-coated magnetic beads sold by Dynal, Inc. Great Neck, NY and Oslo Norway); polar interactions, such as "wetting" associations between two polar surfaces or between oligo/polyethylene glycol; formation of a covalent bond, such as an amide bond, disulfide bond, thioether bond, or via crosslinking agents; and via an acid-labile or  
15 photocleavable linker.

As used herein, "sample" refers to a composition containing a material to be detected. For the purposes herein, sample refers to anything which can contain an biomolecule. The sample can be a biological sample, such as a biological fluid or a biological tissue obtained  
20 from any organism or a cell of or from an organism or a viral particle or portions thereof. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, sperm, amniotic fluid or the like. Biological tissues are aggregates of cells, usually of a particular kind together with their intercellular  
25 substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

Thus, samples include biological samples (*e.g.*, any material

obtained from a source originating from a living being (*e.g.*, human, animal, plant, bacteria, fungi, protist, virus). The biological sample can be in any form, including solid materials (*e.g.*, tissue, cell pellets and biopsies, tissues from cadavers) and biological fluids (*e.g.*, urine, blood, saliva, amniotic fluid and mouth wash (containing buccal cells)). In certain embodiments, solid materials are mixed with a fluid. In embodiments herein, the a sample for mass spectrometric analysis includes samples that contain a mixture of matrix used for mass spectrometric analyses and the capture compound/biomolecule complexes.

As used herein, the term "solid support" means a non-gaseous, non-liquid material having a surface. Thus, a solid support can be a flat surface constructed, for example, of glass, silicon, metal, plastic or a composite; or can be in the form of a bead such as a silica gel, a controlled pore glass, a magnetic or cellulose bead; or can be a pin, including an array of pins suitable for combinatorial synthesis or analysis.

As used herein, a collection refers to combination of two or more members, generally 3, 5, 10, 50, 100, 500, 1000 or more members. In particular a collection refers to such combination of the capture compounds as provided herein.

As used herein, an array refers to a collection of elements, such as the capture compounds, containing three or more members. An addressable array is one in that the members of the array are identifiable, typically by position on a solid phase support but also by virtue of an identifier or detectable label. Hence, in general the members of an array are be immobilized to discrete identifiable loci on the surface of a solid phase. A plurality of of the compounds are attached to a support, such as an array (*i.e.*, a pattern of two or more) on the surface of a support, such as a silicon chip or other surface, generally through binding of the

sorting functionality with a group or compound on the surface of the support. Addressing can be achieved by labeling each each member electronically, such as with an radio-frequency (RF) tag, through the use of color coded beads or other such identifiable and color coded labels and  
5 through molecular weight. These labels for addressing serve as sorting functions "Q." Hence, in general the members of the array are immobilized to discrete identifiable loci on the surface of a solid phase or directly or indirectly linked to or otherwise associated with the identifiable label, such as affixed to a microsphere or other particulate support (herein  
10 referred to as beads) and suspended in solution or spread out on a surface.

As used herein, "substrate" refers to an insoluble support onto which a sample and/or matrix is deposited. Support can be fabricated from virtually any insoluble or solid material. For example, silica gel, glass  
15 (e.g., controlled-pore glass (CPG)), nylon, Wang resin, Merrifield resin, dextran cross-linked with epichlorohydrin (e.g., Sephadex<sup>®</sup>), agarose (e.g., Sepharose<sup>®</sup>), cellulose, magnetic beads, Dynabeads, a metal surface (e.g., steel, gold, silver, aluminum, silicon and copper), a plastic material (e.g., polyethylene, polypropylene, polyamide, polyester, polyvinylidene-  
20 difluoride (PVDF)) Exemplary substrate include, but are not limited to, beads (e.g., silica gel, controlled pore glass, magnetic, dextran cross-linked with epichlorohydrin (e.g., Sephadex<sup>®</sup>), agarose (e.g., Sepharose<sup>®</sup>), cellulose, capillaries, flat supports such as glass fiber filters, glass surfaces, metal surfaces (steel, gold, silver, aluminum, copper and  
25 silicon), plastic materials including multiwell plates or membranes (e.g., of polyethylene, polypropylene, polyamide, polyvinylidenedifluoride), pins (e.g., arrays of pins suitable for combinatorial synthesis or analysis or beads in pits of flat surfaces such as wafers (e.g., silicon wafers) with or without filter plates. The solid support is in any desired form, including,

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but not limited to, a bead, capillary, plate, membrane, wafer, comb, pin, a wafer with pits, an array of pits or nanoliter wells and other geometries and forms known to those of skill in the art. Supports include flat surfaces designed to receive or link samples at discrete loci. In one  
5 embodiment, flat surfaces include those with hydrophobic regions surrounding hydrophilic loci for receiving, containing or binding a sample.

The supports can be particulate or can be in the form of a continuous surface, such as a microtiter dish or well, a glass slide, a silicon chip, a nitrocellulose sheet, nylon mesh, or other such materials.  
10 When particulate, typically the particles have at least one dimension in the 5-10 mm range or smaller. Such particles, referred collectively herein as "beads", are often, but not necessarily, spherical. Reference to "bead," however, does not constrain the geometry of the matrix, which can be any shape, including random shapes, needles, fibers, and  
15 elongated. "Beads", particularly microspheres that are sufficiently small to be used in the liquid phase, are also contemplated. The "beads" can include additional components, such as magnetic or paramagnetic particles (see, *e.g.*, Dyna beads (Dynal, Oslo, Norway)) for separation using magnets, as long as the additional components do not interfere with  
20 the methods and analyses herein.

As used herein, "polymorphism" refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, *e.g.*, two different nucleotide sequences, is referred to as a "polymorphic region of a gene". A  
25 polymorphic region can be a single nucleotide, *e.g.*, a single nucleotide polymorphism (SNP), the identity of which differs in different alleles. A polymorphic region also can be several nucleotides in length.

As used herein, "polymorphic gene" refers to a gene having at least one polymorphic region.

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As used herein, "allele", which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to  
5 be homozygous for the gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions, and insertions of nucleotides. An allele of a gene also can be a form of a gene  
10 containing a mutation.

As used herein, "predominant allele" refers to an allele that is represented in the greatest frequency for a given population. The allele or alleles that are present in lesser frequency are referred to as allelic variants.

15 As used herein, "associated" refers to coincidence with the development or manifestation of a disease, condition or phenotype. Association can be due to, but is not limited to, genes responsible for housekeeping functions whose alteration can provide the foundation for a variety of diseases and conditions, those that are part of a pathway that  
20 is involved in a specific disease, condition or phenotype and those that indirectly contribute to the manifestation of a disease, condition or phenotype.

As used herein, the term "subject" refers to a living organism, such as a mammal, a plant, a fungi, an invertebrate, a fish, an insect, a  
25 pathogenic organism, such as a virus or a bacterium, and, includes humans and other mammals.

As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid molecule containing an open reading frame and including at least one exon and (optionally) an intron sequence. A gene can be either

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RNA or DNA. Genes can include regions preceding and following the coding region.

As used herein, "intron" refers to a DNA fragment present in a given gene that is spliced out during mRNA maturation.

5 As used herein, "nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: x" refers to the nucleotide sequence of the complementary strand of a nucleic acid strand having SEQ ID NO: x. The term "complementary strand" is used herein interchangeably with the term "complement". The complement of a  
10 nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand. When referring to double stranded nucleic acids, the complement of a nucleic acid having SEQ ID NO: x refers to the complementary strand of the strand having SEQ ID NO: x or to any nucleic acid having the nucleotide sequence of the complementary  
15 strand of SEQ ID NO: x. When referring to a single stranded nucleic acid having the nucleotide sequence SEQ ID NO: x, the complement of this nucleic acid is a nucleic acid having a nucleotide sequence that is complementary to that of SEQ ID NO: x.

As used herein, the term "coding sequence" refers to that portion  
20 of a gene that encodes a amino acids that constitute a polypeptide or protein.

As used herein, the term "sense strand" refers to that strand of a double-stranded nucleic acid molecule that has the sequence of the mRNA that encodes the amino acid sequence encoded by the double-  
25 stranded nucleic acid molecule.

As used herein, the term "antisense strand" refers to that strand of a double-stranded nucleic acid molecule that is the complement of the sequence of the mRNA that encodes the amino acid sequence encoded by the double-stranded nucleic acid molecule.

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As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard  
 5 single-letter designations used routinely in the art (see, Table 1).

As used herein, amino acid residue refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are, in certain embodiments, in the "L" isomeric form. Residues in the "D" isomeric  
 10 form can be substituted for any L-amino acid residue, as long as the a desired functional property is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature  
 15 described in *J. Biol. Chem.*, 243:3552-59 (1969) and adopted at 37 C.F.R. § § 1.821 - 1.822, abbreviations for amino acid residues are shown in the following Table:

**Table 1**  
Table of Correspondence

20

25

| SYMBOL   |          | AMINO ACID    |
|----------|----------|---------------|
| 1-Letter | 3-Letter |               |
| Y        | Tyr      | tyrosine      |
| G        | Gly      | glycine       |
| F        | Phe      | phenylalanine |
| M        | Met      | methionine    |
| A        | Ala      | alanine       |
| S        | Ser      | serine        |
| I        | Ile      | isoleucine    |
| L        | Leu      | leucine       |

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| SYMBOL |     |                  |
|--------|-----|------------------|
| T      | Thr | threonine        |
| V      | Val | valine           |
| P      | Pro | proline          |
| K      | Lys | lysine           |
| H      | His | histidine        |
| Q      | Gln | glutamine        |
| E      | Glu | glutamic acid    |
| Z      | Glx | Glu and/or Gln   |
| W      | Trp | tryptophan       |
| R      | Arg | arginine         |
| D      | Asp | aspartic acid    |
| N      | Asn | asparagine       |
| B      | Asx | Asn and/or Asp   |
| C      | Cys | cysteine         |
| X      | Xaa | Unknown or other |

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred to in 37 C.F.R. § § 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as  $\text{NH}_2$  or to a carboxyl-terminal group such as  $\text{COOH}$ .

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In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions

5 in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

Such substitutions can be made in accordance with those set forth in TABLE 2 as follows:

10

TABLE 2

|    |         |               |
|----|---------|---------------|
|    | Ala (A) | Gly; Ser      |
|    | Arg (R) | Lys           |
|    | Asn (N) | Gln; His      |
| 15 | Asp (D) | Glu           |
|    | Cys (C) | Ser           |
|    | Gln (Q) | Asn           |
|    | Glu (E) | Asp           |
|    | Gly (G) | Ala; Pro      |
| 20 | His (H) | Asn; Gln      |
|    | Ile (I) | Leu; Val      |
|    | Leu (L) | Ile; Val      |
|    | Lys (K) | Arg; Gln      |
|    | Met (M) | Leu; Tyr; Ile |
| 25 | Phe (F) | Met; Leu; Tyr |
|    | Ser (S) | Thr           |
|    | Thr (T) | Ser           |
|    | Trp (W) | Tyr           |
|    | Tyr (Y) | Trp; Phe      |
| 30 | Val (V) | Ile; Leu      |

Other substitutions are also permissible and can be determined empirically or in accord with known conservative substitutions.

As used herein, a DNA or nucleic acid homolog refers to a nucleic acid that includes a preselected conserved nucleotide sequence, such as

35 a sequence encoding a therapeutic polypeptide. By the term

"substantially homologous" is meant having at least 80%, at least 90% or at least 95% homology therewith or a less percentage of homology or identity and conserved biological activity or function.

The terms "homology" and "identity" are often used interchangeably. In this regard, percent homology or identity can be determined, for example, by comparing sequence information using a GAP computer program. The GAP program uses the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443 (1970)), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482 (1981)). Briefly, the GAP program defines similarity as the number of aligned symbols (e.g., nucleotides or amino acids) that are similar, divided by the total number of symbols in the shorter of the two sequences. The default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745 (1986), as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Whether any two nucleic acid molecules have nucleotide sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988). Alternatively the BLAST function of the National Center for Biotechnology Information database can be used to determine identity.

In general, sequences are aligned so that the highest order match

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is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. (See, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math* 48:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H. & Lipton, D., *SIAM J Applied Math* 48:1073 (1988). Methods to determine identity and similarity are codified in computer programs. Computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(II):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al.*, *J Molec Biol* 215:403 (1990)).

Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide. For example, a test polypeptide can be defined as any polypeptide that is 90% or more identical to a reference polypeptide.

As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming

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for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (*e.g.*, 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and  
5 reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, *e.g.*, 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or  
10 amino acid substitutions, or deletions.

As used herein: stringency of hybridization in determining percentage mismatch is as follows:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
- 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
- 15 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, *e.g.*, Sambrook, E.F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see  
20 also numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate- buffered, 0.18M NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by  $T_m$ , which is a function of the sodium ion concentration and temperature ( $T_m = 81.5^\circ \text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - 600/l$ ),  
25 so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not

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limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA* 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5),  
5 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100  
10 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and  
15 incubated an additional 1.5 hours at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

20 By way of example and not way of limitation, procedures using conditions of moderate stringency include, for example, but are not limited to, procedures using such conditions of moderate stringency are as follows: filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100  
25 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography.

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Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS.

By way of example and not way of limitation, procedures using  
5 conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization  
10 mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency  
15 which can be used are well known in the art.

The term substantially identical or substantially homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 60% or 70%, preferably means at least 80%, 85% or more preferably at least 90%, and most preferably  
20 at least 95% identity.

It is to be understood that the compounds provided herein can contain chiral centers. Such chiral centers can be of either the (R) or (S) configuration, or can be a mixture thereof. Thus, the compounds provided herein can be enantiomerically pure, or be stereoisomeric or  
25 diastereomeric mixtures. In the case of amino acid residues, such residues can be of either the L- or D-form. In one embodiment, the configuration for naturally occurring amino acid residues is L.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard

methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis, high performance liquid chromatography (HPLC) and mass spectrometry (MS), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not

5 detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers. In such

10 instances, further purification might increase the specific activity of the compound.

As used herein, a cleavable bond or moiety refers to a bond or moiety that is cleaved or cleavable under specific conditions, such as chemically, enzymatically or photolytically. Where not specified herein,

15 such bond is cleavable under conditions of MALDI-MS analysis, such as by a UV or IR laser.

As used herein, a "selectively cleavable" moiety is a moiety that can be selectively cleaved without affecting or altering the composition of the other portions of the compound of interest. For example, a cleavable

20 moiety L of the compounds provided herein is one that can be cleaved by chemical, enzymatic, photolytic, or other means without affecting or altering composition (*e.g.*, the chemical composition) of the conjugated biomolecule, including a protein. "Non-cleavable" moieties are those that cannot be selectively cleaved without affecting or altering the

25 composition of the other portions of the compound of interest.

As used herein, binding with high affinity refers to a binding that has an association constant  $k_a$  of at least  $10^9$  and generally  $10^{10}$ ,  $10^{11}$  liters/mole or greater) or a  $K_{eq}$  of  $10^9$ ,  $10^{10}$ ,  $10^{11}$ ,  $10^{12}$  or greater. For purposes herein, high affinity bonds formed by the reactivity groups are

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those that are stable to the laser (UV and IR) used in MALDI-MS analyses.

As used herein, "alkyl", "alkenyl" and "alkynyl", if not specified, contain from 1 to 20 carbons, or 1 to 16 carbons, and are straight or  
5 branched carbon chains. Alkenyl carbon chains are from 2 to 20 carbons, and, in certain embodiments, contain 1 to 8 double bonds. Alkenyl carbon chains of 1 to 16 carbons, in certain embodiments, contain 1 to 5 double bonds. Alkynyl carbon chains are from 2 to 20 carbons, and, in one embodiment, contain 1 to 8 triple bonds. Alkynyl  
10 carbon chains of 2 to 16 carbons, in certain embodiments, contain 1 to 5 triple bonds. Exemplary alkyl, alkenyl and alkynyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, sec-butyl, tert-butyl, isopentyl, neopentyl, tert-pentylyl and isohexyl. The alkyl, alkenyl and alkynyl groups, unless otherwise specified, can be  
15 optionally substituted, with one or more groups, including alkyl group substituents that can be the same or different.

As used herein, "lower alkyl", "lower alkenyl", and "lower alkynyl" refer to carbon chains having less than about 6 carbons.

As used herein, "alk(en)(yn)yl" refers to an alkyl group containing  
20 at least one double bond and at least one triple bond.

As used herein, an "alkyl group substituent" includes, but is not limited to, halo, haloalkyl, including halo lower alkyl, aryl, hydroxy, alkoxy, aryloxy, alkyloxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxy alkoxycarbonyl, oxo and cycloalkyl.

25 As used herein, "aryl" refers to aromatic groups containing from 5 to 20 carbon atoms and can be a mono-, multicyclic or fused ring system. Aryl groups include, but are not limited to, phenyl, naphthyl, biphenyl, fluorenyl and others that can be unsubstituted or are substituted with one or more substituents.

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As used herein, "aryl" also refers to aryl-containing groups, including, but not limited to, aryloxy, arylthio, arylcarbonyl and arylamino groups.

As used herein, an "aryl group substituent" includes, but is not  
5 limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, heteroaryl optionally substituted with 1 or more, including 1 to 3, substituents selected from halo, halo alkyl and alkyl, aralkyl, heteroaralkyl, alkenyl containing 1 to 2 double bonds, alkynyl containing 1 to 2 triple bonds, alk(en)(yn)yl groups, halo, pseudohalo, cyano,  
10 hydroxy, haloalkyl and polyhaloalkyl, including halo lower alkyl, especially trifluoromethyl, formyl, alkylcarbonyl, arylcarbonyl that is optionally substituted with 1 or more, including 1 to 3, substituents selected from halo, halo alkyl and alkyl, heteroarylcarbonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocar-  
15 bonyl, arylaminocarbonyl, diarylaminocarbonyl, aralkylaminocarbonyl, alkoxy, aryloxy, perfluoroalkoxy, alkenyloxy, alkynyloxy, arylalkoxy, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, arylaminoalkyl, amino, alkylamino, dialkylamino, arylamino, alkylaryl amino, alkylcarbonylamino, arylcarbonylamino, azido, nitro, mercapto, alkylthio, arylthio,  
20 perfluoroalkylthio, thiocyno, isothiocyno, alkylsulfinyl, alkylsulfonyl, arylsulfinyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl and arylaminosulfonyl.

As used herein, "aralkyl" refers to an alkyl group in that one of the hydrogen atoms of the alkyl is replaced by an aryl group.

25 As used herein, "heteroaralkyl" refers to an alkyl group in that one of the hydrogen atoms of the alkyl is replaced by a heteroaryl group.

As used herein, "cycloalkyl" refers to a saturated mono- or multi-cyclic ring system, in one embodiment, of 3 to 10 carbon atoms, or 3 to 6 carbon atoms; cycloalkenyl and cycloalkynyl refer to mono- or

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multicyclic ring systems that respectively include at least one double bond and at least one triple bond. Cycloalkenyl and cycloalkynyl groups can contain, in one embodiment, 3 to 10 carbon atoms, with cycloalkenyl groups, in other embodiments, containing 4 to 7 carbon atoms and

5 cycloalkynyl groups, in other embodiments, containing 8 to 10 carbon atoms. The ring systems of the cycloalkyl, cycloalkenyl and cycloalkynyl groups can be composed of one ring or two or more rings that can be joined together in a fused, bridged or spiro-connected fashion, and can be optionally substituted with one or more alkyl group substituents.

10 "Cycloalk(en)(yn)yl" refers to a cycloalkyl group containing at least one double bond and at least one triple bond.

As used herein, "heteroaryl" refers to a monocyclic or multicyclic ring system, in one embodiment of about 5 to about 15 members where one or more, or 1 to 3, of the atoms in the ring system is a heteroatom,

15 which is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heteroaryl can be optionally substituted with one or more, including 1 to 3, aryl group substituents. The heteroaryl group can be optionally fused to a benzene ring. Exemplary heteroaryl groups include, but are not limited to, pyrroles, porphyrines, furans, thiophenes,

20 selenophenes, pyrazoles, imidazoles, triazoles, tetrazoles, oxazoles, oxadiazoles, thiazoles, thiadiazoles, indoles, carbazoles, benzofurans, benzothiophenes, indazoles, benzimidazoles, benzotriazoles, benzoxatriazoles, benzothiazoles, benzoselenozoles, benzothiadiazoles, benzoselenadiazoles, purines, pyridines, pyridazines, pyrimidines,

25 pyrazines, pyrazines, triazines, quinolines, acridines, isoquinolines, cinnolines, phthalazines, quinazolines, quinoxalines, phenazines, phenanthrolines, imidaziny, pyrrolidinyl, pyrimidinyl, tetrazolyl, thienyl, pyridyl, pyrrolyl, N-methylpyrrolyl, quinolinyl and isoquinolinyl.

As used herein, "heteroaryl" also refers to heteroaryl-containing

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groups, including, but not limited to, heteroaryloxy, heteroarylthio, heteroarylcarbonyl and heteroarylamino.

As used herein, "heterocyclic" refers to a monocyclic or multicyclic ring system, in one embodiment of 3 to 10 members, in another  
5 embodiment 4 to 7 members, including 5 to 6 members, where one or more, including 1 to 3 of the atoms in the ring system is a heteroatom, which is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heterocycle can be optionally substituted with one or more, or 1 to 3 aryl group substituents. In certain embodiments,  
10 substituents of the heterocyclic group include hydroxy, amino, alkoxy containing 1 to 4 carbon atoms, halo lower alkyl, including trihalomethyl, such as trifluoromethyl, and halogen. As used herein, the term heterocycle can include reference to heteroaryl.

As used herein, the nomenclature alkyl, alkoxy, carbonyl, *etc.*, are  
15 used as is generally understood by those of skill in this art. For example, as used herein alkyl refers to saturated carbon chains that contain one or more carbons; the chains can be straight or branched or include cyclic portions or be cyclic.

Where the number of any given substituent is not specified (*e.g.*,  
20 "haloalkyl"), there can be one or more substituents present. For example, "haloalkyl" can include one or more of the same or different halogens. As another example, "C<sub>1-3</sub>alkoxyphenyl" can include one or more of the same or different alkoxy groups containing one, two or three carbons.

Where named substituents such as carboxy or substituents  
25 represented by variables such as W are separately enclosed in parentheses, yet possess no subscript outside the parentheses indicating numerical value and that follow substituents not in parentheses, *e.g.*, "C<sub>1-4</sub>alkyl(W)(carboxy)", "W" and "carboxy" are each directly attached to C<sub>1-4</sub>alkyl.

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As used herein, "halogen" or "halide" refers to F, Cl, Br or I.

As used herein, pseudohalides are compounds that behave substantially similar to halides. Such compounds can be used in the same manner and treated in the same manner as halides ( $X^-$ , in that X is a  
5 halogen, such as Cl or Br). Pseudohalides include, but are not limited to, cyanide, cyanate, isocyanate, thiocyanate, isothiocyanate, selenocyanate, trifluoromethoxy, and azide.

As used herein, "haloalkyl" refers to a lower alkyl radical in that one or more of the hydrogen atoms are replaced by halogen including, but  
10 not limited to, chloromethyl, trifluoromethyl, 1-chloro-2-fluoroethyl and the like.

As used herein, "haloalkoxy" refers to  $RO-$  in that R is a haloalkyl group.

As used herein, "sulfinyl" or "thionyl" refers to  $-S(O)-$ . As used  
15 herein, "sulfonyl" or "sulfuryl" refers to  $-S(O)_2-$ . As used herein, "sulfo" refers to  $-S(O)_2O-$ .

As used herein, "carboxy" refers to a divalent radical,  $-C(O)O-$ .

As used herein, "aminocarbonyl" refers to  $-C(O)NH_2$ .

As used herein, "alkylaminocarbonyl" refers to  $-C(O)NHR$  in that R  
20 is hydrogen or alkyl, including lower alkyl.

As used herein "dialkylaminocarbonyl" as used herein refers to  $-C(O)NR'R$  in that  $R'$  and R are independently selected from hydrogen or alkyl, including lower alkyl.

As used herein, "carboxamide" refers to groups of formula  
25  $-NR'COR$ .

As used herein, "diarylaminocarbonyl" refers to  $-C(O)NRR'$  in that R and  $R'$  are independently selected from aryl, including lower aryl, such as phenyl.

As used herein, "aralkylaminocarbonyl" refers to  $-C(O)NRR'$  in that

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one of R and R' is aryl, including lower aryl, such as phenyl, and the other of R and R' is alkyl, including lower alkyl.

As used herein, "arylamino" refers to  $-C(O)NHR$  in that R is aryl, including lower aryl, such as phenyl.

5 As used herein, "alkoxycarbonyl" refers to  $-C(O)OR$  in that R is alkyl, including lower alkyl.

As used herein, "aryloxycarbonyl" refers to  $-C(O)OR$  in that R is aryl, including lower aryl, such as phenyl.

10 As used herein, "alkoxy" and "alkylthio" refer to  $RO-$  and  $RS-$ , in that R is alkyl, including lower alkyl.

As used herein, "aryloxy" and "arylthio" refer to  $RO-$  and  $RS-$ , in that R is aryl, including lower aryl, such as phenyl.

As used herein, "alkylene" refers to a straight, branched or cyclic, in one embodiment straight or branched, divalent aliphatic hydrocarbon group, in certain embodiments having from 1 to about 20 carbon atoms, 15 in other embodiments 1 to 12 carbons, including lower alkylene. The alkylene group is optionally substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkylene group one or more oxygen, sulphur or substituted or unsubstituted nitrogen 20 atoms, where the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene ( $-CH_2-$ ), ethylene ( $-CH_2CH_2-$ ), propylene ( $-(CH_2)_3-$ ), cyclohexylene ( $-C_6H_{10}-$ ), methylenedioxy ( $-O-CH_2-O-$ ) and ethylenedioxy ( $-O-(CH_2)_2-O-$ ). The term "lower alkylene" refers to alkylene groups having 1 to 6 carbons. In 25 certain embodiments, alkylene groups are lower alkylene, including alkylene of 1 to 3 carbon atoms.

As used herein, "alkenylene" refers to a straight, branched or cyclic, in one embodiment straight or branched, divalent aliphatic hydrocarbon group, in certain embodiments having from 2 to about 20

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carbon atoms and at least one double bond, in other embodiments 1 to 12 carbons, including lower alkenylene. The alkenylene group is optionally substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkenylene group one or more  
5 oxygen, sulphur or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl as previously described. Exemplary alkenylene groups include  $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$  and  $-\text{CH}=\text{CH}-\text{CH}_2-$ . The term "lower alkenylene" refers to alkenylene groups having 2 to 6 carbons. In certain embodiments, alkenylene groups are lower  
10 alkenylene, including alkenylene of 3 to 4 carbon atoms.

As used herein, "alkynylene" refers to a straight, branched or cyclic, in one embodiment straight or branched, divalent aliphatic hydrocarbon group, in certain embodiments having from 2 to about 20 carbon atoms and at least one triple bond, in other embodiments 1 to 12  
15 carbons, including lower alkynylene. The alkynylene group is optionally substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkynylene group one or more oxygen, sulphur or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl as previously described. Exemplary  
20 alkynylene groups include  $-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-$ ,  $-\text{C}\equiv\text{C}-$  and  $-\text{C}\equiv\text{C}-\text{CH}_2-$ . The term "lower alkynylene" refers to alkynylene groups having 2 to 6 carbons. In certain embodiments, alkynylene groups are lower alkynylene, including alkynylene of 3 to 4 carbon atoms.

As used herein, "alk(en)(yn)ylene" refers to a straight, branched or  
25 cyclic, in one embodiment straight or branched, divalent aliphatic hydrocarbon group, in certain embodiments having from 2 to about 20 carbon atoms and at least one triple bond, and at least one double bond; in other embodiments 1 to 12 carbons, including lower alk(en)(yn)ylene. The alk(en)(yn)ylene group is optionally substituted with one or more

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"alkyl group substituents." There can be optionally inserted along the alkynylene group one or more oxygen, sulphur or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl as previously described. Exemplary alk(en)(yn)ylene groups include

5  $-\text{C}=\text{C}-(\text{CH}_2)_n-\text{C}\equiv\text{C}-$ , where  $n$  is 1 or 2. The term "lower alk(en)(yn)ylene" refers to alk(en)(yn)ylene groups having up to 6 carbons. In certain embodiments, alk(en)(yn)ylene groups are lower alk(en)(yn)ylene, including alk(en)(yn)ylene of 4 carbon atoms.

As used herein, "arylene" refers to a monocyclic or polycyclic, in  
10 one embodiment monocyclic, divalent aromatic group, in certain embodiments having from 5 to about 20 carbon atoms and at least one aromatic ring, in other embodiments 5 to 12 carbons, including lower arylene. The arylene group is optionally substituted with one or more "alkyl group substituents." There can be optionally inserted around the  
15 arylene group one or more oxygen, sulphur or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl as previously described. Exemplary arylene groups include 1,2-, 1,3- and 1,4-phenylene. The term "lower arylene" refers to arylene groups having 5 or 6 carbons. In certain embodiments, arylene groups are lower  
20 arylene.

As used herein, "heteroarylene" refers to a divalent monocyclic or multicyclic ring system, in one embodiment of about 5 to about 15 members where one or more, or 1 to 3 of the atoms in the ring system is a heteroatom, which is, an element other than carbon, for example,  
25 nitrogen, oxygen and sulfur atoms. The heteroarylene group can be optionally substituted with one or more, or 1 to 3, aryl group substituents.

As used herein, "alkylidene" refers to a divalent group, such as  $=\text{CR}'\text{R}''$ , which is attached to one atom of another group, forming a

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double bond. Exemplary alkylidene groups are methyldene ( $=\text{CH}_2$ ) and ethylidene ( $=\text{CHCH}_3$ ). As used herein, "aralkylidene" refers to an alkylidene group in that either R' or R" is an aryl group.

As used herein, "amido" refers to the divalent group  $-\text{C}(\text{O})\text{NH}-$ .

5 "Thioamido" refers to the divalent group  $-\text{C}(\text{S})\text{NH}-$ . "Oxyamido" refers to the divalent group  $-\text{OC}(\text{O})\text{NH}-$ . "Thiaamido" refers to the divalent group  $-\text{SC}(\text{O})\text{NH}-$ . "Dithiaamido" refers to the divalent group  $-\text{SC}(\text{S})\text{NH}-$ . "Ureido" refers to the divalent group  $-\text{HNC}(\text{O})\text{NH}-$ . "Thioureido" refers to the divalent group  $-\text{HNC}(\text{S})\text{NH}-$ .

10 As used herein, "semicarbazide" refers to  $-\text{NHC}(\text{O})\text{NHNH}-$ .

"Carbazate" refers to the divalent group  $-\text{OC}(\text{O})\text{NHNH}-$ .

"Isothiocarbazate" refers to the divalent group  $-\text{SC}(\text{O})\text{NHNH}-$ .

"Thiocarbazate" refers to the divalent group  $-\text{OC}(\text{S})\text{NHNH}-$ .

"Sulfonylhydrazide" refers to the group  $-\text{SO}_2\text{NHNH}-$ . "Hydrazide" refers to the divalent group  $-\text{C}(\text{O})\text{NHNH}-$ . "Azo" refers to the divalent group  $-\text{N}=\text{N}-$ . "Hydrazinyl" refers to the divalent group  $-\text{NH}-\text{NH}-$ .

As used herein, the term "amino acid" refers to  $\alpha$ -amino acids that are racemic, or of either the D- or L-configuration. The designation "d" preceding an amino acid designation (*e.g.*, dAla, dSer, dVal, etc.) refers to the D-isomer of the amino acid. The designation "dl" preceding an amino acid designation (*e.g.*, dlAla) refers to a mixture of the L- and D-isomers of the amino acid.

As used herein, when any particular group, such as phenyl or pyridyl, is specified, this means that the group is unsubstituted or is substituted. Substituents where not specified are halo, halo lower alkyl, and lower alkyl.

As used herein, conformationally altered protein disease (or a disease of protein aggregation) refers to diseases associated with a protein or polypeptide that has a disease-associated conformation. The

methods and collections provided herein permit detection of a conformer associated with a disease to be detected. Diseases and associated proteins that exhibit two or more different conformations in which at least one conformation is a conformationally altered protein include, but are not  
5 limited to, amyloid diseases and other neurodegenerative diseases known to those of skill in the art and set forth below.

As used herein, cell sorting refers to an assay in which cells are separated and recovered from suspension based upon properties measured in flow cytometry analysis. Most assays used for analysis can  
10 serve as the basis for sorting experiments, as long as gates and regions defining the subpopulation(s) to be sorted do not logically overlap. Maximum throughput rates are typically 5000 cells/second ( $18 \times 10^6$  cells/hour). The rate of collection of the separated population(s) depends primarily upon the condition of the cells and the percentage of reactivity.

15 As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, *Biochem.* 1972, 11:942). For example, DMF = *N,N*-dimethylformamide, DMAc = *N,N*-di-  
20 methylacetamide; THF = tetrahydrofuran; TRIS = tris(hydroxymethyl)-aminomethane; SSPE = saline-sodium phosphate-EDTA buffer; EDTA = ethylenediaminetetraacetic acid; SDS = sodium dodecyl sulfate.

**B. Collections of capture compounds**

Collections of capture compounds that selectively bind to  
25 biomolecules in samples, such as biomolecules, particularly, although not exclusively, a cell lysate or *in vitro* translated polypeptides from a cell lysate are provided. Each capture compound in the collection can bind to specific groups or classes of biopolymers, and is designed to covalently or tightly (sufficient to sustain mass spectrometric analysis, for

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example) to a subset of all of the biomolecules in the sample. For example, a sample can contain 1000's of members, for example a cell lysate. The collections of compounds permit sufficient selectivity so that, for example, about 10-20 of the components of the sample bind to  
5 each member of the collection. The exact number is a small enough number for routine analyses to identify them, generally in one step, such as by mass spectrometry.

The collections permit a top down holistic approach to analysis of the proteome, including post-translationally modified proteins, and other  
10 biomolecules. Protein and other biomolecule patterns are the starting point for analyses that use these collections; rather than nucleic acids and the genome (bottom up). The collections can be used to assess the biomolecule components of a sample, such as a biological sample, to identify components specific to a particular phenotype, such as a disease  
15 state, to identify structural function, biochemical pathways and mechanisms of action. The collections and methods of use permit an unbiased analysis of biomolecules, since the methods do not necessarily assess specific classes of targets, instead, changes in samples are detected or identified. The collections permit the components of a  
20 complex mixture of biomolecules (*i.e.*, a mixture of 50, 100, 500, 1000, 2000 and more) to be sorted into discrete loci containing reduced numbers, typically by 10%, 50% or greater reduction in complexity, or to about 1 to 50 different biomolecules per locus in an array, so that the components at each spot can be analyzed, such as by mass  
25 spectrometric analysis alone or in combination with other analyses. In some embodiments, such as for phenotypic analyses, homogeneity of the starting sample, such as cells, can be important. To provide homogeneity, cells, with different phenotypes, such as diseased versus healthy, from the same individual are compared. Methods for doing so

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are provided herein.

By virtue of the structure of compounds in the collections, the collections can be used to detect structural changes, such as those from the post-translational processing of proteins, and can be used to detect  
5 changes in membrane proteins, which are involved in the most fundamental processes, such as signal transduction, ion channels, receptors for ligand interaction and cell-to-cell interactions. When cells become diseased, changes associated with disease, such as transformation, often occur in membrane proteins.

10 The collections contain sets of member capture compounds. In general, members of each set differ in at least one functional group, and generally in two or three, from members of the other sets. Thus, for example, if the compounds include a reactivity function, a selectivity  
15 function and a sorting function, each set differs in at least the sorting function, typically in at least in the sorting and selectivity function, and generally in all three functions. The solubility functions, if present, which are selected to permit assaying in a selected environment, can differ among the compounds, or can be the same among all sets.

In practicing methods, the collections are contacted with a sample  
20 or partially purified or purified components thereof to effect binding of biomolecules to capture compounds in the collection. The capture compounds can be in an addressable array, such as bound to a solid support prior to contacting, or can be arrayed after contacting with the sample. The resulting array is optionally treated with a reagent that  
25 specifically cleaves the bound polymers, such as a protease, and is subjected to analysis, particularly mass spectrometric analysis to identify components of the bound biomolecules at each locus. Once a molecular weight of a biomolecule, such as a protein or portion thereof of interest is determined, the biomolecule can be identified. Methods for identification

include comparison of the molecular weights with databases, for example protein databases that include protease fragments and their molecular weights.

The capture compounds that include functional groups that confer  
5 reactivity, selective and separative properties, depending on the specificity of separation and analysis required (which depends on the complexity of the mixture to be analyzed). As more functional groups are added to the compounds, the compounds can exhibit increased selectivity and develop a signature for target molecules similar to an antigen (Ag)  
10 binding site on an antibody. In general, the compounds provided herein include at least two functional groups (functions) selected from four types of functions: a reactivity function, which binds to biopolymers either covalently or with a high  $k_s$  (generally greater than about  $10^9$ ,  $10^{10}$ ,  $10^{12}$  liters/mole and/or such that the binding is substantially irreversible or  
15 stable under conditions of mass spectrometric analyses, such as MALDI-MS conditions); a selectivity function, which by virtue of non-covalent interactions alters, generally increases, the specificity of the reactivity function; a sorting function, which permits the compounds to be addressed (arrayed or otherwise separated based according to the  
20 structure of the capture compound; and a solubility function, which when selected alters the solubility of the compounds depending upon the environment in which reactions are performed, permitting the conditions to simulate physiological conditions. In general, the reactivity function is the reactive group that specifically interacts, typically covalently or with  
25 high binding affinity ( $k_s$ ), with particular biomolecules, such as proteins, or portions thereof; and the other functionality, the selectivity functions, alters, typically increasing, the specificity of the reactivity function. In general, the reactive function covalently interacts with groups on a particular biomolecule, such as amine groups on the surface of a protein.

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The reactivity function interacts with biomolecules to form a covalent bond or a non-covalent bond that is stable under conditions of analysis, generally with a  $k_s$  of greater than  $10^9$  liters/mole or greater than  $10^{10}$  liters/mole. Conditions of analysis include, but are not limited to, mass spectrophotometric analysis, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The selectivity function influences the types of biomolecules that can interact with the reactivity function through a non-covalent interaction. The selectivity function alters the specificity for the particular groups, generally reducing the number of such groups with which the reactivity functions react. A goal is to reduce the number of proteins or biomolecules bound at a locus, so that the proteins can then be separated, such as by mass spectrometry.

Included among the capture compounds provided herein are those that can, the compounds for use in the methods herein can be classified in at least two sets: one for reactions in aqueous solution (*e.g.*, for reaction with hydrophilic biomolecules), and the other for reaction in organic solvents (*e.g.*, chloroform) (*e.g.*, for reaction with hydrophobic biomolecules). Thus, in certain embodiments, the compounds provided herein discriminate between hydrophilic and hydrophobic biomolecules, including, but not limited to, proteins, and allow for analysis of both classes of biomolecules.

### C. Capture Compounds

Capture compounds (also referred to as capture agents) are provided. The capture compounds include a core "Z" that presents one or more reactivity functions "X" and optionally at least a selectivity function "Y" and/or a sorting function "Q", and also optionally one or more solubility functions "W." Additionally, cleavable linkers and other functions are included in the molecules. The particular manner in which

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the functions are presented on the core or scaffold is a matter of design choice, but are selected such that the resulting molecule has the property that it captures biomolecules, particularly proteins, with sufficient specificity and either covalently or with bonds of sufficient stability or affinity to permit analysis, such as by mass spectrometry, including MALDI mass spectrometric analysis, so that at least a portion of bound biomolecules remain bound (generally a binding affinity of  $10^9$ ,  $10^{10}$ ,  $10^{11}$  liters/mole or greater, or a  $K_{eq}$  of  $10^9$ ,  $10^{10}$ ,  $10^{11}$ ,  $10^{12}$  or greater).

X, the reactivity functionality, is selected to be anything that forms such a covalent bond or a bond of high affinity that is stable under conditions of mass spectrometric analysis, particularly MALDI analysis. The selectivity functionality Y, is a group that "looks" at the topology of the protein around reactivity binding sites and functions to select particular groups on biomolecules from among those with which a reactivity group can form a covalent bond (or high affinity bond). For example, a selectivity group can cause steric hindrance, or permit specific binding to an epitope, or anything in between. It can be a substrate for a drug, lipid or peptide. It selects the environment of the groups with which the reactivity function interacts. The selectivity functionality Y, can be one whereby a capture compound forms a covalent bond with a biomolecule in a mixture or interacts with high stability such that the affinity of binding of the capture compound to the biomolecule through the reactive functionality in the presence of the selectivity functionality is at least ten-fold or 100-fold greater than in the absence of the selectivity functionality.

Q is a sorting function that can be anything that provides a means for separating each set of capture compounds from the others, such as by arraying, and includes groups such as biotin, generally a spacer binding to an avidin on a surface (or vice versa) array, oligonucleotides

for binding oligonucleotide arrays or any molecule that has a cognate binding partner to which it binds with sufficient affinity to survive mass spectrometric analysis, such as MALDI-MS analysis, can be selected. For any collection a variety of different sorting groups can be used; each set  
5 of capture compounds should have unique Q compared to the other sets. In addition, labeling means that can be sorted by virtue of the label, such as RF tags, fluorescent tags, color-coded tags or beads, bar-coded or other symbology labeled tags and other such labels can be used. For example, the capture compounds or the X, Y, Z, W functionalities can be  
10 on a surface that is attached to an RF tag or a colored tag. These can be readily sorted after reaction so that each set can be separately analyzed to identify bound biomolecules. Thus, the collections can include capture compounds that have a variety of sorting groups.

The solubility function, W, permits alteration in properties of the  
15 capture compound components of the collection. For example, W can be selected so that the capture compounds are soluble or not in a particular reaction medium or environment, such as a hydrophobic environment, thereby permitting reactions with membrane components. The collections include sets of capture compounds, each of which set differs in Q and at  
20 least one or both X and Y.

As noted, among the capture compounds provided are those with at least three functionalities: reactivity, sorting and solubility. The sorting function can be selectively cleavable to permit its removal. These compounds also can include a selectivity function to alter the range of  
25 binding of the reactivity function, which binds either covalently or with high affinity ( $k_d$  greater than  $10^9$  to biomolecules, and optionally one or both of a sorting and solubility function.

More detailed description and discussion of each functionality and non-limiting exemplary embodiments follow.

### 1. Z, the Core

Generally all compounds include a function, even if it is one atom, such as carbon, for presenting the functional groups. In certain embodiments herein, in the capture compounds for use in the methods  
5 provided herein, Z is a moiety that is cleavable prior to or during analysis of the biomolecule, including mass spectral analysis, without altering the chemical structure of the biomolecule, including, but not limited to, a protein.

For example, in some embodiments, the methods provided herein  
10 include a step of mass spectral analysis of biomolecules, including proteins, which are displayed in an addressable format. In certain embodiments, the compounds are then bound to an array of single oligonucleotides that include single-stranded portions (or portions that can be made single-stranded) that are complementary to the oligonucleotide  
15 portions, or oligonucleotide analog portions, (Q, the sorting function) of the capture compounds. In these embodiments, Z can be selected to be a group that is (i) stable to the reaction conditions required for reaction of the compounds provided herein with the biomolecule, such as a protein, (ii) stable to the conditions required for hybridization of the Q moiety with  
20 the single stranded oligonucleotides, and (iii) cleavable prior to or during analysis of the biomolecule.

In another embodiment, Z with the linked functional groups can be designed so that with the Q, X, W and/or Y it dissolved into lipid bilayers of a cell membrane, thereby contacting internal portions of cell membrane  
25 proteins through the X and Y functions. In this embodiment, the support captures proteins, such as membrane proteins and organelle proteins, including proteins within cell membranes. The capture compounds and functional group can be selected so that the resulting capture compounds function under selected physiological conditions. Thus, the choice of Z,

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Q, X, W and/or Y allows for design of surfaces and supports that mimic cell membranes and other biological membranes.

In some embodiments, a lipid bilayer, such as as those used for forming liposomes and other micelles, can be provided on the surface of a support as a way of maintaining the structures of membrane proteins to make a lipid bilayer on the surface. This can be employed where the support is the "Z" function and the other functions are linked thereto, or where the compounds are linked to a support through a Q group, such as by double-stranded oligonucleotides. The resulting immobilized capture compounds can be coated with or dissolved in a lipid coating. As a result, the compounds and collections provided herein can act as an artificial membrane, dendrimer polymer chemistry can be employed for controlled synthesis of membranes having consistent pore dimensions and membrane thicknesses, through synthesis of amphiphilic dendrimeric or hyperbranched block copolymers that can be self-assembled to form ultrathin organic film membranes on porous supports. In one embodiment, an organic film membrane is composed of a linear-dendritic diblock copolymer composed of polyamidoamine (PAMAM) dendrimer attached to one end of a linear polyethylene oxide (PEO) block.

**Z is cleavable under the conditions of mass spectrometric analysis**

In one such embodiment, Z is a photocleavable group that is cleaved by a laser used in MALDI-TOF mass spectrometry. In another embodiment, Z is an acid labile group that is cleaved upon application of a matrix for mass spectrometric analysis to arrayed, such as hybridized compound-biomolecule conjugates, or by exposure to acids (e.g., trifluoroacetic or hydrochloric acids) in a vapor or liquid form, prior to analysis. In this embodiment, the matrix maintains the spacial integrity of the array, allowing for addressable analysis of the array.

**Z is not cleavable under the conditions of mass spectrometric analysis**

In certain embodiments, the capture compounds for use in the methods provided herein have a Z moiety that is not cleavable under conditions used for analysis of biomolecules, including, but not limited to, mass spectrometry, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Capture compounds of these embodiments can be used, for example, in methods provided herein for identifying biomolecules in mixtures thereof, for determining biomolecule-biomolecule, including protein-protein, interactions, and for determining biomolecule-small molecule, including protein-drug or protein-drug candidate, interactions. In these embodiments, it is not necessary for the Z group to be cleaved for the analysis.

Thus, as noted, Z can be virtually any moiety that serves as a core to present the binding (the selectivity and reactivity functions) and the solubility and sorting functions. A variety are exemplified herein, but others may be substituted. The precise nature can be a matter of design choice in view of the disclosure herein and the skill of the skilled artisan

**a. Divalent Z moieties**

In one embodiment, Z is a cleavable or non-cleavable divalent group that contains, generally 50 or fewer, or less than 20 members, and is selected from straight or branched chain alkylene, straight or branched chain alkenylene, straight or branched chain alkynylene, straight or branched chain alkyleneoxy, straight or branched chain alkylenthio, straight or branched chain alkylencarbonyl, straight or branched chain alkylenamino, cycloalkylene, cycloalkenylene, cycloalkynylene, cycloalkyleneoxy, cycloalkylenthio, cycloalkylencarbonyl, cycloalkylenamino, heterocyclylene, arylene, aryleneoxy, arylenthio, arylencarbonyl, arylenamino, heteroarylene, heteroaryleneoxy,

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heteroarylentio, heteroarylencarbonyl, heteroarylenamino, oxy, thio, carbonyl, carbonyloxy, ester, amino, amido, phosphino, phosphineoxido, phosphoramidato, phosphinamidato, sulfonamido, sulfonyl, sulfoxido, carbamato, ureido, and combinations thereof, and is optionally

- 5 substituted with one or more, including one, two, three or four, substituents each independently selected from Y, as described elsewhere herein.

- In other embodiments, Z is a divalent cleavable or non-cleavable group selected from straight or branched chain alkyl, straight or branched
- 10 chain alkenyl, straight or branched chain alkynyl,  $-(C(R^{15})_2)_d-$ ,  $-O-$ ,  $-S-$ ,  $-(CH_2)_d-$ ,  $-(CH_2)_dO-$ ,  $-(CH_2)_dS-$ ,  $>N(R^{15})$ ,  $-(S(O)_u)-$ ,  $-(S(O)_2)_w-$ ,  $>C(O)$ ,  $-(C(O))_w-$ ,  $-(C(S(O)_u))_w-$ ,  $-(C(O)O)_w-$ ,  $-(C(R^{15})_2)_dO-$ ,  $-(C(R^{15})_2)_dS(O)_u-$ ,  $-O(C(R^{15})_2)_d-$ ,  $-S(O)_u(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_dO(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_dS(O)_u(C(R^{15})_2)_d-$ ,  $-N(R^{15})(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_dNR^{15}-$ ,
- 15  $-(C(R^{15})_2)_dN(R^{15})(C(R^{15})_2)_d-$ ,  $-(S(R^{15})(O)_u)_w-$ ,  $-(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_dO(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_d(C(O)O)_w(C(R^{15})_2)_d-$ ,  $-(C(O)O)_w(C(R^{15})_2)_d-$ ,  $-(C(R^{15})_2)_d(C(O)O)_w-$ ,  $-(C(S)(R^{15}))_w-$ ,  $-(C(O))_w(CR^{15})_2)_d-$ ,  $-(CR^{15})_d(C(O))_w(CR^{15})_d-$ ,  $-(C(R^{15})_2)_d(C(O))_w-$ ,  $-N(R^{15})(C(R^{15})_2)_w-$ ,  $-OC(R^{15})_2C(O)-$ ,  $-O((R^{15})_2C(O)N(R^{15})-$ ,  $-(C(R^{15})_2)_wN(R^{15})(C(R^{15})_2)_w-$ ,
- 20  $-(C(R^{15})_2)_wN(R^{15})-$ ,  $>P(O)_v(R^{15})_x$ ,  $>P(O)_u(R^{15})_3$ ,  $>P(O)_u(C(R^{15})_2)_d$ ,  $>Si(R^{15})_2$  and combinations of any of these groups;

where u, v and x are each independently 0 to 5;

each d is independently an integer from 1 to 20, or 1 to 12, or 1-6, or 1 to 3;

- 25 each w is independently an integer selected from 1 to 6, or 1 to 3, or 1 to 2; and

each  $R^{15}$  is independently a monovalent group selected from straight or branched chain alkyl, straight or branched chain alkenyl, straight or branched chain alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl,

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- heterocyclyl, straight or branched chain heterocyclylalkyl, straight or branched chain heterocyclylalkenyl, straight or branched chain heterocyclylalkynyl, aryl, straight or branched chain arylalkyl, straight or branched chain arylalkenyl, straight or branched chain arylalkynyl,
- 5 heteroaryl, straight or branched chain heteroarylalkyl, straight or branched chain heteroarylalkenyl, straight or branched chain heteroarylalkynyl, halo, straight or branched chain haloalkyl, pseudohalo, azido, cyano, nitro,  $\text{OR}^{60}$ ,  $\text{NR}^{60}\text{R}^{61}$ ,  $\text{COOR}^{60}$ ,  $\text{C(O)R}^{60}$ ,  $\text{C(O)NR}^{60}\text{R}^{61}$ ,  $\text{S(O)}_q\text{R}^{60}$ ,  $\text{S(O)}_q\text{OR}^{60}$ ,  $\text{S(O)}_q\text{NR}^{60}\text{R}^{61}$ ,  $\text{NR}^{60}\text{C(O)R}^{61}$ ,  $\text{NR}^{60}\text{C(O)NR}^{60}\text{R}^{61}$ ,  $\text{NR}^{60}\text{S(O)}_q\text{R}^{60}$ ,  $\text{SiR}^{60}\text{R}^{61}\text{R}^{62}$ ,
- 10  $\text{P(R}^{60})_2$ ,  $\text{P(O)(R}^{60})_2$ ,  $\text{P(OR}^{60})_2$ ,  $\text{P(O)(OR}^{60})_2$ ,  $\text{P(O)(OR}^{60})(\text{R}^{61})$  and  $\text{P(O)NR}^{60}\text{R}^{61}$ , where  $q$  is an integer from 0 to 2;
- each  $\text{R}^{60}$ ,  $\text{R}^{61}$ , and  $\text{R}^{62}$  is independently hydrogen, straight or branched chain alkyl, straight or branched chain alkenyl, straight or branched chain alkynyl, aryl, straight or branched chain aralkyl, straight or
- 15 branched chain aralkenyl, straight or branched chain aralkynyl, heteroaryl, straight or branched chain heteroaralkyl, straight or branched chain heteroaralkenyl, straight or branched chain heteroaralkynyl, heterocyclyl, straight or branched chain heterocyclylalkyl, straight or branched chain heterocyclylalkenyl or straight or branched chain heterocyclylalkynyl.
- 20 In other embodiments,  $Z$  is a cleavable or non-cleavable divalent group having any combination of the following groups: arylene, heteroarylene, cycloalkylene,  $>\text{C(R}^{15})_2$ ,  $-\text{C(R}^{15})=\text{C(R}^{15})-$ ,  $>\text{C}=\text{C(R}^{23})(\text{R}^{24})$ ,  $>\text{C(R}^{23})(\text{R}^{24})$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{O}-$ ,  $>\text{S(A)}_u$ ,  $>\text{P(D)}_v(\text{R}^{15})$ ,  $>\text{P(D)}_v(\text{ER}^{15})$ ,  $>\text{N(R}^{15})$ ,  $>\text{N}^+(\text{R}^{23})(\text{R}^{24})$ ,  $>\text{Si(R}^{15})_2$  or  $>\text{C(E)}$ ; where  $u$  is 0, 1 or 2;  $v$  is 0, 1, 2 or 3;
- 25  $A$  is  $-\text{O}-$  or  $-\text{NR}^{15}$ ;  $D$  is  $-\text{S}-$  or  $-\text{O}-$ ; and  $E$  is  $-\text{S}-$ ,  $-\text{O}-$  or  $-\text{NR}^{15}$ ; that groups can be combined in any order;
- each  $\text{R}^{15}$  is a monovalent group independently selected from the group consisting of hydrogen and  $\text{V-R}^{18}$ ;
- each  $V$  is a divalent group independently having any combination of

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the following groups: a direct link, arylene, heteroarylene, cycloalkylene,  
 $>C(R^{17})_2$ ,  $-C(R^{17})=C(R^{17})-$ ,  $>C=C(R^{23})(R^{24})$ ,  $>C(R^{23})(R^{24})$ ,  $-C\equiv C-$ ,  $-O-$ ,  
 $>S(A)_u$ ,  $>P(D)_v(R^{17})$ ,  $>P(D)_v(ER^{17})$ ,  $>N(R^{17})$ ,  $>N(COR^{17})$ ,  $>N^+(R^{23})(R^{24})$ ,  
 $>Si(R^{17})_2$  and  $>C(E)$ ; where u is 0, 1 or 2; v is 0, 1, 2 or 3; A is  $-O-$  or  
5  $-NR^{17}$ ; D is  $-S-$  or  $-O-$ ; and E is  $-S-$ ,  $-O-$  or  $-NR^{17}$ ; that groups can be  
combined in any order;

$R^{17}$  and  $R^{18}$  are each independently selected from the group  
consisting of hydrogen, halo, pseudohalo, cyano, azido, nitro,  
 $-SiR^{27}R^{28}R^{25}$ , alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl,  
10 aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl,  
heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl,  
heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy  
and  $-NR^{19}R^{20}$ ;

$R^{19}$  and  $R^{20}$  are each independently selected from hydrogen, alkyl,  
15 alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl and  
heterocyclyl;

$R^{23}$  and  $R^{24}$  are selected from (i) or (ii) as follows:

(i)  $R^{23}$  and  $R^{24}$  are independently selected from the group consisting  
of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl; or  
20 (ii)  $R^{23}$  and  $R^{24}$  together form alkylene, alkenylene or cycloalkylene;

$R^{25}$ ,  $R^{27}$  and  $R^{28}$  are each independently a monovalent group  
selected from hydrogen, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl,  
aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl,  
heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl,  
25 heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy  
and  $-NR^{19}R^{20}$ ;

$R^{15}$ ,  $R^{17}$ ,  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$ ,  $R^{23}$ ,  $R^{24}$ ,  $R^{25}$ ,  $R^{27}$  and  $R^{28}$  can be substituted  
with one or more substituents each independently selected from  $Z^2$ , in  
that  $Z^2$  is selected from alkyl, alkenyl, alkynyl, aryl, cycloalkyl,

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cycloalkenyl, hydroxy,  $-S(O)_hR^{35}$  in that h is 0, 1 or 2,  $-NR^{35}R^{36}$ ,  $-COOR^{35}$ ,  $-COR^{35}$ ,  $-CONR^{35}R^{36}$ ,  $-OC(O)NR^{35}R^{36}$ ,  $-N(R^{35})C(O)R^{36}$ , alkoxy, aryloxy, heteroaryl, heterocyclyl, heteroaryloxy, heterocyclyloxy, aralkyl, aralkenyl, aralkynyl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, aralkoxy, heteroaralkoxy, alkoxycarbonyl, carbamoyl, thiocarbamoyl, alkoxycarbonyl, carboxyaryl, halo, pseudohalo, haloalkyl and carboxamido;

$R^{35}$  and  $R^{36}$  are each independently selected from among hydrogen, halo, pseudohalo, cyano, azido, nitro, trialkylsilyl, dialkylarylsilyl, alkyl diarylsilyl, triarylsilyl, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy, amino, amido, alkylamino, dialkylamino, alkylaryl amino, diarylamino and arylamino.

In certain embodiments herein, the compounds are selected with the proviso that Z is cleavable prior to or during analysis, including mass spectral analysis, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, of the biomolecule.

In certain embodiments, Z is at least a trivalent moiety selected from the divalent moieties disclosed herein absent at least one hydrogen. The capture compounds in the collections provided herein include a core Z that has a variety of valencies. Among the capture compounds are those in which Z is at least trivalent. Also among the compounds in the collections are those where Z is divalent and linked to either a Q and an X, or a Q and a Y, or an X and a Y, or other combination of the functionalities provided herein.

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**(i) Cleavable divalent Z moieties**

In one embodiment, Z is a cleavable divalent moiety and has the formula:  $-(S^1)_t-M(R^{15})_a-(S^2)_b-L-$ ,

where  $S^1$  and  $S^2$  are spacer moieties; t and b are each independently 0 or

5 1; M is a central moiety possessing two or more points of attachment

(*i.e.*, divalent or higher valency); in certain embodiments, two to six

points of attachment (*i.e.*, divalent to hexavalent), in other embodiments,

2, 3, 4 or 5 points of attachment (*i.e.*, divalent, trivalent, tetravalent or

pentavalent);  $R^{15}$  is as described above; a is 0 to 4, in certain

10 embodiments, 0, 1 or 2; and L is a bond that is cleavable prior to or

during analysis, including mass spectral analysis, of a biomolecule

without altering the chemical structure of the biomolecule, such as a protein.

**(a) M**

15 In certain embodiments, M is alkylene, phenylene, biphenylene or a divalent heterobifunctional trityl derivative. M is unsubstituted or is substituted with 1 to 4 groups, each independently selected from  $R^{15}$ .

In other embodiments, M is selected from  $-(CH_2)_r-$ ,  $-(CH_2O)_r-$ ,

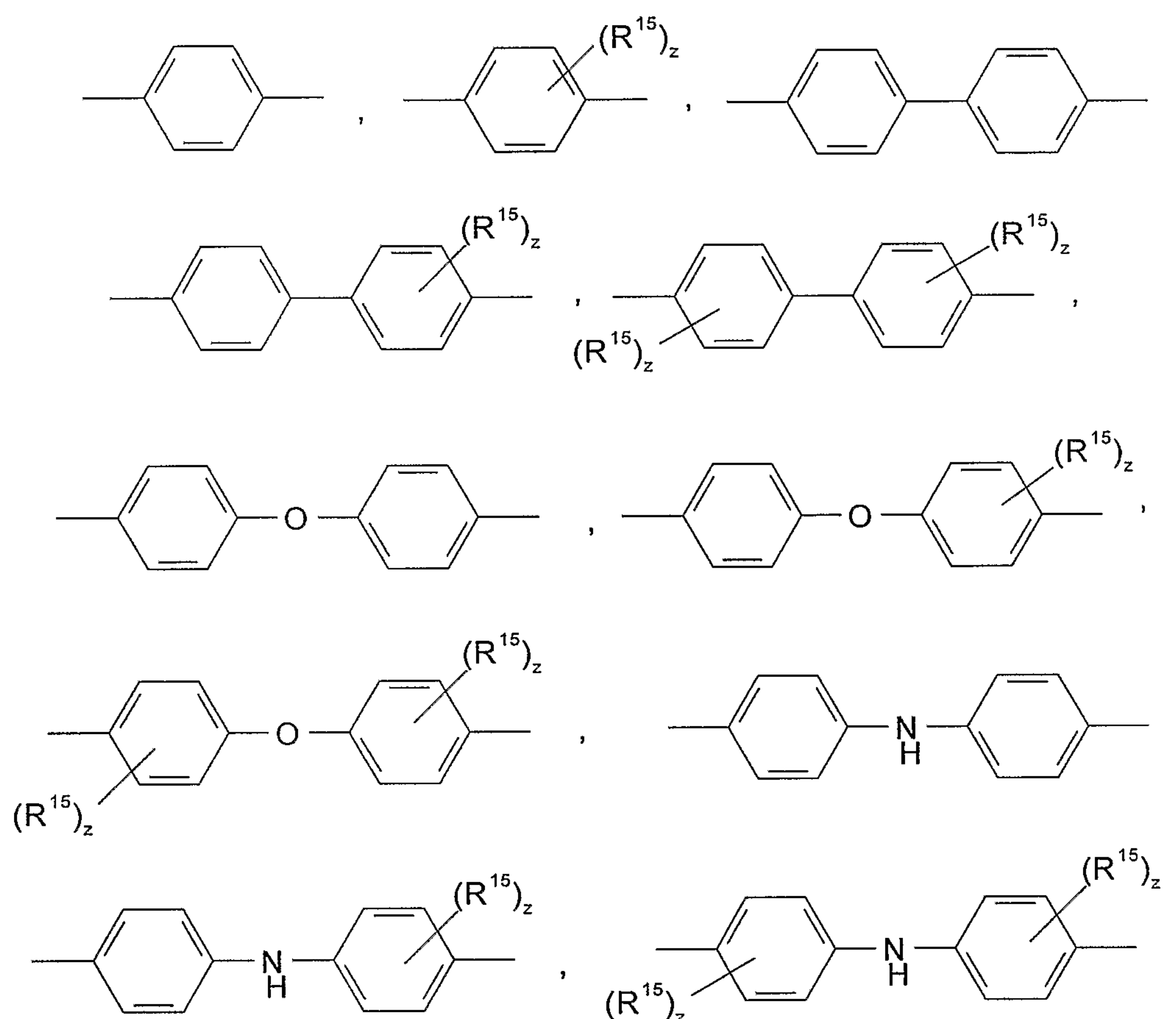
$-(CH_2CH_2-O)_r-$ ,  $-(NH-(CH_2)_r-C(=O))_s-$ ,  $-(NH-CH(R^{52})-C(=O))_r-$ ,

20  $-(O-(CH)_r-C(=O))_s-$ ,

25

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where  $R^{15}$  is as defined above;  $r$  and  $s$  are each independently an integer from 1 to 10;  $R^{52}$  is the side chain of a natural  $\alpha$ -amino acid; and  $z$  is an integer from 1 to 4. In one embodiment,  $z$  is 1.

- In certain embodiments,  $R^{15}$  is -H, -OH, -OR<sup>51</sup>, -SH, -SR<sup>51</sup>, -NH<sub>2</sub>,  
 5 -NHR<sup>51</sup>, -N(R<sup>51</sup>)<sub>2</sub>, -F, -Cl, -Br, -I, -SO<sub>3</sub>H, -PO<sub>4</sub><sup>-2</sup>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -CH(CH<sub>3</sub>)<sub>2</sub>  
 or -C(CH<sub>3</sub>)<sub>3</sub>; where  $R^{51}$  is straight or branched chain alkyl, straight or  
 branched chain alkenyl, straight or branched chain alkynyl, aryl,  
 heteroaryl, cycloalkyl, heterocyclyl, straight or branched chain aralkyl,  
 straight or branched chain aralkenyl, straight or branched chain aralkynyl,  
 10 straight or branched chain heteroaralkyl, straight or branched chain  
 heteroaralkenyl, straight or branched chain heteroaralkynyl, straight or  
 branched chain cycloalkylalkyl, straight or branched chain

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cycloalkylalkenyl, straight or branched chain cycloalkylalkynyl, straight or branched chain heterocyclalkyl, straight or branched chain heterocyclalkenyl or straight or branched chain heterocyclalkynyl.

(b)  $S^1$  and  $S^2$

5            Optionally, a spacer region  $S^1$  and/or  $S^2$  can be present on either or both sides of the central moiety M (linked to Z) of the compounds, for example, to reduce steric hindrance in reactions with the surface of large biomolecules and/or for facilitating sorting. These can be any groups that provide for spacing, typically without altering desired functional properties  
10 of the capture compounds and/or capture compound/biomolecule complexes. Those of skill in the art in light of the disclosure herein, can readily select suitable spacers. Exemplary spacers are set forth below.

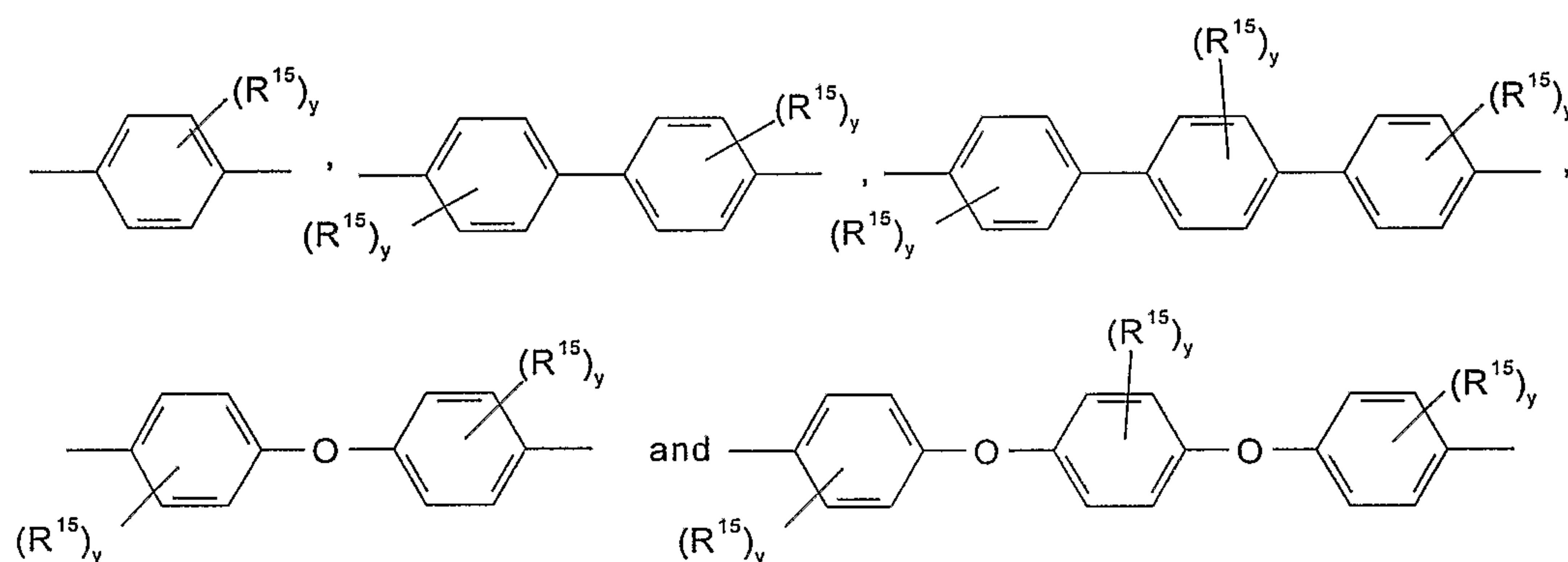
For embodiments, for example, where the biomolecule and the sorting function possess low steric hinderance, a spacer is optional. In  
15 certain embodiments, steric hindrance also can enhance selectivity in conjunction with Y (or in the absence of a Y). This enhanced selectivity can be achieved either by the presence of a selectivity function, Y, that is attached to M or by the selection of the appropriate spacer molecules for  $S^1$  and/or  $S^2$ .

20            If  $S^2$  is not required, the reactivity of the cleavable bond L can be influenced by one or more substituted functionalities, for example,  $R^{15}$  on M. Electronic (*e.g.*, mesomeric, inductive) and/or steric effects can be used to modulate the stability of the cleavable bond L. For example, if M is a trityl derivative, the linkage to the biomolecule, including, but not  
25 limited to, a protein, is in one embodiment a trityl ether bond. The sensitivity of this bond to mild acids, such as acetic acid or the vapor of trifluoroacetic acid, can be significantly enhanced by having as  $R^{15}$  one or two electron donating groups, including, but not limited to, alkoxy groups, such as methoxy groups, in the para positions of the aryl rings.

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Alternatively, the trityl ether bond can be stabilized by the introduction of electron withdrawing groups, including, but not limited to, either halogen, including bromo and chloro, groups, nitro groups or ester moieties, in the para and/or ortho positions of the aromatic rings.

- 5 In certain embodiments,  $S^1$  and  $S^2$  are each independently selected from  $-(CH_2)_r-$ ,  $-(CH_2O)-$ ,  $-(CH_2CH_2-O)_r-$ ,  $-(NH-(CH_2)_r-C(=O))_s-$ ,  $-(NH-CH(R^{52})-C(=O))_s-$ ,  $-(O-(CH)_r-C(=O))_s-$ ,



where  $R^{15}$  is selected as above;  $r$  and  $s$  are each independently an integer from 1 to 10;  $R^{52}$  is the side chain of a natural  $\alpha$ -amino acid; and  $y$  is an

- 10 integer from 0 to 4. In one embodiment,  $y$  is 0 or 1.

In certain embodiments,  $R^{15}$  is  $-H$ ,  $-OH$ ,  $-OR^{51}$ ,  $-SH$ ,  $-SR^{51}$ ,  $-NH_2$ ,  $-NHR^{51}$ ,  $-NR^{51}_2$ ,  $-F$ ,  $-Cl$ ,  $-Br$ ,  $-I$ ,  $-SO_3H$ ,  $-PO_4^{-2}$ ,  $-CH_3$ ,  $-CH_2CH_3$ ,  $-CH(CH_3)_2$  or  $-C(CH_3)_3$ ; where  $R^{51}$  is straight or branched chain alkyl, straight or

- 15 branched chain alkenyl, straight or branched chain alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, straight or branched chain aralkyl, straight or branched chain aralkenyl, straight or branched chain aralkynyl, straight or branched chain heteroaralkyl, straight or branched chain heteroaralkenyl, straight or branched chain heteroaralkynyl, straight or branched chain cycloalkylalkyl, straight or branched chain
- 20 cycloalkylalkenyl, straight or branched chain cycloalkylalkynyl, straight or branched chain heterocyclylalkyl, straight or branched chain

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heterocyclalkenyl or straight or branched chain heterocyclalkynyl.

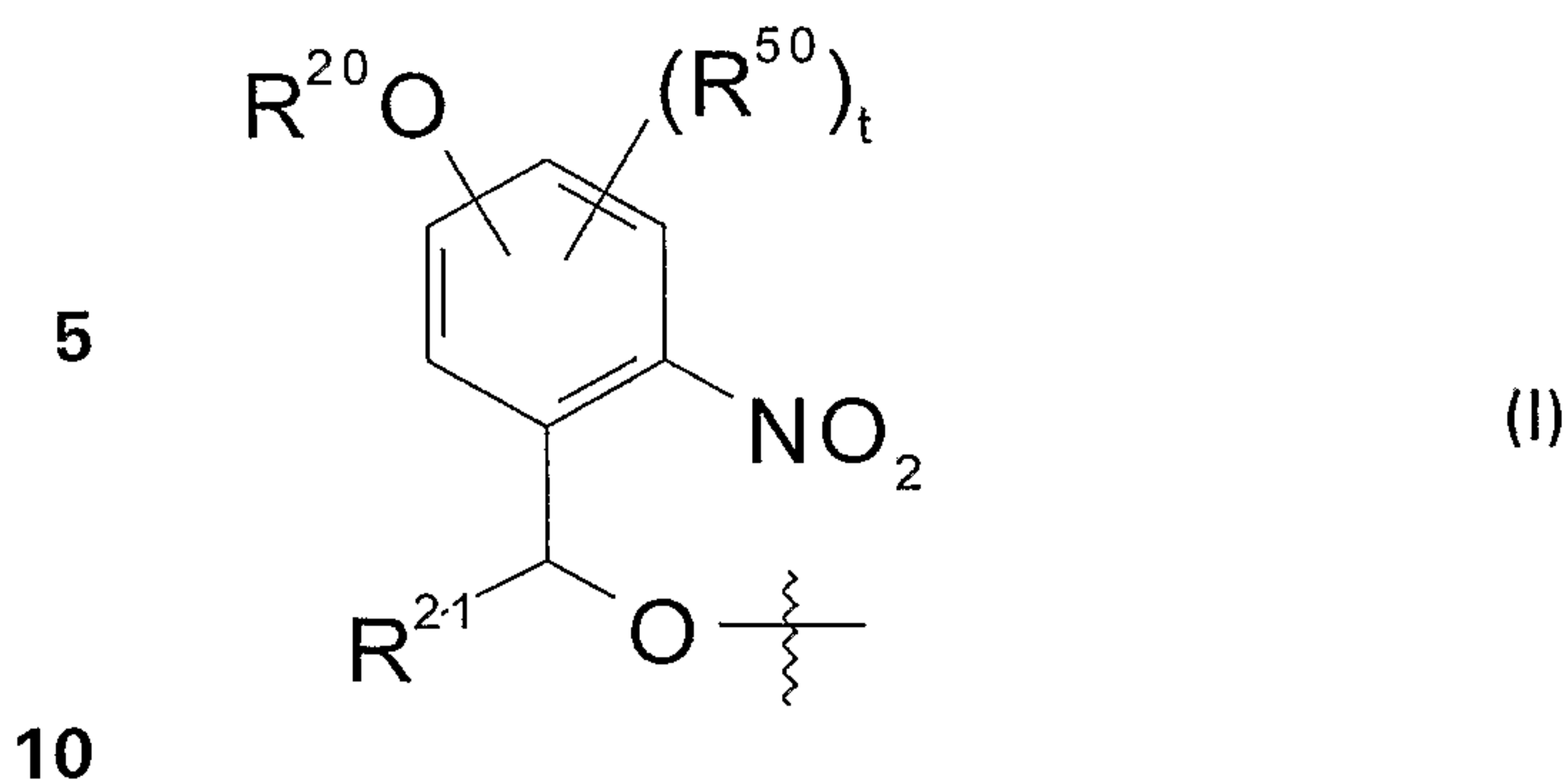
(c) L

In certain embodiments, the cleavable group L is cleaved either prior to or during analysis of the biomolecule, such as a protein. The analysis can include mass spectral analysis, for example MALDI-TOF mass spectral analysis. The cleavable group L is selected so that the group is stable during conjugation to a biomolecule, and sorting, such as hybridization of a single stranded oligonucleotide Q moiety to a complementary sequence, and washing of the hybrid; but is susceptible to cleavage under conditions of analysis of the biomolecule, including, but not limited to, mass spectral analysis, for example MALDI-TOF analysis. In certain embodiments, the cleavable group L can be a disulfide moiety, created by reaction of the compounds where  $X = -SH$ , with the thiol side chain of cysteine residues on the surface of biomolecules, including, but not limited to, proteins. The resulting disulfide bond can be cleaved under various reducing conditions including, but not limited to, treatment with dithiothreitol and 2-mercaptoethanol.

In another embodiment, L is a photocleavable group, which can be cleaved by a short treatment with UV light of the appropriate wave length either prior to or during mass spectrometry. Photocleavable groups, including those bonds that can be cleaved during MALDI-TOF mass spectrometry by the action of a laser beam, can be used. For example, a trityl ether or an ortho nitro substituted aralkyl, including benzyl, group are susceptible to laser induced bond cleavage during MALDI-TOF mass spectrometry. Other useful photocleavable groups include, but are not limited to, o-nitrobenzyl, phenacyl, and nitrophenylsulfenyl groups.

Other photocleavable groups for use herein include those disclosed in International Patent Application Publication No. WO 98/20166. In one embodiment, the photocleavable groups have formula I:

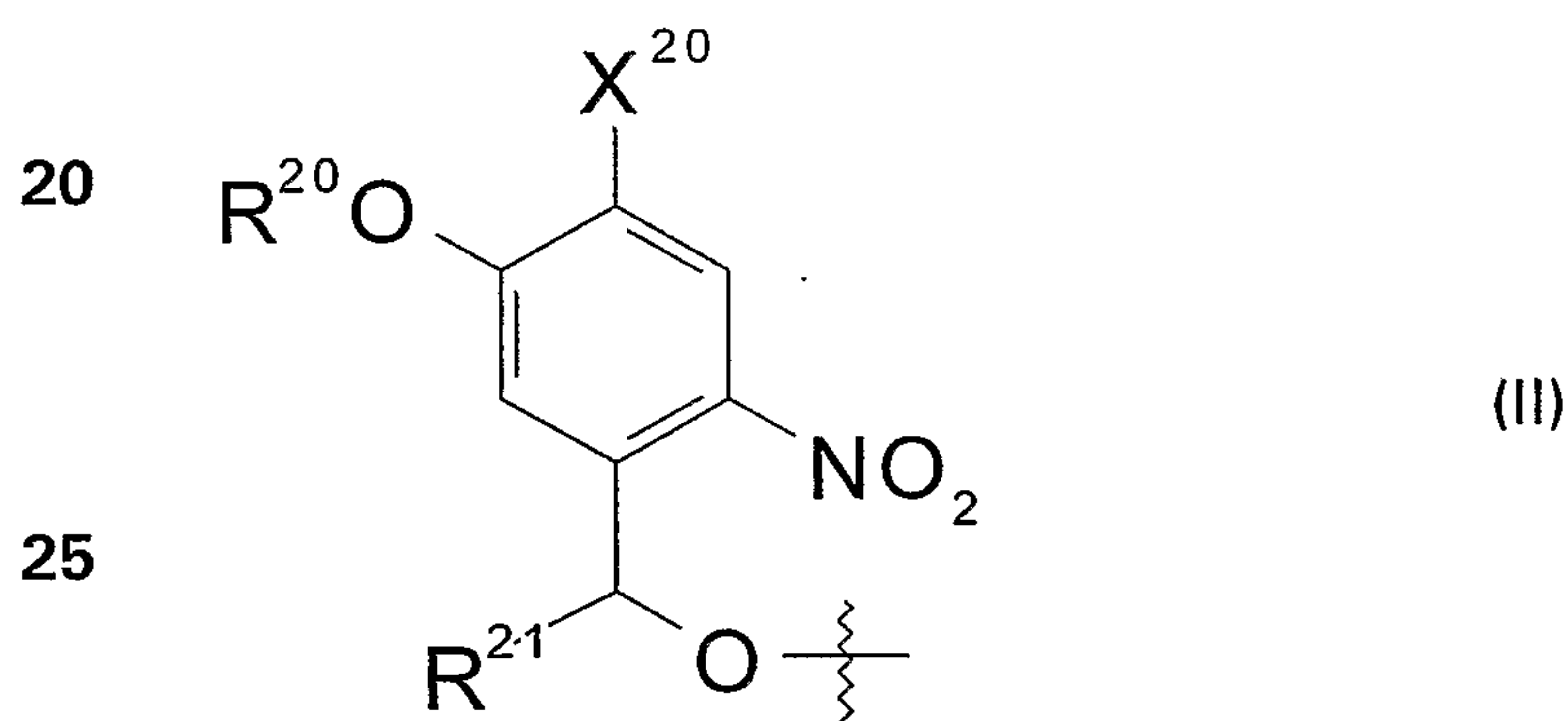
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where  $R^{20}$  is  $\omega$ -O-alkylene-;  $R^{21}$  is selected from hydrogen, alkyl, aryl, alkoxy, carbonyl, aryloxy, carbonyl and carboxy;  $t$  is 0-3; and  $R^{50}$  is alkyl, alkoxy, aryl or aryloxy. In one embodiment,  $Q$  is attached to  $R^{20}$  through

15  $(S^1)_t-M(R^{15})_a-(S^2)_b$ ; and the biomolecule of interest is captured onto the  $R^{21}CH-O-$  moiety via a reactive derivative of the oxygen (*e.g.*,  $X$ ).

In another embodiment, the photocleavable groups have formula II:



30 where  $R^{20}$  is  $\omega$ -O-alkylene- or alkylene;  $R^{21}$  is selected from hydrogen, alkyl, aryl, alkoxy, carbonyl, aryloxy, carbonyl and carboxy; and  $X^{20}$  is hydrogen, alkyl or  $OR^{21}$ . In one embodiment,  $Q$  is attached to  $R^{20}$  through  $(S^1)_t-M(R^{15})_a-(S^2)_b$ ; and the biomolecule of interest is captured

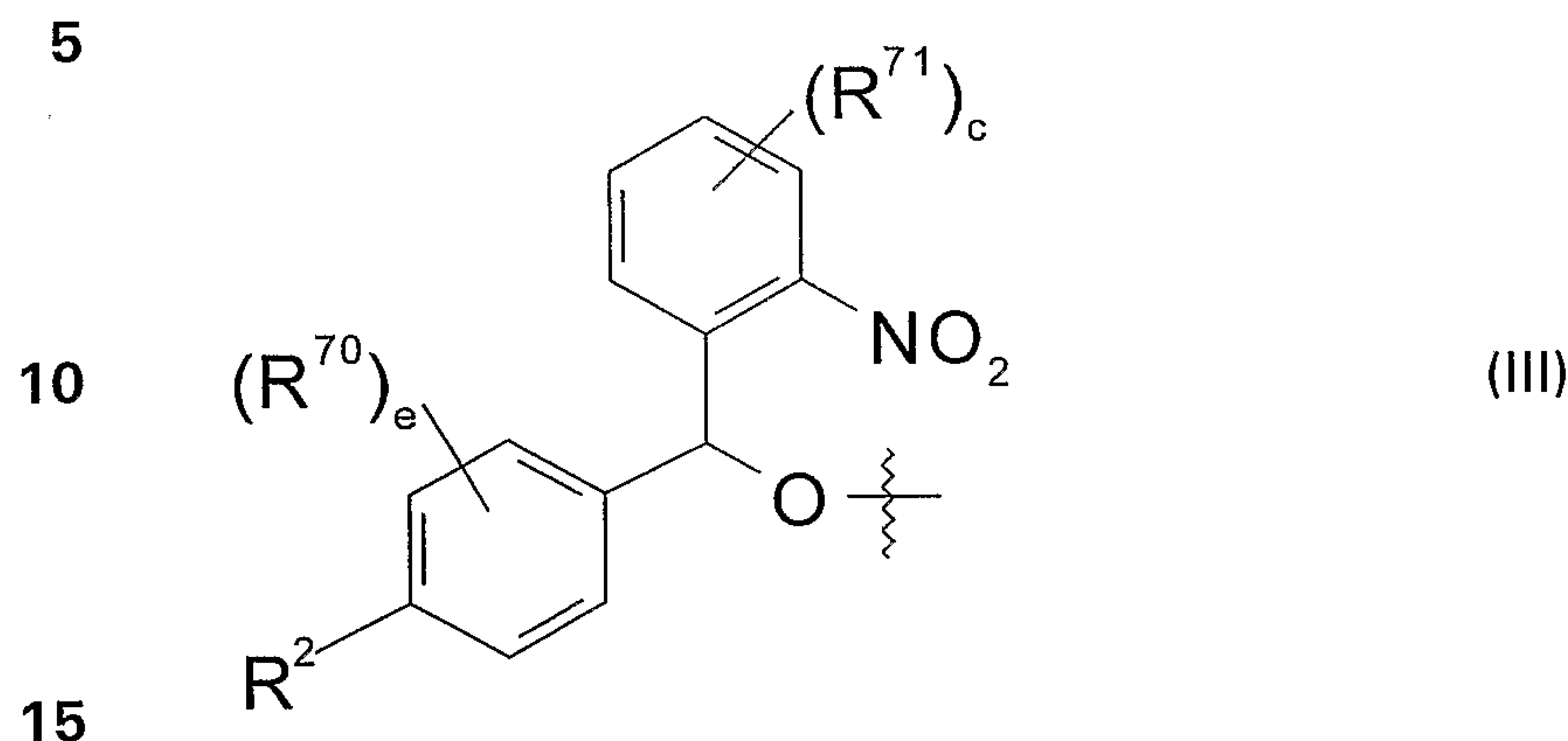
35 onto the  $R^{21}CH-O-$  moiety via a reactive derivative of the oxygen (*e.g.*,  $X$ ).

In further embodiments,  $R^{20}$  is  $-O-(CH_2)_3-$  or methylene;  $R^{21}$  is selected from hydrogen, methyl and carboxy; and  $X^{20}$  is hydrogen, methyl

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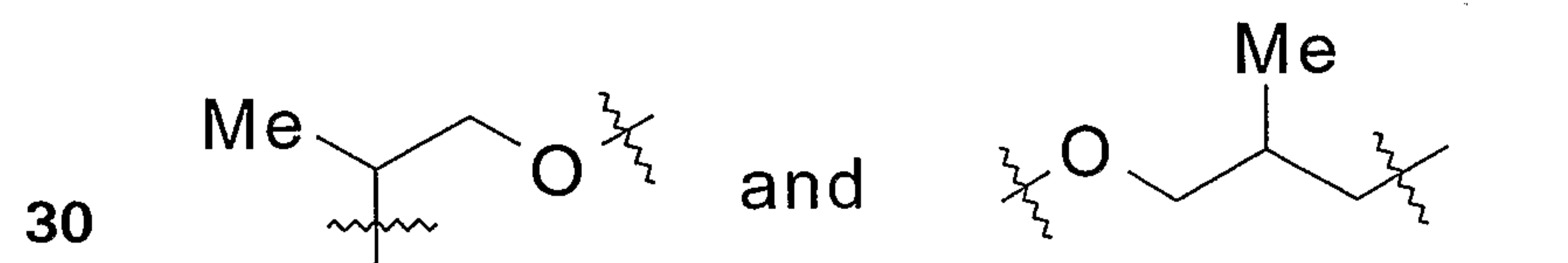
or  $\text{OR}^{21}$ . In another embodiment,  $\text{R}^{21}$  is methyl; and  $\text{X}^{20}$  is hydrogen. In certain embodiments,  $\text{R}^{20}$  is methylene;  $\text{R}^{21}$  is methyl; and  $\text{X}^{20}$  is 3-(4,4'-dimethoxytrityloxy)propoxy.

In another embodiment, the photocleavable groups have formula III:



where  $\text{R}^2$  is selected from  $\omega$ -O-alkylene-O and  $\omega$ -O-alkylene-, and is unsubstituted or substituted on the alkylene chain with one or more alkyl groups; c and e are each independently 0-4; and  $\text{R}^{70}$  and  $\text{R}^{71}$  are each independently alkyl, alkoxy, aryl or aryloxy. In certain embodiments,  $\text{R}^2$  is  $\omega$ -O-alkylene-, and is substituted on the alkylene chain with a methyl group. In one embodiment, Q is attached to  $\text{R}^2$  through  $(\text{S}^1)_t\text{-M}(\text{R}^{15})_a\text{-(S}^2)_b$ ; and the biomolecule of interest is captured onto the  $\text{Ar}_2\text{CH-O-}$  moiety via a reactive derivative of the oxygen (*e.g.*, X).

25 In further embodiments,  $\text{R}^2$  is selected from 3-O-( $\text{CH}_2$ )<sub>3</sub>-O-, 4-O-( $\text{CH}_2$ )<sub>4</sub>-, 3-O-( $\text{CH}_2$ )<sub>3</sub>-, 2-O- $\text{CH}_2\text{CH}_2$ -, - $\text{OCH}_2$ -,



In other embodiments, c and e are 0.

35 Other cleavable groups L include acid sensitive groups, where bond cleavage is promoted by formation of a cation upon exposure to mild to strong acids. For these acid-labile groups, cleavage of the group L can be

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effected either prior to or during analysis, including mass spectrometric analysis, by the acidity of the matrix molecules, or by applying a short treatment of the array with an acid, such as the vapor of trifluoroacetic acid. Exposure of a trityl group to acetic or trifluoroacetic acid produces  
5 cleavage of the ether bond either before or during MALDI-TOF mass spectrometry.

The capture compound-biomolecule array can be treated by either chemical, including, but not limited to, cyanogen bromide, or enzymatic, including, but not limited to, in embodiments where the biomolecule is a  
10 protein, trypsin, chymotrypsin, an exopeptidase (*e.g.*, aminopeptidase and carboxypeptidase) reagents to effect cleavage. For the latter, all but one peptide fragment will remain hybridized when digestion is quantitative. Partial digestion also can be of advantage to identify and characterize proteins following desorption from the array. The cleaved protein/peptide  
15 fragments are desorbed, analyzed, and characterized by their respective molecular weights.

In certain embodiments herein, L is selected from -S-S-,  
-O-P(=O)(OR<sup>51</sup>)-NH-, -O-C(=O)-,

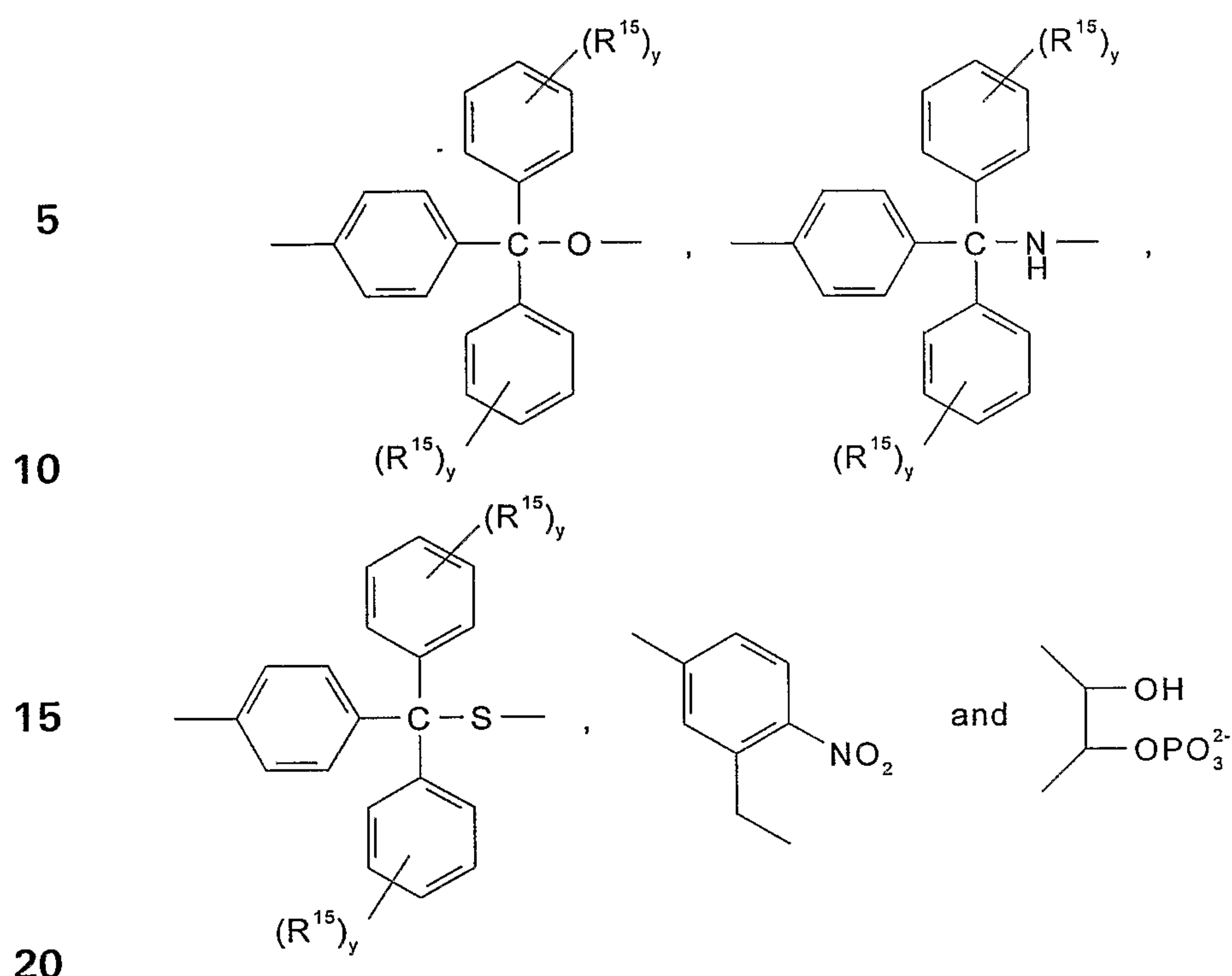
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where  $R^{15}$ ,  $R^{51}$  and  $y$  are as defined above. In certain embodiments,  $R^{15}$  is -H, -OH, -OR<sup>51</sup>, -SH, -SR<sup>51</sup>, -NH<sub>2</sub>, -NHR<sup>51</sup>, -N(R<sup>51</sup>)<sub>2</sub>, -F, -Cl, -Br, -I, -SO<sub>3</sub>H, -PO<sub>4</sub><sup>2-</sup>, -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, -CH(CH<sub>3</sub>)<sub>2</sub> or -C(CH<sub>3</sub>)<sub>3</sub>; where  $R^{51}$  is straight or branched chain alkyl, straight or branched chain alkenyl, straight or branched chain alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, straight or branched chain aralkyl, straight or branched chain aralkenyl, straight or branched chain aralkynyl, straight or branched chain heteroaralkyl, straight or branched chain heteroaralkenyl, straight or branched chain heteroaralkynyl, straight or branched chain cycloalkylalkyl, straight or branched chain cycloalkylalkenyl, straight or branched chain cycloalkylalkynyl, straight or branched chain heterocyclylalkyl, straight or branched chain heterocyclylalkenyl or straight or branched chain heterocyclylalkynyl

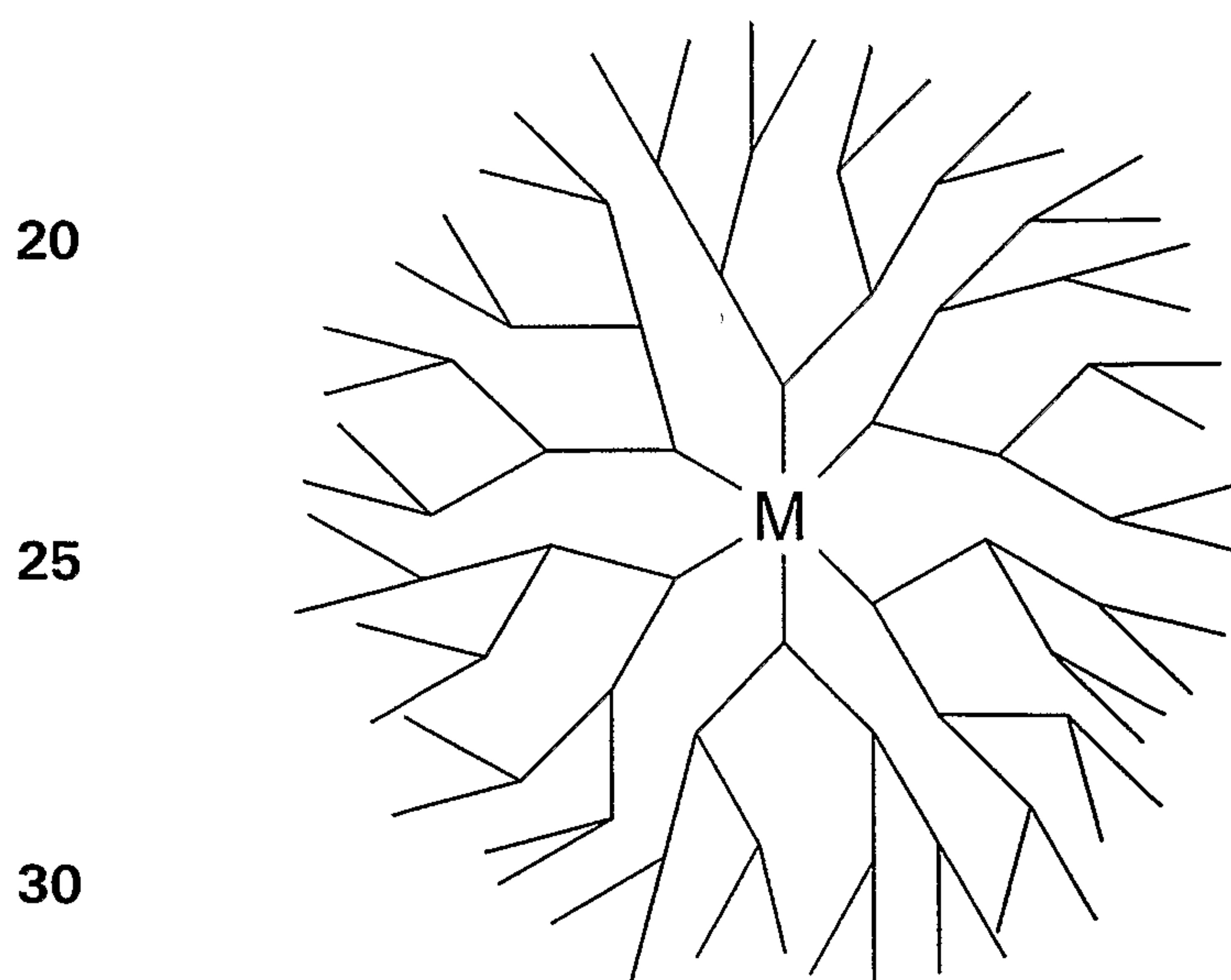
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**(ii) Non-cleavable divalent Z moieties**

In another embodiment, Z is a non-cleavable divalent moiety and has the formula:  $-(S^1)_t-M(R^{15})_a-(S^2)_b-$ , where  $S^1$ , M,  $R^{15}$ ,  $S^2$ , t, a and b are as defined above.

**5                    b.        Z has a dendrimeric structure**

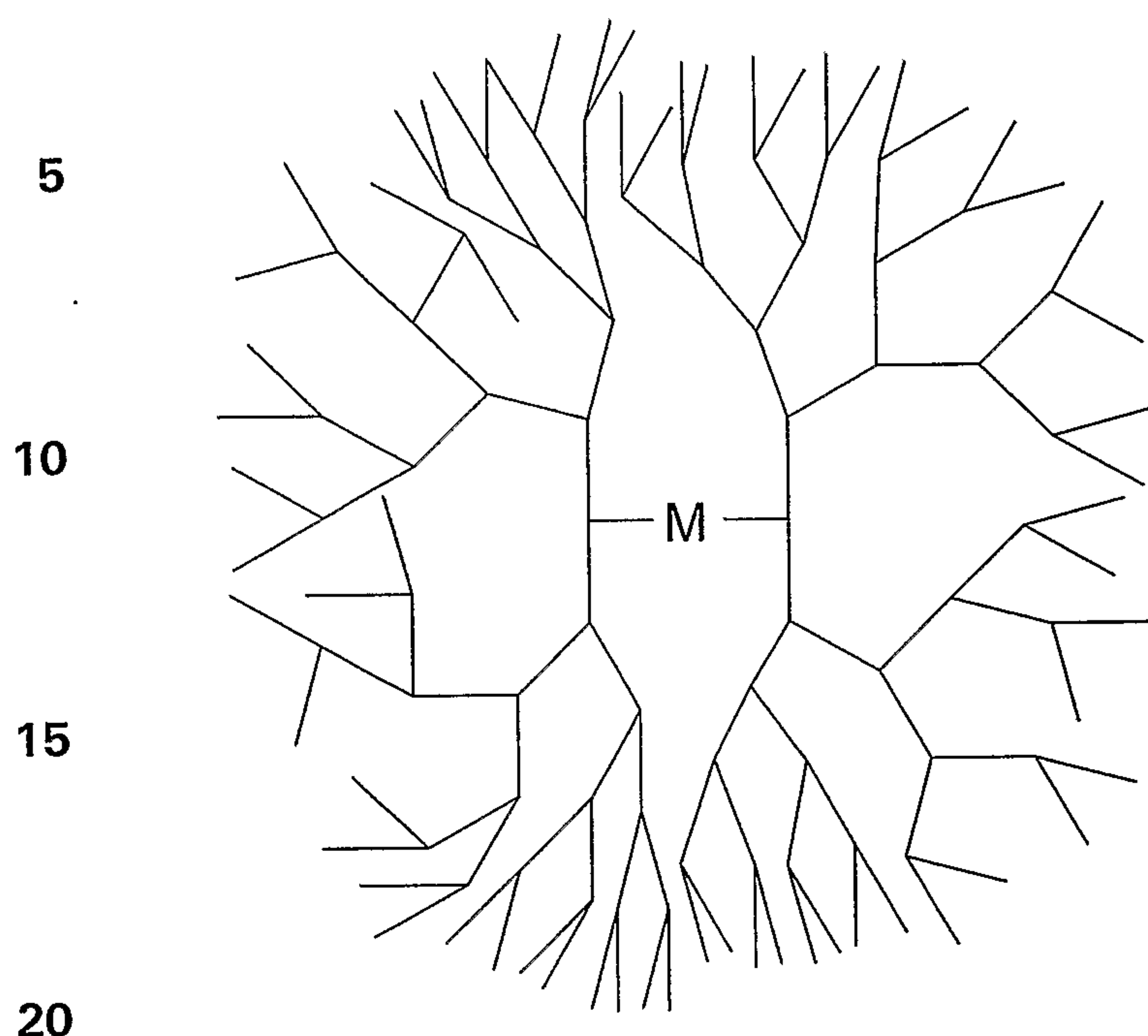
In another embodiment, Z has a dendritic structure (*i.e.*, Z is a multivalent dendrimer) that is linked to a plurality of Q and X moieties. Z, in certain embodiments, has about 4 up to about 6, about 8, about 10, about 20, about 40, about 60 or more points of attachment (*i.e.*, Z is  
 10 tetravalent up to hexavalent, octavalent, decavalent, didecavalent, tetradecavalent, hexadecavalent, etc.). In these embodiments, the dendritic moiety Z is based on a multivalent core M, as defined above. The number of points of attachment on M may vary from about 2 up to about 4, about 6, about 8, or more. Thus, in one embodiment, Z has the  
 15 structure:



where M is as defined above, and is linked to a plurality of Q, Y, W and X  
 35 moieties.

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In another embodiment, Z has the structure:



where M is as defined above, and is linked to a plurality of Q, Y, W and X moieties.

In other embodiments, the dendritic Z moieties may optionally possess a plurality of spacer groups  $S^1$  and/or  $S^2$ , or for embodiments where Z is a cleavable linkage, a plurality of L groups. The  $S^1$ ,  $S^2$  and/or L moieties are attached to the end of the dendritic chain(s).

In these embodiments, the density of the biopolymer to be analyzed, and thus signal intensity of the subsequent analysis, is increased relative to embodiments where Z is a divalent group.

**c. Z is an insoluble support or a substrate**

In other embodiments, Z can be an insoluble support or a substrate, such as a particulate solid support, such as a silicon or other "bead" or microsphere, or solid surface so that the surface presents the functional groups (X, Y, Q and, as needed W). In these embodiments, Z has bound to it one or a plurality of X moieties (typically, 1 to 100,

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generally 1 to 10) and optionally to at least one Q and/or Y moiety, and also optionally to one or more W moieties. Z, in these embodiments, can have tens up to hundreds, thousands, millions, or more functional moieties (groups) on its surface. For example, the capture compound can  
5 be a silicon particule or a agarose or other paricle with groups presented on it. As discussed below, it further can be coated with a hydrophobic material, such as lipid bilayers or other lipids that are used, for example to produce liposomes. In such embodiments, the resulting particles with a hydrophobic surface and optional hydrophobic W groups are used in  
10 methods for probing cell membrane environments and other intracellular environments. Gentle lysis of cells, can expose the intracellular compartments and organelles, and hydrophobic capture compounds, such as these, can be reacted with them, and the bound biomolecules assessed by, for example, mass spectrometry or further treated to release  
15 the contents of the compartments and organelles and reacted with the capture compounds or other capture compounds.

In embodiments in which Z is an insoluble support, the insoluble support or substrate moiety Z can be based on a flat surface constructed, for example, of glass, silicon, metal, plastic or a composite or other  
20 suitable surface; or can be in the form of a "bead" or particle, such as a silica gel, a controlled pore glass, a magnetic or cellulose bead; or can be a pin, including an array of pins suitable for combinatorial synthesis or analysis. Substrates can be fabricated from virtually any insoluble or solid material. For example, silica gel, glass (*e.g.*, controlled-pore glass  
25 (CPG)), nylon, Wang resin, Merrifield resin, dextran cross—linked with epichlorohydrin (*e.g.*, Sephadex<sup>®</sup>), agarose (*e.g.*, Sepharose<sup>®</sup>), cellulose, magnetic beads, Dynabeads, a metal surface (*e.g.*, steel, gold, silver, aluminum, silicon and copper), a plastic material (*e.g.*, polyethylene, polypropylene, polyamide, polyester, polyvinylidenedifluoride (PVDF))

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Exemplary substrate include, but are not limited to, beads (*e.g.*, silica gel, controlled pore glass, magnetic, dextran cross—linked with epichlorohydrin (*e.g.*, Sephadex<sup>®</sup>), agarose (*e.g.*, Sepharose<sup>®</sup>), cellulose, capillaries, flat supports such as glass fiber filters, glass surfaces, metal  
5 surfaces (steel, gold, silver, aluminum, copper and silicon), plastic materials including multiwell plates or membranes (*e.g.*, of polyethylene, polypropylene, polyamide, polyvinylidenedifluoride), pins (*e.g.*, arrays of pins suitable for combinatorial synthesis or analysis or beads in pits of flat surfaces such as wafers (*e.g.*, silicon wafers) with or without plates. The  
10 solid support is in any desired form, including, but not limited to, a bead, capillary, plate, membrane, wafer, comb, pin, a wafer with pits, an array of pits or nanoliter wells and other geometries and forms known to those of skill in the art. Supports include flat surfaces designed to receive or link samples at discrete loci.

15 In one embodiment, the solid supports or substrates Z are "beads" (*i.e.*, particles, typically in the range of less than 200  $\mu\text{m}$  or less than 50  $\mu\text{m}$  in their largest dimension) including, but not limited to, polymeric, magnetic, colored, R<sub>f</sub>-tagged, and other such beads. The beads can be made from hydrophobic materials, including, but not limited to,  
20 polystyrene, polyethylene, polypropylene or teflon, or hydrophilic materials, including, but not limited to, cellulose, dextran cross—linked with epichlorohydrin (*e.g.*, Sephadex<sup>®</sup>), agarose (*e.g.*, Sepharose<sup>®</sup>), polyacrylamide, silica gel and controlled pore glass beads or particles. These types of capture compounds can be reacted in liquid phase in  
25 suspension, and the spun down or other removed from the reaction medium, and the resulting complexes analyzed, such as by mass spectrometry. They can be sorted using the Q function to bind to distinct loci on a solid support, or they can include a label to permit addressing, such as an radio frequency tag or a colored label or bar code or other

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symbolism imprinted thereon. These can be sorted according to the label, which serves as "Q" function, and then analyzed by mass spectrometry.

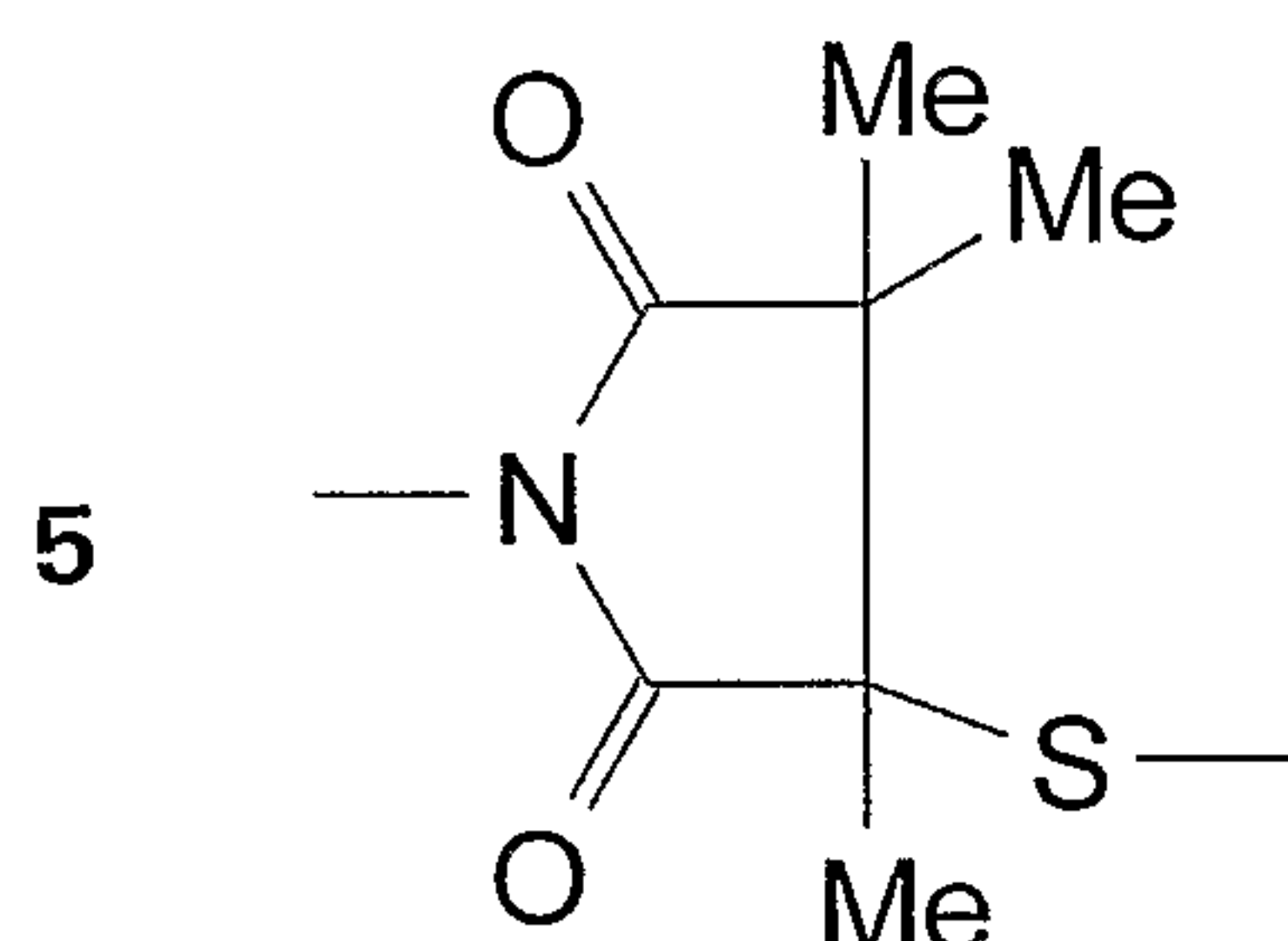
In further embodiments, the insoluble support or substrate Z  
 5 moieties optionally can possess spacer groups  $S^1$  and/or  $S^2$ , or for embodiments where Z is a cleavable linkage, L. The  $S^1$ ,  $S^2$  and/or L moieties are attached to the surface of the insoluble support or substrate.

In these embodiments, the density of the biomolecule to be analyzed, and thus signal intensity of the subsequent analysis, is  
 10 increased relative to embodiments where Z is a divalent group. In certain embodiments, an appropriate array of single stranded oligonucleotides or oligonucleotide analogs that are complementary to the single stranded oligonucleotide or oligonucleotide analog sorting functions Q will be employed in the methods provided herein.

#### 15 d. Mass modified Z moieties

In other embodiments, including embodiments where Z is a cleavable moiety, Z includes a mass modifying tag. In certain embodiments, the mass modifying tag is attached to the cleavable linker L. In one embodiment, the mass modified Z moiety has the formula:  
 20  $-(S^1)_t-M(R^{15})_a-(S^2)_b-L-T-$ , where  $S^1$ , t, M,  $R^{15}$ , a,  $S^2$ , b and L are selected as above; and T is a mass modifying tag. Mass modifying tags for use herein include, but are not limited to, groups of formula  $-X^1R^{10}-$ , where  $X^1$  is a divalent group such as  $-O-$ ,  $-O-C(O)-(CH_2)_y-C(O)O-$ ,  $-NH-C(O)-$ ,  $-C(O)-NH-$ ,  $-NH-C(O)-(CH_2)_y-C(O)O-$ ,  $-NH-C(S)-NH-$ ,  $-O-P(O-alkyl)-O-$ ,  
 25  $-O-SO_2-O-$ ,  $-O-C(O)-CH_2-S-$ ,  $-S-$ ,  $-NH-$  and

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- 10 and  $R^{10}$  is a divalent group including  $-(CH_2CH_2O)_z-CH_2CH_2O-$ ,  $-(CH_2CH_2O)_z-CH_2CH_2O$ -alkylene, alkylene, alkenylene, alkynylene, arylene, heteroarylene,  $-(CH_2)_z-CH_2-O-$ ,  $-(CH_2)_z-CH_2-O$ -alkylene,  $-(CH_2CH_2NH)_z-CH_2CH_2NH-$ ,  $-CH_2-CH(OH)-CH_2O-$ ,  $-Si(R^{12})(R^{13})-$ ,  $-CHF-$  and  $-CF_2-$ ; where  $y$  is an integer from 1 to 20;  $z$  is an integer from 0 to 200;
- 15  $R^{11}$  is the side chain of an  $\alpha$ -amino acid; and  $R^{12}$  and  $R^{13}$  are each independently selected from alkyl, aryl and aralkyl.

- In other embodiments,  $-X^1R^{10}-$  is selected from  $-S-S-$ ,  $-S-$ ,  $-(NH-(CH_2)_y-NH-C(O)-(CH_2)_y-C(O))_z-NH-(CH_2)_y-NH-C(O)-(CH_2)_y-C(O)O-$ ,  $-(NH-(CH_2)_y-C(O))_z-NH-(CH_2)_y-C(O)O-$ ,
- 20  $-(NH-CH(R^{11})-C(O))_z-NH-CH(R^{11})-C(O)O-$ , and  $-(O-(CH_2)_y-C(O))_z-NH-(CH_2)_y-C(O)O-$ .

- In the above embodiments, where  $R^{10}$  is an oligo-/polyethylene glycol derivative, the mass-modifying increment is 44, *i.e.*, five different mass-modified species can be generated by changing  $z$  from 0 to 4, thus
- 25 adding mass units of 45 ( $z = 0$ ), 89 ( $z = 1$ ), 133 ( $z = 2$ ), 177 ( $z = 3$ ) and 221 ( $z = 4$ ) to the compounds. The oligo/polyethylene glycols also can be monoalkylated by a lower alkyl such as methyl, ethyl, propyl, isopropyl, *t*-butyl and the like.

- Other mass modifying tags include, but are not limited to  $-CHF-$ ,
- 30  $-CF_2-$ ,  $-Si(CH_3)_2-$ ,  $-Si(CH_3)(C_2H_5)-$  and  $-Si(C_2H_5)_2-$ . In other embodiments, the mass modifying tags include homo- or heteropeptides. A non-limiting example that generates mass-modified species with a mass increment of

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57 is an oligoglycine, which produce mass modifications of, *e.g.*, 74 ( $y = 1, z = 0$ ), 131 ( $y = 1, z = 2$ ), 188 ( $y = 1, z = 3$ ) or 245 ( $y = 1, z = 4$ ). Oligoamides also can be used, *e.g.*, mass-modifications of 74 ( $y = 1, z = 0$ ), 88 ( $y = 2, z = 0$ ), 102 ( $y = 3, z = 0$ ), 116 ( $y = 4, z = 0$ ),  
5 etc., are obtainable. Those skilled in the art will appreciate that there are numerous possibilities in addition to those exemplified herein for introducing, in a predetermined manner, many different mass modifying tags to the compounds provided herein.

In other embodiments,  $R^{15}$  and/or  $S^2$  can be functionalized with  
10  $-X^1R^{10}H$  or  $-X^1R^{10}$ -alkyl, where  $X^1$  and  $R^{10}$  are defined as above, to serve as mass modifying tags.

## 2. Reactivity Functions "X"

Reactivity functions ("X") confer the ability on the compounds the ability to bind either covalently or with a high affinity (greater than  $10^9$ ,  
15 generally greater than  $10^{10}$  or  $10^{11}$  liters/mole, typically greater than a monoclonal antibody, and typically stable to mass spectrometric analysis, such as MALDI-MS) to a biomolecule, particularly proteins, including functional groups thereon, which include post-translationally added groups. Generally the binding is covalent or is of such affinity that it is  
20 stable under conditions of analysis, such as mass spectral, including MALDI-TOF, analysis. Exemplary groups are set forth herein (see, *e.g.*, Figure 16, and the discussion below).

In the compounds provided herein, X is a moiety that binds to or interacts with the surface of a biomolecule, including, but not limited to,  
25 the surface of a protein; an amino acid side chain of a protein; or an active site of an enzyme (protein) or to functional groups of other biomolecule, including lipids and polysaccharides.

Thus, for example, X is a group that reacts or interacts with functionalities on the surface of a protein to form covalent or non-

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covalent bonds with high affinity. A wide selection of different functional groups are available for X to interact with a protein. For example, X can act either as a nucleophile or an electrophile to form covalent bonds upon reaction with the amino acid residues on the surface of a protein.

- 5 Exemplary reagents that bind covalently to amino acid side chains include, but are not limited to, protecting groups for hydroxyl, carboxyl, amino, amide, and thiol moieties, including, for example, those disclosed in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed. (1999, Wiley Interscience); photoreactive groups,
- 10 Diels Alder couples (*i.e.*, a diene on one side and a single double bond on the other side).

Hydroxyl protecting groups for use as X groups herein include, but are not limited to:

- (i) ethers such as methyl, substituted methyl (methoxymethyl,
- 15 methylthiomethyl, (phenyldimethylsilyl)methoxymethyl, benzyloxymethyl, *p*-methoxybenzyloxymethyl, *p*-nitrobenzyloxymethyl, *o*-nitrobenzyloxymethyl, (4-methoxyphenoxy)methyl, guaiacolmethyl, *t*-butoxymethyl, 4-pentenylloxymethyl, siloxymethyl, 2-methoxyethoxymethyl, 2,2,2-trichloroethoxymethyl, bis(2-
- 20 chloroethoxymethyl), 2-(trimethylsilyl)ethoxymethyl, menthoxymethyl, tetrahydropyranyl, 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl, 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl *S,S*-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl, 1-(2-
- 25 fluorophenyl)-4-methoxypiperidin-4-yl, 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl), substituted ethyl (1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-[2-(trimethylsilyl)ethoxy]ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-

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fluoroethyl, 1-methyl-1-phenoxyethyl, 2,2,2-trichloroethyl, 1,1-dianisyl-  
 2,2,2-trichloroethyl, 1,1,1,3,3,3-hexafluoro-2-phenylisopropyl, 2-  
 trimethylsilylethyl, 2-(benzylthio)ethyl, 2-(phenylselenyl)ethyl), *t*-butyl,  
 allyl, propargyl, *p*-chlorophenyl, *p*-methoxyphenyl, *p*-nitrophenyl, 2,4-  
 5 dinitrophenyl, 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl, benzyl,  
 substituted benzyl (*p*-methoxybenzyl, 3,4,-dimethoxybenzyl, *o*-  
 nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-  
 phenylbenzyl, *p*-phenylenzyl, 2,6-difluorobenzyl, *p*-acylaminobenzyl, *p*-  
 azidobenzyl, 4-azido-3-chlorobenzyl, 2-trifluoromethylbenzyl, *p*-  
 10 (methylsulfinyl)benzyl), 2- and 4-picolyl, 3-methyl-2-picolyl *N*-oxido, 2-  
 quinolinylmethyl, 1-pyrenylmethyl, diphenylmethyl, *p,p'*-dinitrobenzhydryl,  
 5-dibenzosuberyl, triphenylmethyl,  $\alpha$ -naphthyldiphenylmethyl, *p*-  
 methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-  
 methoxyphenyl)methyl, 4-(4'-bromophenacyloxy)phenyldiphenylmethyl,  
 15 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-  
 tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl,  
 4,4'-dimethoxy-3''-[*N*-(imidazolylmethyl)]trityl, 4,4'-dimethoxy-3''-[*N*-  
 (imidazolethyl)carbamoyl]trityl, 1,1-bis(4-methoxyphenyl-1'-  
 pyrenylmethyl, 4-(17-tetrabenzo[*a,c,g,i*]fluorenylmethyl)-4,4''-  
 20 dimethoxytrityl, 9-Anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-  
 oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl *s,s*-dioxido, silyl  
 ethers (trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl,  
 diethylisopropylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, *t*-  
 butyldiphenylsilyl, tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl,  
 25 diphenylmethylsilyl, di-*t*-butylmethylsilyl, tris(trimethylsilyl)silyl (sisyl), (2-  
 hydroxystyryl)dimethylsilyl, (2-hydroxystyryl)diisopropylsilyl, *t*-  
 butylmethoxyphenylsilyl, *t*-butoxydiphenylsilyl);

(ii) esters such as formate, benzoylformate, acetate, substituted  
 acetate (chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate,

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- methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, phenylacetate, *p*-*P*-phenylacetate, diphenylacetate), nicotinate, 3-phenylpropionate, 4-pentenoate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate, 5-[3-bis(4-methoxyphenyl)hydroxymethylphenoxy]levulinate, pivaloate, 1-adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), carbonates (methyl, methoxymethyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 1,1,-dimethyl-2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, 2-(triphenylphosphonio)ethyl, isobutyl, vinyl, allyl, *p*-nitrophenyl, benzyl, *p*-methoxybenzyl, 3,4,-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, 2-dansylethyl, 2-(4-nitrophenyl)ethyl, 2-(2,4-dinitrophenyl)ethyl, 2-cyano-1-phenylethyl, *S*-benzyl thiocarbonate, 4-ethoxy-1-naphthyl, methyl dithiocarbonate), 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl carbonate, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2-(chloroacetoxymethyl)benzoate, 2-[(2-chloroacetoxy)ethyl]benzoate, 2-[2-(benzyloxy)ethyl]benzoate, 2-[2-(4-methoxybenzyloxy)ethyl]benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccionoate, (*E*)-2-methyl-2-butenate (tigloate), *o*-(methoxycarbonyl)benzoate, *p*-*P*-benzoate,  $\alpha$ -naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, 2-chlorobenzoate, 4-bromobenzoate, 4-nitrobenzoate, 3'5'-dimethoxybenzoin, a wild and woolly photolabile fluorescent ester, *N*-phenylcarbamate, borate, dimethylphosphinothioyl, 2,4-dinitrophenylsulfenate; and
- (iii) sulfonates (sulfate, allylsulfonate, methanesulfonate (mesylate),

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benzylsulfonate, tosylate, 2-[(4-nitrophenyl)ethyl]sulfonate).

Carboxyl protecting groups for use as X groups herein include, but are not limited to:

- (i) esters such as enzymatically cleavable esters (heptyl, 2-*N*-
- 5 (morpholino)ethyl, choline, (methoxyethoxy)ethyl, methoxyethyl), methyl, substituted methyl (9-fluorenylmethyl, methoxymethyl, methylthiomethyl, tetrahydropyranyl, tetrahydrofuranyl, methoxyethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, benzyloxymethyl, pivaloyloxymethyl, phenylacetoxymethyl, triisopropylsilylmethyl, cyanomethyl, acetol,
- 10 phenacyl, *p*-bromophenacyl,  $\alpha$ -methylphenacyl, *p*-methoxyphenacyl, desyl, carboxamidomethyl, *p*-azobenzenecarboxamidomethyl, *N*-phthalimidomethyl), 2-substituted ethyl (2,2,2-trichloroethyl, 2-haloethyl,  $\omega$ -chloroalkyl, 2-(trimethylsilyl)ethyl, 2-methylthioethyl, 1,3-dithianyl-2-methyl, 2-(*p*-nitrophenylsulfenyl)ethyl, 2-(*p*-toluenesulfonyl)ethyl, 2-(2'-
- 15 pyridyl)ethyl, 2-(*p*-methoxyphenyl)ethyl, 2-(diphenylphosphino)ethyl, 1-methyl-1-phenylethyl, 2-(4-acetyl-2-nitrophenyl)ethyl, 2-cyanoethyl), *t*-butyl, 3-methyl-3-pentyl, dicyclopropylmethyl, 2,4-dimethyl-3-pentyl, dicyclopropylmethyl, cyclopentyl, cyclohexyl, allyl, methallyl, 2-methylbut-3-en-2-yl, 3-methylbut-2-(prenyl), 3-buten-1-yl, 4-
- 20 (trimethylsilyl)-2-buten-1-yl, cinnamyl,  $\alpha$ -methylcinnamyl, prop-2-ynyl (propargyl), phenyl, 2,6-dialkylphenyl (2,6,-dimethylphenyl, 2,6-diisopropylphenyl, 2,6-di-*t*-butyl-4-methylphenyl, 2,6-di-*t*-butyl-4-methoxyphenyl, *p*-(methylthio)phenyl, pentafluorophenyl, benzyl, substituted benzyl (triphenylmethyl, diphenylmethyl, bis(*o*-
- 25 nitrophenyl)methyl, 9-anthrylmethyl, 2-(9,10-dioxo)anthrylmethyl, 5-dibenzosuberyl, 1-pyrenylmethyl, 2-(trifluoromethyl)-6-chromonylmethyl, 2,4,6-trimethylbenzyl, *p*-bromobenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-methoxybenzyl, 2,6-dimethoxybenzyl, 4-(methylsulfinyl)benzyl, 4-sulfobenzyl, 4-azidomethoxybenzyl, 4-{*N*-[1-(4,4,-dimethyl-2,6-

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dioxocyclohexylidene)-3-methylbutyl]amino}benzyl, piperonyl, 4-picolyl, *p-P*-benzyl), silyl (trimethylsilyl, triethylsilyl, *t*-butyldimethylsilyl, *i*-propyldimethylsilyl, phenyldimethylsilyl, di-*t*-butylmethylsilyl, triisopropylsilyl), activated (thiol), oxazoles, 2-alkyl-1,3-oxazoline, 4-alkyl-5-oxo-1,3-oxazolidine, 2,2,-bistrifluoromethyl-4-alkyl-5-oxo-1-,3-oxazolidine, 5-alkyl-4-oxo-1,3-dioxolane, dioxanones, ortho esters, Braun ortho ester, pentaaminocobalt(iii) complex, stannyl (triethylstannyl, tri-*N*-butylstannyl);

(ii) amides (*N,N*-dimethyl, pyrrolidinyl, piperidinyl, 5,6-dihydrophenanthridinyl, *o*-nitroanilide, *N*-7-nitroindolyl, *N*-8-nitro-1,2,3,4-tetrahydroquinolyl, 2-(2-aminophenyl)acetaldehyde dimethyl acetal amide, *p-P*-benzenesulfonamide;

(iii) hydrazides (*N*-phenyl, *N,N'*-diisopropyl); and

(iv) tetraalkylammonium salts.

Thiol protecting groups for use as X groups herein include, but are not limited to:

(i) thioethers (*S*-alkyl, *S*-benzyl, *S-p*-methoxybenzyl, *S-o*- or *p*-hydroxy- or acetoxybenzyl, *S-p*-nitrobenzyl, *S*-2,4,6-trimethylbenzyl, *S*-2,4,6-trimethoxybenzyl, *S*-4-picolyl, *S*-2-quinolinylmethyl, *S*-2-picolyl *N*-oxido, *S*-9-anthrylmethyl, *S*-9-fluorenylmethyl, *S*-xanthenyl, *S*-ferrocenyilmethyl); *S*-diphenylmethyl, substituted *S*-diphenylmethyl and *S*-triphenylmethyl (*S*-diphenylmethyl, *S*-bis(4-methoxyphenyl)methyl, *S*-5-dibenzosuberyl, *S*-triphenylmethyl, *S*-diphenyl-4-pyridylmethyl), *S*-phenyl, *S*-2,4-dinitrophenyl, *S-t*-butyl, *S*-1-adamantyl, substituted *S*-methyl including monothio, dithio and aminothioacetals (*S*-methoxymethyl, *S*-isobutoxymethyl, *S*-benzyloxymethyl, *S*-2-tetrahydropyranyl, *S*-benzylthiomethyl, *S*-phenylthiomethyl, thiazolidine, *S*-acetamidomethyl, *S*-trimethylacetamidomethyl, *S*-benzamidomethyl, *S*-allyloxycarbonylaminomethyl, *S*-phenylacetamidomethyl, *S*-

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phthalimidomethyl, *S*-acetyl-, *S*-carboxyl-, and *S*-cyanomethyl), substituted *S*-ethyl (*S*-(2-nitro-1-phenyl)ethyl, *S*-2-(2,4-dinitrophenyl)ethyl, *S*-2-(4'-pyridyl)ethyl, *S*-2-cyanoethyl, *S*-2-(trimethylsilyl)ethyl, *S*-(1-*m*-nitrophenyl-2-benzoyl)ethyl, *S*-2-phenylsulfonyl, *S*-1-(4-

5 methylphenylsulfonyl)-2-methylprop-2-yl, silyl;

(ii) thioesters (*S*-acetyl, *S*-benzoyl, *S*-trifluoroacetyl, *S*-*N*-[[*p*-biphenyl]isopropoxy]carbonyl]-*N*-methyl- $\gamma$ -aminothiobutyrate, *S*-*N*-(*t*-butoxycarbonyl)-*N*-methyl- $\gamma$ -aminothiobutyrate), thiocarbonates (*S*-2,2,2-trichloroethoxycarbonyl, *S*-*t*-butoxycarbonyl, *S*-benzyloxycarbonyl, *S*-*p*-methoxybenzyloxycarbonyl), thiocarbamates (*S*-(*N*-ethyl), *S*-(*N*-methoxymethyl));

10

(iii) unsymmetrical disulfides (*S*-ethyl, *S*-*t*-butyl, substituted *S*-phenyl disulfides);

(iv) sulfenyl derivatives (*S*-sulfonate, *S*-sulfenylthiocarbonate, *S*-3-nitro-2-pyridinesulfenyl sulfide, *S*-[tricarbonyl[1,2,3,4,5- $\eta$ ]-2-,4-cyclohexadien-1-yl]-iron(1+), oxathiolone); and

15

(v) *S*-methylsulfonium salt, *S*-benzyl- and *S*-4-methoxybenzylsulfonium salt, *S*-1-(4-phthalimidobutyl)sulfonium salt, *S*-(dimethylphosphinol)thioyl, *S*-(diphenylphosphino)thioyl.

20 Amino protecting groups for use as X groups herein include, but are not limited to:

(i) carbamates (methyl, ethyl, 9-fluorenylmethyl, 9-(2-sulfo)fluorenylmethyl, 9-(2,7-dibromo)fluorenylmethyl, 17-tetrabenzo[*a,c,g,i*]fluorenylmethyl, 2-Chloro-3-indenylmethyl, benz[*f*]inden-3-ylmethyl, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothiox, 1,1-dioxobenzo[*b*]thiophene-2-yl)methyl, substituted ethyl (2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-phenylethyl, 1-(1-adamantyl)-1-methylethyl, 2-chloroethyl, 1,1-dimethyl-2-haloethyl, 1,1-dimethyl-2,2-dibromoethyl, 1,1-dimethyl-2,2,2-trichloroethyl, 1-methyl-1-(4-

25

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biphenyl)ethyl, 1-(3,5-di-*t*-butylphenyl)-1-methylethyl, 2-(2'- and 4'-  
 pyridyl)ethyl, 2,2-bis(4'-nitrophenyl)ethyl, *N*-(2-pivaloylamino)-1,1-  
 dimethylethyl, 2-[(2-nitrophenyl)dithio]-1-phenylethyl, 2-(*N,N*-  
 dicyclohexylcarboxamido)ethyl), *t*-butyl, 1-adamantyl, 2-adamantyl, vinyl,  
 5 allyl, 1-isopropylallyl, cinnamyl, 4-nitrocinnamyl, 3-(3'-pyridyl)prop-2-enyl,  
 8-quinolyl, *N*-hydroxypiperidinyl, alkylidithio, benzyl, *p*-methoxybenzyl, *p*-  
 nitrobenzyl, *p*-bromobenzyl, *p*-chlorobenzyl, 2,4-dichlorobenzyl, 4-  
 methylsulfinylbenzyl, 9-anthrylmethyl, diphenylmethyl, 2-methylthioethyl,  
 2-methylsulfonyl, 2-(*p*-toluenesulfonyl)ethyl, [2-(1,3-dithianyl)methyl],  
 10 4-methylthiophenyl, 2,4-dimethylthiophenyl, 2'-phosphonioethyl, 1-  
 methyl-1-(triphenylphosphonio)ethyl, 1,1-dimethyl-2-cyanoethyl, 2-  
 dansylethyl, 2-(4-nitrophenyl)ethyl, 4-phenylacetoxymethyl, 4-azidobenzyl,  
 4-azidomethoxybenzyl, *m*-chloro-*p*-acyloxybenzyl, *p*-  
 (dihydroxyboryl)benzyl, 5-benzisoxazolylmethyl, 2-(trifluoromethyl)-6-  
 15 chromonylmethyl, *m*-nitrophenyl, 3,5-dimethoxybenzyl, 1-methyl-1-(3,5-  
 dimethoxyphenyl)ethyl,  $\alpha$ -methylnitropiperonyl, *o*-nitrobenzyl, 3,4-  
 dimethoxy-6-nitrobenzyl, phenyl(*o*-nitrophenyl)methyl, 2-(2-  
 nitrophenyl)ethyl, 6-nitroveratryl, 4-methoxyphenacyl, 3',5'-  
 dimethoxybenzoin, ureas (phenothiazinyl-(10)-carbonyl derivative, *N'*-*p*-  
 20 toluenesulfonylamino-carbonyl, *N'*-phenylaminothiocarbonyl), *t*-amyl, *S*-  
 benzyl thiocarbamate, butynyl, *p*-cyanobenzyl, cyclobutyl, cyclohexyl,  
 cyclopentyl, cyclopropylmethyl, *p*-decyloxybenzyl, diisopropylmethyl, 2,2-  
 dimethoxycarbonylvinyl, *o*-(*N',N'*-dimethylcarboxamido)benzyl, 1,1-  
 dimethyl-3-(*N',N'*-dimethylcarboxamido)propyl, 1,1-dimethylpropynyl,  
 25 di(2-pyridyl)methyl, 2-furanylmethyl, 2-iodoethyl, isobornyl, isobutyl,  
 isonicotinyl, *p*-(*p'*-methoxyphenylazo)benzyl, 1-methylcyclobutyl, 1-  
 methylcyclohexyl, 1-methyl-1-cyclopropylmethyl, 1-methyl-1-(*p*-  
 phenylazophenyl)ethyl, 1-methyl-1-phenylethyl, 1-methyl-1-(4'-  
 pyridyl)ethyl, phenyl, *p*-(phenylazo)benzyl, 2,4,6-tri-*t*-butylphenyl, 4-

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(trimethylammonium)benzyl, 2,4,6-trimethylbenzyl);

(ii) amides (*N*-formyl, *N*-acetyl, *N*-chloroacetyl, *N*-trichloroacetyl, *N*-trifluoroacetyl, *N*-phenylacetyl, *N*-3-phenylpropionyl, *N*-4-pentenoyl, *N*-picolinoyl, *n*-3-pyridylcarboxamido, *N*-benzoylphenylalanyl  
 5 derivative, *N*-benzoyl, *N*-*p*-phenylbenzoyl, *N*-*o*-nitrophenylacetyl, *N*-*o*-nitrophenoxyacetyl, *N*-3-(*o*-nitrophenyl)propionyl, *N*-2-methyl-2-(*o*-nitrophenoxy)propionyl, *N*-3-methyl-3-nitrobutyryl, *N*-*o*-nitrocinnamoyl, *N*-*o*-nitrobenzoyl, *N*-3-(4-*t*-butyl-2,6-dinitrophenyl)-2,2-dimethylpropionyl, *N*-*o*-(benzoyloxymethyl)benzoyl, *N*-(2-acetoxymethyl)benzoyl, *N*-2-[(*t*-  
 10 butyldiphenylsiloxy)methyl]benzoyl, *N*-3-(3',6'-dioxo-2',4',5'-trimethylcyclohexa-1',4'-diene)-3,3-dimethylpropionyl, *N*-*o*-hydroxy-*trans*-cinnamoyl, *N*-2-methyl-2-(*o*-phenylazophenoxy)propionyl, *N*-4-chlorobutyryl, *N*-acetoacetyl, *N*-3-(*p*-hydroxyphenyl)propionyl, (*N*'-dithiobenzoyloxycarbonylamino)acetyl, *N*-acetylmethionine derivative, 4,5-  
 15 diphenyl-3-oxazolin-2-one), cyclic imides (*N*-phthaloyl, *N*-tetrachlorophthaloyl, *N*-4-nitrophthaloyl, *N*-dithiasuccinoyl, *N*-2,3-diphenylmaleoyl, *N*-2,5-dimethylpyrrolyl, *N*-2,5-bis(triisopropylsiloxy)pyrrolyl, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct, *N*-1,1,3,3-tetramethyl-1,3-disilaisoindolyl, 5-substituted 1,3-  
 20 dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridonyl, 1,3,5-dioxazinyl);

(iii) *N*-alkyl and *N*-aryl amines (*N*-methyl, *N*-*t*-butyl, *N*-allyl, *N*-[2-(trimethylsilyl)ethoxy]methyl, *N*-3-acetoxypropyl, *N*-cyanomethyl, *N*-(1-  
 25 isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl), *N*-2,4-dimethoxybenzyl, *N*-2-azanorbornenyl, *N*-2,4-dinitrophenyl, quaternary ammonium salts, *N*-benzyl, *N*-4-methoxybenzyl, *N*-2,4-dimethoxybenzyl, *N*-2-hydroxybenzyl, *N*-diphenylmethyl, *N*-bis(4-methoxyphenyl)methyl, *N*-5-dibenzosuberyl, *N*-triphenylmethyl, *N*-(4-methoxyphenyl)diphenylmethyl, *N*-9-

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phenylfluorenyl, *N*-ferrocenylmethyl, *N*-2-picolylamine *N'*-oxide);

(iv) imines (*N*-1,1-dimethylthiomethylene, *N*-benzylidene, *N*-*p*-methoxybenzylidene, *N*-diphenylmethylene, *N*-[(2-pyridyl)mesityl]methylene, *N*-(*N'*,*N'*-dimethylaminomethylene), *N*-(*N'*,*N'*-dibenzylaminomethylene), *N*-(*N'*-*t*-butylaminomethylene), *N*,*N'*-isopropylidene, *N*-*p*-nitrobenzylidene, *N*-salicylidene, *N*-5-chlorosalicylidene, *N*-(5-chloro-2-hydroxyphenyl)phenylmethylene, *N*-cyclohexylidene, *N*-*t*-butylidene);

(v) enamines (*N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl, *N*-2,7-dichloro-9-fluorenylmethylene, *N*-2-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, *N*-4,4,4-trifluoro-3-oxo-1-buteryl, *N*-1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl);

(vi) *N*-heteroatom derivatives (*N*-borane derivatives, *N*-diphenylborinic acid derivative, *N*-diethylborinic acid derivative, *N*-difluoroborinic acid derivative, *N*,*N'*-3,5-bis(trifluoromethyl)phenylboronic acid derivative, *N*-[phenyl(pentacarbonylchromium- or -tungsten)]carbenyl, *N*-copper or *N*-zinc chelate, 18-crown-6 derivative, *N*-nitro, *N*-nitroso, *N*-oxide, triazene derivative, *N*-diphenylphosphinyl, *N*-dimethyl- and diphenylthiophosphinyl, *N*-dialkyl phosphoryl, *N*-dibenzyl and diphenyl phosphoryl, iminotriphenylphosphorane derivative, *N*-benzenesulfonyl, *N*-*o*-nitrobenzenesulfonyl, *N*-2,4-dinitrobenzenesulfonyl, *N*-pentachlorobenzenesulfonyl, *N*-2-nitro-4-methoxybenzenesulfonyl, *N*-triphenylmethylsulfonyl, *N*-1-(2,2,2-trifluoro-1,1-diphenyl)ethylsulfonyl, *N*-3-nitro-2-pyridinesulfonyl, *N*-*p*-toluenesulfonyl, *N*-benzenesulfonyl, *N*-2,3-6-trimethyl-4-methoxybenzenesulfonyl, *N*-2,4,6-trimethoxybenzenesulfonyl, *N*-2,6-dimethyl-4-methoxybenzenesulfonyl, *N*-pentamethylbenzenesulfonyl, *N*-2,3,5,6-tetramethyl-4-methoxybenzenesulfonyl, *N*-4-methoxybenzenesulfonyl, *N*-2,4,6-trimethylbenzenesulfonyl, *N*-2,6-dimethoxy-4-methylbenzenesulfonyl, *N*-

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3-methoxy-4-*t*-butylbenzenesulfonyl, *N*-2,2,5,7,8-pentamethylchroman-6-sulfonyl, *N*-2- and 4-nitrobenzenesulfonyl, *N*-2,4-dinitrobenzenesulfonyl, *N*-benzothiazole-2-sulfonyl, *N*-pyridine-2-sulfonyl, *N*-methanesulfonyl, *N*-2-(trimethylsilyl)ethanesulfonyl, *N*-9-anthracenesulfonyl, *N*-4-(4',8'-  
 5 dimethoxynaphthylmethyl)benzenesulfonyl, *N*-benzylsulfonyl, *N*-trifluoromethylsulfonyl, *N*-phenacylsulfonyl, *N*-*t*-butylsulfonyl);

(vii) imidazole protecting groups including *N*-sulfonyl derivatives (*N,N*-dimethylsulfonyl, *N*-mesitylenesulfonyl, *N*-*p*-methoxyphenylsulfonyl, *N*-benzenesulfonyl, *N*-*p*-toluenesulfonyl); carbamates (2,2,2-trichloroethyl, 10 2-(trimethylsilyl)ethyl, *t*-butyl, 2,4-dimethylpent-3-yl, cyclohexyl, 1,1-dimethyl-2,2,2-trichloroethyl, 1-adamantyl, 2-adamantyl); *N*-alkyl and *N*-aryl derivatives (*N*-vinyl, *N*-2-chloroethyl, *N*-(1-ethoxy)ethyl, *N*-2-(2'-pyridyl)ethyl, *N*-2-(4'-pyridyl)ethyl, *N*-2-(4'-nitrophenyl)ethyl), *N*-trialkyl silyl derivatives (*N*-*t*-butyldimethylsilyl, *N*-triisopropylsilyl), *N*-allyl, *N*-  
 15 benzyl, *N*-*p*-methoxybenzyl, *N*-3,4-dimethoxybenzyl, *N*-3-methoxybenzyl, *N*-3,5-dimethoxybenzyl, *N*-2-nitrobenzyl, *N*-4-nitrobenzyl, *N*-2,4-dinitrophenyl, *N*-phenacyl, *N*-triphenylmethyl, *N*-diphenylmethyl, *N*-(diphenyl-4-pyridylmethyl), *N*-(*n'*,*n'*-dimethylamino)), amino acetal derivatives (*N*-hydroxymethyl, *N*-methoxymethyl, *N*-diethoxymethyl, *N*-  
 20 ethoxymethyl, *N*-(2-chloroethoxy)methyl, *N*-[2-(trimethylsilyl)ethoxy]methyl, *N*-*t*-butoxymethyl, *N*-*t*-butyldimethylsiloxymethyl, *N*-pivaloyloxymethyl, *N*-benzyloxymethyl, *N*-dimethylaminomethyl, *N*-2-tetrahydropyranyl), amides (carbon dioxide adduct, *N*-formyl, *N*-(*n'*,*n'*-diethylureidyl), *N*-dichloroacetyl, *N*-pivaloyl, *N*-  
 25 diphenylthiophosphinyl); and

(viii) amide -NH protecting groups including amides (*N*-allyl, *N*-*t*-butyl, *N*-dicyclopropylmethyl, *N*-methoxymethyl, *N*-methylthiomethyl, *N*-benzyloxymethyl, *N*-2,2,2-trichloroethoxymethyl, *N*-*t*-butyldimethylsiloxymethyl, *N*-pivaloyloxymethyl, *N*-cyanomethyl, *N*-

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pyrrolidinomethyl, *N*-methoxy, *N*-benzyloxy, *N*-methylthio, *N*-triphenylmethylthio, *N*-*t*-butyldimethylsilyl, *N*-triisopropylsilyl, *N*-4-methoxyphenyl, *N*-3,4-dimethoxyphenyl, *N*-4-(methoxymethoxy)phenyl, *N*-2-methoxy-1-naphthyl, *N*-benzyl, *N*-4-methoxybenzyl, *N*-2,4-

5 dimethoxybenzyl, *N*-3,4-dimethoxybenzyl, *N*-*o*-nitrobenzyl, *N*-bis(4-methoxyphenyl)methyl, *N*-bis(4-methoxyphenyl)phenylmethyl, *N*-bis(4-methylsulfinylphenyl)methyl, *N*-triphenylmethyl, *N*-9-phenylfluorenyl, *N*-bis(trimethylsilyl)methyl, *N*-*t*-butoxycarbonyl, *N*-benzyloxycarbonyl, *N*-methoxycarbonyl, *N*-ethoxycarbonyl, *N*-*p*-toluenesulfonyl, *N*,*O*-

10 isopropylidene ketal, *N*,*O*-benzylidene acetal, *N*,*O*-formylidene acetal, *N*-butenyl, *N*-ethenyl, *N*-[(*e*)-(2-methoxycarbonyl)vinyl], *N*-diethoxymethyl, *N*-(1-methoxy-2,2-dimethylpropyl), *N*-2-(4-methylphenylsulfonyl)ethyl).

These protecting groups react with amino acid side chains such as hydroxyl (serine, threonine, tyrosine); amino (lysine, arginine, histidine,

15 proline); amide (glutamine, asparagine); carboxylic acid (aspartic acid, glutamic acid); and sulfur derivatives (cysteine, methionine), and are readily adaptable for use in the capture compounds as the reactive moiety X.

It is in addition to the wide range of group-specific reagents that

20 are known to persons of skill in the art, reagents that are known in natural product chemistry also can serve as a basis for X in forming covalent linkages. Other choices for, X include protein purification dyes, such as acridine or methylene blue, which have a strong affinity for certain proteins.

25 Alternatively, X can act as an electron donor or an electron acceptor to form non-covalent bonds or a complex, such as a charge-transfer complex, with a biomolecule, including, but not limited to, a protein, such that the resulting bond has a high stability (*i.e.*, stable under conditions of mass spectrometric analysis, such as MALDI-TOF, as

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defined above). These reagents include those that interact strongly and with high specificity with biomolecules, including, but not limited to, proteins, without forming covalent bonds through the interaction of complementary affinity surfaces. For example, well known binding pairs, such as biotin and streptavidin, antibody and antigen, receptor and ligand, lectin and carbohydrate or other similar types of reagents are readily adaptable for use in these compounds as the reactive moiety X that will react with high affinity to biomolecules with surfaces similar to or identical to the other member of the binding pair. These moieties are selected so that the resulting conjugates (also referred to herein as complexes) have strong interactions that are sufficiently stable enough for suitable washing of the unbound biomolecules, including, but not limited to, proteins, out of the complexed biological mixtures.

The reactivity of X can be influenced by one or more selectivity functions Y on the core, *i.e.*, M in the formula above, particularly where  $S^2$  is not present.

The Y function, discussed below is employed for electronic (*e.g.*, mesomeric, inductive) and/or steric effects to modulate the reactivity of X and the stability of the resulting X-biomolecule linkage. In these embodiments, biomolecule mixtures, including, but not limited to, protein mixtures, can react and be analyzed due to the modulation by Y, which changes the electronic or steric properties of X and, therefore, increases the selectivity of the reaction of X with the biomolecule.

In certain embodiments, X is an active ester, such as  $-C(=O)O-Ph-pNO_2$ ,  $-C(=O)O-C_6F_5$  or  $-C(=O)-O-(N-succinimidyl)$ ; an active halo moiety, such as an  $\alpha$ -halo ether or an  $\alpha$ -halo carbonyl group, including, but not limited to,  $-OCH_2-I$ ,  $-OCH_2-Br$ ,  $-OCH_2-Cl$ ,  $-C(O)CH_2I$ ,  $-C(O)CH_2Br$  and  $-C(O)CH_2Cl$ ; amino acid side chain-specific functional groups, such as maleimido (for cysteine), a metal complex, including gold

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or mercury complexes (for cysteine or methionine), an epoxide or isothiocyanate (for arginine or lysine); reagents that bind to active sites of enzymes, including, but not limited to, transition state analogs; antibodies, *e.g.*, against phosphorylated peptides; antigens, such as a  
5 phage display library; haptens; biotin; avidin; or streptavidin.

### 3. Selectivity Functions "Y"

The selectivity functions ("Y") serves to modulate the reactivity function by reducing the number of groups to which the reactivity functions bind, such as by steric hindrance and other interactions. It is a  
10 group that modifies the steric and/or electronic (*e.g.*, mesomeric, inductive effects) properties as well as the resulting affinities of the capture compound. Selectivity functions include any functional groups that increase the selectivity of the reactivity group so that it binds to fewer different biomolecules than in the absence of the selectivity  
15 function or binds with greater affinity to biomolecules than in its absence. In the capture compounds provided herein, Y is allowed to be extensively varied depending on the goal to be achieved regarding steric hindrance and electronic factors as they relate to modulating the reactivity of the cleavable bond L, if present, and the reactive  
20 functionality X. For example, a reactivity function X can be selected to bind to amine groups on proteins; the selectivity function can be selected to ensure that only groups exposed on the surface can be accessed. The selectivity function is such that the compounds bind to or react with (via the reactivity function) fewer different biomolecules when it is part of the  
25 molecule than when it is absent and/or the compounds bind with greater specificity and higher affinity. The selectivity function can be attached directly to a compound or can be attached via a linker, such as  $\text{CH}_2\text{CO}_2$  or  $\text{CH}_2\text{-O-(CH}_2\text{)}_n\text{-O}$ , where *n* is an integer from 1 to 12, or 1 to 6, or 2 to 4. See, *e.g.*, Figure 17 and the discussion below for exemplary selectivity

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functions.

In certain embodiments, each Y is independently a group that modifies the affinity properties and/or steric and/or electronic (*e.g.*, mesomeric, inductive effects) properties of the resulting capture  
5 compound. For example, Y, in certain embodiments, is selected from ATP analogs and inhibitors; peptides and peptide analogs; polyethyleneglycol (PEG); activated esters of amino acids, isolated or within a peptide; cytochrome C; and hydrophilic trityl groups.

In another embodiment, Y is a small molecule moiety, a natural  
10 product, a protein agonist or antagonist, a peptide or an antibody (see, *e.g.*, Figure 17). In another embodiment, Y is a hydrophilic compound or protein (*e.g.*, PEG or trityl ether), a hydrophobic compound or protein (*e.g.*, polar aromatics, lipids, glycolipids, phosphotriesters, oligosaccharides), a positive or negatively charged group, a small  
15 molecule, a pharmaceutical compound or a biomolecule that creates defined secondary or tertiary structures.

In other embodiments, Y is a group that is a component of a luminescent, including fluorescent, phosphorescent, chemiluminescent and bioluminescent system, or is a group that can be detected in a  
20 colorimetric assay; in certain embodiments, Y is a monovalent group selected from straight or branched chain alkyl, straight or branched chain alkenyl, straight or branched chain alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocyclyl, straight or branched chain heterocyclylalkyl, straight or branched chain heterocyclylalkenyl, straight or branched chain  
25 heterocyclylalkynyl, aryl, straight or branched chain arylalkyl, straight or branched chain arylalkenyl, straight or branched chain arylalkynyl, heteroaryl, straight or branched chain heteroarylalkyl, straight or branched chain heteroarylalkenyl, straight or branched chain heteroarylalkynyl, halo, straight or branched chain haloalkyl, pseudohalo, azido, cyano, nitro,

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$OR^{60}$ ,  $NR^{60}R^{61}$ ,  $COOR^{60}$ ,  $C(O)R^{60}$ ,  $C(O)NR^{60}R^{61}$ ,  $S(O)_qR^{60}$ ,  $S(O)_qOR^{60}$ ,  $S(O)_qNR^{60}R^{61}$ ,  $NR^{60}C(O)R^{61}$ ,  $NR^{60}C(O)NR^{60}R^{61}$ ,  $NR^{60}S(O)_qR^{60}$ ,  $SiR^{60}R^{61}R^{62}$ ,  $P(R^{60})_2$ ,  $P(O)(R^{60})_2$ ,  $P(OR^{60})_2$ ,  $P(O)(OR^{60})_2$ ,  $P(O)(OR^{60})(R^{61})$  and  $P(O)NR^{60}R^{61}$ , where  $q$  is an integer from 0 to 2;

- 5 each  $R^{60}$ ,  $R^{61}$ , and  $R^{62}$  is independently hydrogen, straight or branched chain alkyl, straight or branched chain alkenyl, straight or branched chain alkynyl, aryl, straight or branched chain aralkyl, straight or branched chain aralkenyl, straight or branched chain aralkynyl, heteroaryl, straight or branched chain heteroaralkyl, straight or branched chain
- 10 heteroaralkenyl, straight or branched chain heteroaralkynyl, heterocyclyl, straight or branched chain heterocyclylalkyl, straight or branched chain heterocyclylalkenyl or straight or branched chain heterocyclylalkynyl.

- Fluorescent, colorimetric and phosphorescent groups are known to those of skill in the art (see, *e.g.*, U.S. Patent No. 6,274,337; Sapan *et al.* (1999) *Biotechnol. Appl. Biochem.* 29 (Pt. 2):99-108; Sittampalam *et al.* (1997) *Curr. Opin. Chem. Biol.* 1(3):384-91; Lakowicz, J. R., Principles of Fluorescence Spectroscopy, New York: Plenum Press (1983); Herman, B., Resonance Energy Transfer Microscopy, in: Fluorescence Microscopy of Living Cells in Culture, Part B, Methods in
- 15 Cell Biology, vol. 30, ed. Taylor, D. L. & Wang, Y. -L., San Diego: Academic Press (1989), pp. 219-243; Turro, N. J., Modern Molecular Photochemistry, Menlo Park: Benjamin/Cummings Publishing Co., Inc. (1978), pp. 296-361 and the Molecular Probes Catalog (1997), OR, USA). Fluorescent moieties include, but are not limited to, 1- and 2-
- 25 aminonaphthalene, p,p'-diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, bis-benzoxazole, bis-p-oxazolyl benzene, 1,2-benzophenazin, retinol, bis-3-aminopyridinium salts, hellebrigenin, tetracycline, sterophenol,

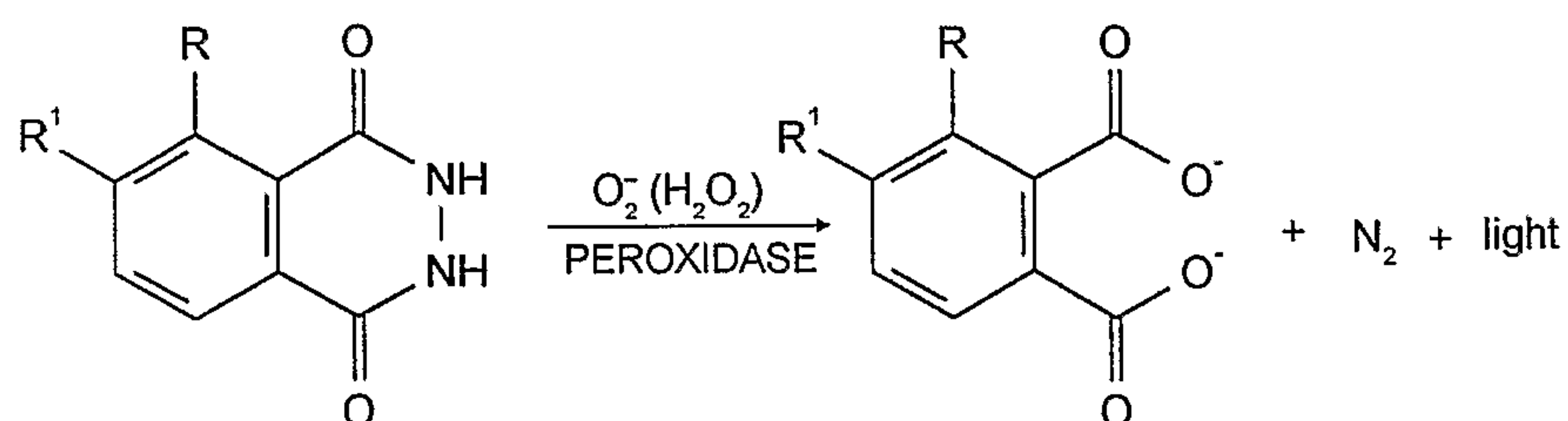
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benzimidazolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, calicylate, strophanthidin, porphyrins, triarylmethanes and flavin. Fluorescent compounds that have functionalities for linking to a compound provided herein, or that can be modified to incorporate such functionalities include, *e.g.*, dansyl chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthhydrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanato-stilbene-2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6-sulfonate; N-phenyl-N-methyl-2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine; N,N'-dihexyl oxacarbocyanine; merocyanine, 4-(3'pyrenyl)stearate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylantracene; 9-vinylanthracene; 2,2'(vinylene-p-phenylene)bisbenzoxazole; p-bis(2-(4-methyl-5-phenyl-oxazolyl))benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N-(7-dimethylamino-4-methyl-2-oxo-3-chromenyl)maleimide; N-(p-(2-benzimidazolyl)-phenyl)maleimide; N-(4-fluoranthyl)maleimide; bis(homovanillic acid); resazarin; 4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3(2H)-furanone. Many fluorescent tags are commercially available from SIGMA chemical company (Saint Louis, Mo.), Molecular Probes, R&D systems (Minneapolis, Minn.), Pharmacia LKB Biotechnology. (Piscataway, N.J.), CLONTECH Laboratories, Inc. (Palo Alto, Calif.), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, Wis.), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), and

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Applied Biosystems (Foster City, Calif.) as well as other commercial sources known to one of skill in the art.

Chemiluminescent groups intended for use herein include any components of light generating systems that are catalyzed by a  
 5 peroxidase and require superoxide anion ( $O_2^-$ ) (and/or hydrogen peroxide ( $H_2O_2$ ))(see, *e.g.*, Musiani *et al.* (1998) *Histol. Histopathol.* 13(1):243-8). Light-generating systems include, but are not limited to, luminol, isoluminol, peroxyoxalate-fluorophore, acridinium ester, lucigenin, dioxetanes, oxalate esters, acridan, hemin, indoxyl esters including 3-O-  
 10 indoxyl esters, naphthalene derivatives, such as 7-dimethylamino-naphthalene-1,2-dicarboxylic acid hydrazide and cypridina luciferin analogs, including 2-methyl-6-[p-methoxyphenyl]-3,7-dihydroimidazo[1,2- $\alpha$ ]pyrazin-3-one, 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2- $\alpha$ ]pyrazin-3-one and 2-methyl-6-[p-[2-[sodium 3-carboxylato-4-(6-hydroxy-3-xanthenon-9-  
 15 yl]phenylthioureylene]ethyleneoxy]phenyl]-3,7-dihydroimidazo[1,2- $\alpha$ ]pyrazin-3-one. In other embodiments, the chemiluminescent moieties intended for use herein include, but are not limited to, luminol, isoluminol, N-(4-aminobutyl)-N-ethyl isoluminol (ABEI), N-(4-aminobutyl)-N-methyl isoluminol (ABMI), which have the following structures and participate in  
 20 the following reactions:



where luminol is represented, when R is  $NH_2$  and R<sup>1</sup> is H; isoluminol, when R is H and R<sup>1</sup> is  $NH_2$ ; for ABEI ((6-[N-(4-aminobutyl)-N-ethylamino]-2,3-dihydrophthalazine-1,4-dione), when R is H and R<sup>1</sup> is  $C_2H_5-N-(CH_2)_4NH_2$ ; and for ABMI ((6-[N-(4-aminobutyl)-N-methylamino]-2,3-

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dihyrophthalazine-1-4-dione), when R is H and R<sup>1</sup> is CH<sub>3</sub>-N-(CH<sub>2</sub>)<sub>4</sub>NH<sub>2</sub>.

Bioluminescent groups for use herein include luciferase/luciferin couples, including firefly [*Photinus pyralis*] luciferase, the *Aequorin* system (*i.e.*, the purified jellyfish photoprotein, aequorin). Many

- 5 luciferases and substrates have been studied and well-characterized and are commercially available (*e.g.*, firefly luciferase is available from Sigma, St. Louis, MO, and Boehringer Mannheim Biochemicals, Indianapolis, IN; recombinantly produced firefly luciferase and other reagents based on this gene or for use with this protein are available from Promega Corporation,
- 10 Madison, WI; the aequorin photoprotein luciferase from jellyfish and luciferase from *Renilla* are commercially available from Sealite Sciences, Bogart, GA; coelenterazine, the naturally-occurring substrate for these luciferases, is available from Molecular Probes, Eugene, OR]. Other bioluminescent systems include crustacean, such as *Cypridina* (*Vargula*),
- 15 systems; insect bioluminescence generating systems including fireflies, click beetles, and other insect systems; bacterial systems; dinoflagellate bioluminescence generating systems; systems from molluscs, such as *Latia* and *Pholas*; earthworms and other annelids; glow worms; marine polychaete worm systems; South American railway beetle; fish (*i.e.*,
- 20 those found in species of *Aristostomias*, such as *A. scintillans* (see, *e.g.*, O'Day *et al.* (1974) *Vision Res.* 14:545-550), *Pachystomias*, and *Malacosteus*, such as *M. niger*; blue/green emitters include cyclthone, myctophids, hatchet fish (*agryropelecus*), *vinciguerria*, *howella*, *florenciella*, and *Chauliodus*); and fluorescent proteins, including green
- 25 (*i.e.*, GFPs, including those from *Renilla* and from *Ptilosarcus*), red and blue (*i.e.*, BFPs, including those from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*) fluorescent proteins (including *Renilla mulleri* luciferase, *Gaussia* species luciferase and *Pleuromamma* species luciferase) and phycobiliproteins.

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Exemplary selectivity functions include, but are not limited to, ligands that bind to receptors such as insulin and other receptors (see, *e.g.*, the Table of ligands below); cyclodextrins; enzyme substrates; lipid structures; prostaglandins; antibiotics; steroids; therapeutic drugs; enzyme inhibitors; transition state analogs; specific peptides that bind to biomolecule surfaces, including glue peptides; lectins (*e.g.*, mannose type, lactose type); peptide mimetics; statins; functionalities, such as dyes and other compounds and moieties employed for protein purification and affinity chromatraphy. See *e.g.*, Figure 17, and the following table of peptide ligands:

| Exemplary peptide ligands    |                                                                 |        |
|------------------------------|-----------------------------------------------------------------|--------|
| Designation                  | Sequence                                                        | SEQ ID |
| Adrenocorticotrophic hormone | SYSMEHFRWG KPVGKKRRPV<br>KVYPNGAEDE SAEAFPLEF                   | 1      |
| Adrenomedullin               | YRQSMNNFQG LRSFGCRFGT<br>CTVOKLAHQI YQFTDKDKDN VAPRSKISPO<br>GY | 2      |
| Allatostatin I-IV            | APSGAQRLYGFGL                                                   | 3      |
| alpha MSH                    | WGKPV(ac)SYSMEHFR                                               | 4      |
| alpha-Bag Cell Peptide       | APRERFYSE                                                       | 5      |
| alpha-Neo-endorphin          | YGGFLRKYPK                                                      | 6      |
| Alytesin                     | E*GRLGTQWAV GHLM-NH <sub>2</sub>                                | 7      |
| Amylin                       | KCNTATCATN RLANFLVHSS NNFGAILSST<br>NVGSNTY                     | 8      |
| Angiotensin-1                | DRVYIHPFHL                                                      | 9      |
| Angiotensin-2                | DRVYIHPF                                                        | 10     |
| Angiotensin-3                | RVYIHPF                                                         | 11     |
| Apelin-13                    | NRPRLSHLGMPMF                                                   | 12     |
| Astressin                    | *FHLLREVLE*IARAEQLAQEAHKNRL*IEII                                | 13     |
| Atrial Natriuretic Peptide   | SLRRSSCFGG RMDRIGAQSG LGCNSFRY                                  | 14     |
| Autocamtide 2                | KKALRRQETV DAL                                                  | 15     |
| BAM12                        | YGGFMRRVGR PE                                                   | 16     |
| BAM18                        | YGGFMRRVGR PEWW                                                 | 17     |
| BAM22                        | YGGFMRRVGR PE                                                   | 18     |
| Beta Endorphins ("44")       | YGGFMTSEKS QTPLVTLFKN AIIKNAYKKG<br>E                           | 19     |
| beta MSH                     | AEKKDEGPYR MEHFRWGSPK KD                                        | 20     |
| beta- Neo-endorphin          | YGGFLRKYP                                                       | 21     |
| BetaAmyloid                  | DAEFRHASGYE VHHQKLVFFAE<br>DVGSNLGAIIG LMVGGVVIAT               | 22     |
| Beta-Bag Cell Peptide        | RLRFH                                                           | 23     |

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|    |                          |                                                                  |    |
|----|--------------------------|------------------------------------------------------------------|----|
| 5  | BNP                      | SPKMVQSGSGC FGRKMDRISS<br>SSGLGCKVLR RH                          | 24 |
|    | Bradykinin               | RPPGFSPFR                                                        | 25 |
|    | Buccalin                 | GMDSLAFSGG L-NH <sub>2</sub>                                     | 26 |
|    | Bursin                   | KHG-NH <sub>2</sub>                                              | 27 |
|    | C3 (undeca peptide)      | ASKKPKRNIKA                                                      | 28 |
| 10 | Caerulein                | *EQDY(SO3H)TGWMDF                                                | 29 |
|    | Calcineurin              | AIP ITSFEEAKGL DRINERMPPR RDAMP                                  | 30 |
|    | Calcitonin               | CGNLSTCMLG TYTQDFNKFH<br>TFPQTAIGVG AP                           | 31 |
|    | Calpain Inhibitor ("42") | DPMSSTYIEE LGKREVTIPP KYRELLA                                    | 32 |
|    | CAP-37                   | NOGRHFCCGA EIHFVMTA ASCFN                                        | 33 |
| 15 | Cardiodilatin            | * NPMYNAVSNA DLMDFKNLLD<br>HLEEKMPLED                            | 34 |
|    | CD36peptideP (139-155)   | CNLAVAAASH IYQNQFVQ                                              | 35 |
|    | Cecropin B               | KWKVFKKIEK MGRNIRNGIV KAGPAIAVLG<br>EAKAL                        | 36 |
|    | Cerebellin               | SGSAKVAFSA IRSTNH                                                | 37 |
|    | CGRP-1                   | ACDTATCVTH RLAGLLSRSG<br>GVVKNNFVPT NVGSKAF                      | 38 |
| 20 | CGRP-2                   | ACNTATCVTH RLAGLLSRSG<br>GMVKS NFVPT NVGSKAF                     | 39 |
|    | CKS17                    | LQNRRLDLL FLKEGGL                                                | 40 |
|    | Cortistatins             | QEGAPQOSA RRDRMPCRN FFWKTFSSCK                                   | 41 |
|    | Crystalline              | WG                                                               | 42 |
|    | Defensin 1 HNP1          | ACYCRIPACI AGERRYGTCT YQGR LWAFCC                                | 43 |
| 25 | Defensin HNP2            | CYCRIPACIA GERRYGTCTY QGR LWAFCC                                 | 44 |
|    | Dermaseptin              | ALWKTMLKKL GTMALHAGKA<br>ALGAAADTIS QTQ                          | 45 |
|    | Dynorphin-A              | YGGFLRRIRP KLKWDNQ                                               | 46 |
|    | Dynorphin-B              | YGGFLRRQFK VVT                                                   | 47 |
|    | Eledoisin                | E*PSKDAFIGLM-NH <sub>2</sub>                                     | 48 |
| 30 | Endomorphin-1            | YPWF                                                             | 49 |
|    | Endomorphin-2            | YPFF                                                             | 50 |
|    | Endothelin-1             | CSCSSLMDKE CVYFCHLDII W                                          | 51 |
|    | Exendin-4                | HSDGTFTSDL SKQMEEEAVR LFIEWLKNNGG<br>PSSGAPPPS(NH <sub>2</sub> ) | 52 |
|    | Fibrinopeptide           | AADSGEGDFLA EGGGVR                                               | 53 |
| 35 | Fibrinopeptide           | BQGVNDNEEGF FSAR                                                 | 54 |
|    | Fibronectin CS1          | EILDVPST                                                         | 55 |
|    | FMRF                     | FMRF                                                             | 56 |
|    | Galanin                  | GWTLNSAGYL LGPHAVGNHR<br>SFSDKNGLTS                              | 57 |
|    | Galantide                | GWTLNSAGYL LGPQQFFGLM(NH <sub>2</sub> )                          | 58 |
|    | gamma-Bag Cell Peptide   | RLRFD                                                            | 59 |
|    | Gastrin                  | EGPWLEEEEE AYGWMDF                                               | 60 |
|    | Gastrin Releasing        | VPLPAGGGTV LTKMYPRGNH WAVGHLM                                    | 61 |

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|    |                                    |                                                           |     |
|----|------------------------------------|-----------------------------------------------------------|-----|
| 5  | Ghrelin                            | GSSFLSPEHQ RVOQRKESKK PPAKLQPR                            | 62  |
|    | GIP                                | YAEGTFISDY SIAMDKIHQQ DFNWLLAQK<br>GKKNDWKHNI TQ          | 63  |
|    | Glucagon                           | HSQGTFTSDY SKYLDSTRRAQ<br>DFVDWLMNT                       | 64  |
|    | Grb-7 SH2 domain-1                 | RRFA C DPDG YDN YFH C VPGG                                | 65  |
|    | Grb-7 SH2 domain-10                | TGSW C GLMH YDN AWL C NTQG                                | 66  |
| 10 | Grb-7 SH2 domain-11                | RSKW C RDGY YAN YPO C WTQG                                | 67  |
|    | Grb-7 SH2 domain-18                | RSTL C WFEG YDN TFP C KYFR                                | 68  |
|    | Grb-7 SH2 domain-2                 | RVOE C KYLY YDN DYI C KDDG                                | 69  |
|    | Grb-7 SH2 domain-23                | GLRR C LYGP YDN AWV C NIHE                                | 70  |
|    | Grb-7 SH2 domain-3                 | KLFW C TYED YAN EWP C PGYS                                | 71  |
| 15 | Grb-7 SH2 domain-34                | FCAV C NEEL YEN CGG C SCGK                                | 72  |
|    | Grb-7 SH2 domain-46                | RTSP C GYIG YDN IFE C TYLG                                | 73  |
|    | Grb-7 SH2 domain-5                 | TGEW C AOSV YAN YDN C KSAW                                | 74  |
|    | Grb-7 SH2 domain-6                 | NVSR C TYIH YDN WSL C GVEV                                | 75  |
|    | Grb-7 SH2 domain-8                 | GVSN C VFWG YAN DWL C SDYS                                | 76  |
| 20 | Growth hormone<br>releasing factor | YADAIFTNSY RQVLGQLSAR KLLQDIMSRO<br>QGESNQERGA RARL       | 77  |
|    | Guanylin                           | PGTCEICAYA ACTGC                                          | 78  |
|    | Helodermin                         | HSDAIFTEEY SKLLAKLALQ KYLASILGSR<br>TSPPP-NH <sub>2</sub> | 79  |
|    | Helospectin-1                      | HSDATFTAAY SKLLAKLALQ KYLESILGSS<br>TSPRPPSS              | 80  |
|    | Helospectin-2                      | HSDATFTAAY SKLLAKLALQ KYLESILGSS<br>TSPRPPS               | 81  |
| 25 | Histatin 5                         | DSHAKRHHGY KRKFHEKHHS HRGY                                | 82  |
|    | ICE inhibitor(III)                 | ac-YVAD-fluoroacyloxymethylketone                         | 83  |
|    | Immunostimulating<br>Peptide       | VEPIPY                                                    | 84  |
|    | Insulin (A-chain)                  | GIVEQCCTSI CSLYQLENYC N                                   | 85  |
|    | Insulin (B-chain)                  | FVNOHLCGSH LVEALYLVCG ERGFFYTPKT                          | 86  |
| 30 | Insulin (whole molecule)           | see above                                                 | 87  |
|    | Kinetensin                         | IARRHPYFL                                                 | 88  |
|    | Leu-Enkephalin                     | YGGFL                                                     | 89  |
|    | Litorin                            | E*QWAVGHFM-NH <sub>2</sub>                                | 90  |
|    | Malantide                          | RTKRSGSVYE PLKI                                           | 91  |
| 35 | Met-Enkephalin                     | YGGFM                                                     | 92  |
|    | Metorphamide                       | YGGGFMRRV-NH <sub>2</sub>                                 | 93  |
|    | Motilin                            | FVPIFTYGEL QRMQEKERNK GO                                  | 94  |
|    | Myomodulin                         | PMSMLRL-NH <sub>2</sub>                                   | 95  |
|    | Myosin Kinase                      | IPKKRAARATS-NH <sub>2</sub>                               | 96  |
| 40 | Necrofibrin                        | GAVSTA                                                    | 97  |
|    | Neurokinin A                       | HKTDSFVGLM-NH <sub>2</sub>                                | 98  |
|    | Neurokinin B                       | DMHDFVGLM-NH <sub>2</sub>                                 | 99  |
|    | Neuromedin B                       | GNLWATGHFM-NH <sub>2</sub>                                | 100 |

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|    |                                       |                                                            |     |
|----|---------------------------------------|------------------------------------------------------------|-----|
| 5  | Neuropeptide Y                        | YPSKPDNPGE DAPAEDMARY<br>YSAKRHYINL ITRQRY-NH <sub>2</sub> | 101 |
|    | Neurotensin                           | E*LYENKPRRPUIL                                             | 102 |
|    | Nociceptin                            | FGGFTGARKS ARKLANQ                                         | 103 |
|    | Nociceptin/Orphanin FQ                | FAEPLPSEEE GESYSKEVPE MEKRYGGFMR<br>F                      | 104 |
|    | Nocistatin                            | EOKOLO                                                     | 105 |
| 10 | Orexin A                              | E*PLPDCCRQKTCSCRLYELLHGAGNHAAGI<br>LTL-NH <sub>2</sub>     | 106 |
|    | Orexin B                              | RSGPPGLQGR LQRLLOASGN HAAGILTM-<br>NH <sub>2</sub>         | 107 |
|    | Osteocalcin                           | YLYQWLGAHV PYPDPLEPRR EVCELNPDCD<br>ELADHIGFQE AYRRFYGPV   | 108 |
|    | Oxytocin                              | CYIQNCPLG-NH <sub>2</sub>                                  | 109 |
|    | PACAP                                 | HSDGIFTDSY SRYRKQMAVK KYLA AVL                             | 110 |
| 15 | PACAP-RP                              | DVAHGILNEA YRKVLDQLSA GKHLOSLVA                            | 111 |
|    | Pancreatic Polypeptide                | APLEPVYPGD NATPEQMAQY<br>AADLRRYINM LTRPRY-NH <sub>2</sub> | 112 |
|    | Papain Inhibitor                      | GGYR                                                       | 113 |
|    | Peptide E                             | YGGFMRRVGR PE                                              | 114 |
|    | Peptide YY                            | YPIKPEAPGE DASPEELNRY YASLRHYLNL<br>VTRQRY-NH <sub>2</sub> | 115 |
| 20 | Phosphate acceptor                    | RRKASGPPV                                                  | 116 |
|    | Physalaemin                           | E*ADPNKFYGLM-NH <sub>2</sub>                               | 117 |
|    | Ranatensin                            | E*VPQWAVGHFM-NH <sub>2</sub>                               | 118 |
|    | RGD peptides                          | X-RGD-X                                                    | 119 |
|    | Rigin                                 | GQPR                                                       | 120 |
| 25 | RR-SRC                                | RRLIEDAEYA ARG                                             | 121 |
|    | Schizophrenia                         | RPTVL                                                      | 122 |
|    | Secretin                              | HSDGTFTSEL SRLREGARLO RLLOGLV                              | 123 |
|    | Serum Thymic Factor                   | E*AKSQGGSN                                                 | 124 |
|    | structural-site zinc<br>ligands-alpha | PQCGKCRICK NPESNYCLK                                       | 125 |
| 30 | structural-site zinc<br>ligands-beta  | PQCGKCRVCK NPESNYCLK                                       | 126 |
|    | structural-site zinc<br>ligands-gamma | PQCGKCRICK NPESNYCLK                                       | 127 |
|    | structural-site-zinc<br>ligands-pi    | PLCRKCKFCLSPLTNLCGK                                        | 128 |
|    | structural-site-zinc<br>ligands-X     | PQGECKFCLNPKTNLCQK                                         | 129 |
|    | Substance P                           | RPKPQQFFGL M-NH <sub>2</sub>                               | 130 |
| 35 | Syntide 2                             | PLARTLSVAG LPGKK                                           | 131 |
|    | Systemin                              | AVQSKPPSKR DPPKMOTD                                        | 132 |
|    | Thrombin-light chain                  | TFGSGEADCG LRPLFEKKSL EDKTERELLE<br>SYIDGR                 | 133 |
|    | Thymopentin                           | RKDVY                                                      | 134 |
|    | Thymus Factor                         | QAKSQGGSN                                                  | 135 |

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|    |                                     |                                               |     |
|----|-------------------------------------|-----------------------------------------------|-----|
| 5  | TRH                                 | E*HP                                          | 136 |
|    | Tuftsia                             | TKPR                                          | 137 |
|    | Uperoleia                           | E*PDPNAFYGLM-NH <sub>2</sub>                  | 138 |
|    | Uremic Pentapeptide                 | DLWQK                                         | 139 |
|    | Urocortia                           | DNPSLSIDLT FHLLRTLLEL ARTQSQRERA<br>EQNRIFDSV | 140 |
| 10 | Uroguanylin                         | NDDCELCVNV ACTGCL                             | 141 |
|    | Vasonatrin                          | GLSKGCFGLK LDRIGSMSGL GCNSFRY                 | 142 |
|    | Vasopressin                         | CYFQNCPRG                                     | 143 |
|    | Vasotocia                           | CYIQNCPRG                                     | 144 |
|    | VIP                                 | HSDAVFTDNY TRLRKQMAVK KYLNSILN                | 145 |
| 15 | Xenin                               | MLTKFETKSA RVKGLSFHPK RPWIL                   | 146 |
|    | YXN motif                           | Tyr-X-Asn.                                    | 147 |
|    | Zinc ligand of carbonic anhydrase I | FQFHFHWGS                                     | 148 |
|    | Zinc ligand of carbonic anhydrase   | IIIQFHFHWGS                                   | 149 |

Other selections for Y are can be identified by those of skill in the art and include, for example, those disclosed in *Techniques in Protein Chemistry, Vol. 1* (1989) T. Hugli ed. (Academic Press); *Techniques in Protein Chemistry, Vol. 5* (1994) J.W. Crabb ed. (Academic Press); Lundblad *Techniques in Protein Modification* (1995) (CRC Press, Boca Raton, FL); Glazer *et al.* (1976) *Chemical Modification of Proteins* (North Holland (Amsterdam))(American Elsevier, New York); and Hermanson (1996) *Bioconjugate Techniques* (Academic Press, San Diego, CA).

#### 4. Sorting Functions "Q"

The compounds provided herein can include a sorting function ("Q"), which permits the compounds to be addressed, such as by capture in a 2-D array. The sorting functions are "tags", such as oligonucleotide tags, such that when the compounds are bathed over an array of complementary oligonucleotides linked to solid supports, such as beads, chips, under suitable binding conditions, the oligonucleotides hybridize. The identity of the capture compound can be known by virtue of its position in the array. Other sorting functions can be optically coded, including as color coded or bar coded beads that can be separated, or an

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electronically-tagged, such as by providing microreactor supports with electronic tags or bar coded supports (see, *e.g.*, U.S. Patent No. 6,025,129; U.S. Patent No. 6,017,496; U.S. Patent No. 5,972,639; U.S. Patent No. 5,961,923; U.S. Patent No. 5,925,562; U.S. Patent No. 5,874,214; U.S. Patent No. 5,751,629; U.S. Patent No. 5,741,462), or chemical tags (see, *e.g.*, U.S. Patent No. 5,432,018; U.S. Patent No. 5,547,839) or colored tags or other such addressing methods that can be used in place of physically addressable arrays. The sorting function is selected to permit physical arraying or other addressable separation method suitable for analysis, particularly mass spectrometric, including MALDI, analysis.

Other sorting functions for use in the compounds provided herein include biotin, 6 x His, BODIPY, oligonucleotides, nucleosides, nucleotides, antibodies, immunotoxin conjugates, adhesive peptides, lectins, liposomes, PNA, activated dextrans and peptides. In one embodiment, the sorting function is an oligonucleotide, particularly, either a single-stranded or partially single-stranded oligonucleotide to permit hybridization to single-stranded regions on complementary oligonucleotides on solid supports.

In one embodiment of the capture compounds provided herein, Q is a single stranded unprotected or suitably protected oligonucleotide or oligonucleotide analog (*e.g.*, PNA) of up to 50 building blocks, which is capable of hybridizing with a base-complementary single stranded nucleic acid molecule. In certain embodiments, Q contains from about 5 up to about 10, 15, 25, 30, 35, 40, 45 or 50 building blocks.

Biomolecule mixtures, including, but not limited to, protein mixtures, can have different hydrophobicities (solubility) than the compounds provided herein. In certain embodiments, in order to achieve high reaction yields between the functionality X on the compounds

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provided herein and the protein surface, the reaction is performed in solution. In other embodiments, the reaction is performed at a solid/liquid or liquid/liquid interface. In certain embodiments, the solubility properties of the compounds provided herein are dominated by the Q moiety. A  
5 change in the structure of Q can, in these embodiments, accommodate different solubilities. For example, if the protein mixture is very water soluble, Q can have natural phosphodiester linkages; if the bimolecular mixture is very hydrophobic (lipids, glycolipids, membrane proteins, lipoproteins), Q can have its phosphodiester bonds protected as  
10 phosphotriesters, or alternatively, these bonds can be methylphosphonatediesters or peptide nucleic acids (PNAs). If the biomolecule mixture is of an intermediate hydrophobicity, solubility is achieved, *e.g.*, with phosphothioate diester bonds. Intermediate solubility also can be attained by mixing phosphodiester with phosphotriester linkages. Those  
15 skilled in the art can easily conceive of other means to achieve this goal, including, but not limited to, addition of substituents on Z, as described elsewhere herein, or use of beads for Z that are hydrophobic, including, but not limited to, polystyrene, polyethylene, polypropylene or teflon, or hydrophilic, including, but not limited to, cellulose, dextran cross-linked  
20 with epichlorohydrin (*e.g.*, Sephadex<sup>®</sup>), agarose (*e.g.*, Sepharose<sup>®</sup>), lectins, adhesive polypeptides, and polyacrylamides.

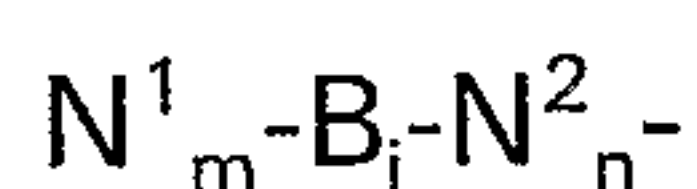
The flexibility of being able to change the solubility of the compounds is a significant advantage over current methods. In contrast, 2D gel electrophoresis is useful only for analysis of water soluble proteins  
25 with the result that about 30 to 35% of all cellular proteins, such as those residing in the cell membrane, cannot be analyzed by this method. This is a severe limitation of 2D gel electrophoresis since many proteins, including, but not limited to, those involved in tissue specific cell-cell contacts, signal transduction, ion channels and receptors, are localized in

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the cell membrane.

In one embodiment, after reaction or complexation of the compounds provided herein with a biomolecule, including, but not limited to, a protein, the compounds are brought into contact with a set of  
 5 spatially resolved complementary sequences on a flat support, beads or microtiter plates under hybridization conditions.

In certain embodiments, Q is a monovalent oligonucleotide or oligonucleotide analog group that is at least partially single stranded or includes a region that can be single-stranded for hybridization to  
 10 complementary oligonucleotides on a support. Q can have the formula:



where  $N^1$  and  $N^2$  are regions of conserved sequences; B is a region of sequence permutations; m, i and n are the number of building blocks in  
 15  $N^1$ , B and  $N^2$ , respectively; and the sum of m, n and i is a number of units able to hybridize with a complementary nucleic acid sequence to form a stable hybrid. Thus, in embodiments where B is a single stranded DNA or RNA, the number of sequence permutations is equal to  $4^i$ . In one embodiment, the sum of m, n and i is about 5 up to about 10, 15, 25,  
 20 30, 35, 40, 45 or 50. In certain embodiments m and n are each independently 0 to about 48, or are each independently about 1 to about 25, or about 1 to about 10 or 15, or about 1 to about 5. In other embodiments, i is about 2 to about 25, or is about 3 to about 12, or is about 3 to about 5, 6, 7 or 8.

25 The oligonucleotide portion, or oligonucleotide analog portion, of the compounds ( $N^1_m-B_i-N^2_n-$ ), can be varied to allow optimal size for binding and sequence recognition. The diversity of the sequence permutation region B can be relatively low if the biomolecule mixture, including, but not limited to, protein mixtures, is of low complexity. If

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the mixture is of high complexity, the sequence region B has to be of high diversity to afford sufficient resolving power to separate all the species.

The flanking conserved regions  $N^1_m$  and  $N^2_n$ , need only be long enough to provide for efficient and stable hybrid formation. There is, however,

5 flexibility in designing these regions:  $N^1_m$  and  $N^2_n$  can be of the same length and same sequence, of the same length and different sequence or of different length and different sequence. In certain embodiments, including those where B is of sufficient length to provide stable hybrid formation,  $N^1$  and/or  $N^2$  are absent. In these embodiments, the

10 oligonucleotide portion of the compounds, or oligonucleotide analog portion of the compounds, has the formula  $N^1_m-B_i-$ , or  $B_i-N^2_n-$ , or  $B_i-$ .

In an exemplary embodiment (see, *e.g.*, EXAMPLE 1.a.), B has a trinucleotide sequence embedded within a 11-mer oligonucleotide sequence, where the  $N^1_m$  and  $N^2_n$  tetranucleotide sequences provide

15 flanking identical (conserved) regions. This arrangement for  $N^1_m-B_i-N^2_n-$  affords 64 different compounds where each compound carries the same reactive functionality X. In another exemplary embodiment (see, *e.g.*,

EXAMPLE 1.b.), B has a tetranucleotide sequence embedded within a 12-mer oligonucleotide sequence, where the  $N^1_m$  and  $N^2_n$  oligonucleotide

20 sequences provide flanking but not identical octanucleotide sequences.

This arrangement for  $N^1_m-B_i-N^2_n-$  affords 256 different compounds where each carry the same reactive functionality X. In a further exemplary

embodiment (see, *e.g.*, EXAMPLE 1.c.), B has an octanucleotide

sequence embedded within a 23-mer oligonucleotide sequence, where the

25  $N^1_m$  and  $N^2_n$  oligonucleotide sequences provide flanking but not identical octanucleotide sequences. This arrangement for  $N^1_m-B_i-N^2_n-$  affords

65,536 different compounds where each carries the same reactive

functionality X, and exceeds the estimated complexity of the human

proteome (*e.g.*, 30,000-35,000 different proteins). In certain

embodiments, use of a B with excess permutations for the complexity of the protein mixture, as the oligonucleotides with the best hybridization properties can be used for analysis to reduce mismatching.

#### 5. Solubility Functions "W"

5 The compounds provided herein can include a solubility function, W, to confer desired solubility properties, such as solubility in hydrophobic environments or hydrophilic environments to permit probing of biomolecules in physiological environments, such as in membranes. Exemplary solubility functions for use in the compounds provided herein  
10 include polyethylene glycols, sulfates, polysulfates, phosphates, sulfonates, polysulfonates, carbohydrates, dextrin, polyphosphates, polycarboxylic acids, triethanolamine, alcohols, water soluble polymers, salts of alkyl and aryl carboxylic acids and glycols.

Amphiphilic compounds, such as quaternary ammonium salts (*i.e.*,  
15 betain, choline, sphingomyelin, tetramethyl (or tetrabutyl) alkyl ammonium salts, cationic, ionic and neutral tensides may also be used as the solubility function W.

In other embodiments, W also can be used to modulate the solubility of the compounds to achieve homogeneous solutions, if desired,  
20 when reacting with biomolecule mixtures, including, but not limited to, protein mixtures. In certain embodiments, W is a sulfonate, a polar functionality that can be used to make the compounds more water-soluble. In other embodiments, W is a hydrophobic group, including lower alkyl, such as tert-butyl, tert-amyl, isoamyl, isopropyl, n-hexyl, sec-  
25 hexyl, isohexyl, n-butyl, sec-butyl, iso-butyl and n-amyl, or an aryl group, including phenyl or naphthyl.

#### 6. Exemplary Embodiments

The following provides exemplary capture compounds that exhibit the above-described properties. It is understood that these are exemplary

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only and that any compounds that can react covalently with a biomolecule or by other highly stable interaction that is stable to analytic conditions, such as those of mass spectrometric analysis, and that can sorted or otherwise identified are contemplated for use in the collections.

**5 a. Exemplary embodiment 1**

In one embodiment, the compounds for use in the methods provided herein have formulae:

Q-Z-X or Q-Z-Y,

where Q is a sorting function that contains a single stranded unprotected  
**10** or suitably protected oligonucleotide or oligonucleotide analog (*e.g.*, peptide nucleic acid (PNA)) of up to 50 building blocks, which is capable of hybridizing with a base-complementary single-stranded nucleic acid molecule;

Z is a moiety that is cleavable prior to or during analysis of a  
**15** biomolecule, including mass spectral analysis, without altering the structure of the biomolecule, including, but not limited to, a protein;

X is a reactivity functional group that interacts with and/or reacts with functionalities on the surface of a biomolecule, including, but not limited to, a protein, to form covalent bonds or bonds that are stable  
**20** under conditions of mass spectrometric analysis, particularly MALDI analysis; and

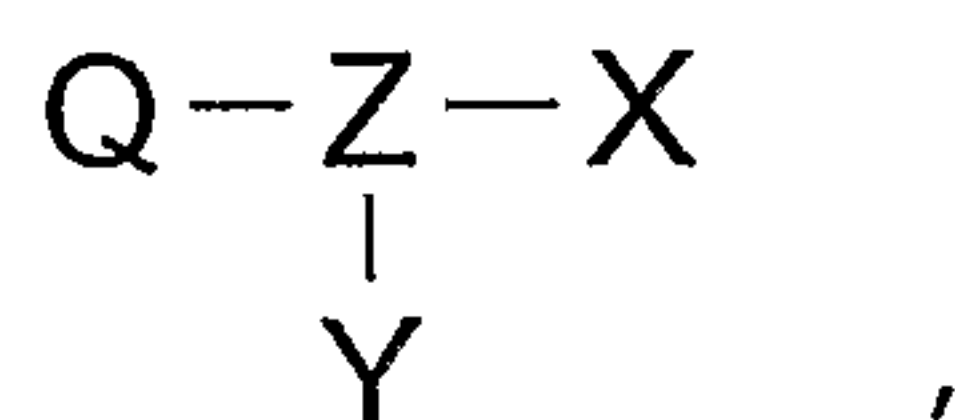
Y is a selectivity functional group that interacts with and/or reacts by imposing unique selectivity by introducing functionalities that interact noncovalently with target proteins.

**25 b. Exemplary embodiment 2**

In another embodiment, the compounds for use in the methods provided herein have formula:

**30**

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5            where Q is a single-stranded unprotected or suitably protected oligonucleotide or oligonucleotide analog (*e.g.*, peptide nucleic acid (PNA)) of up to 50 building blocks, which is capable of hybridizing with a base-complementary single stranded nucleic acid molecule;

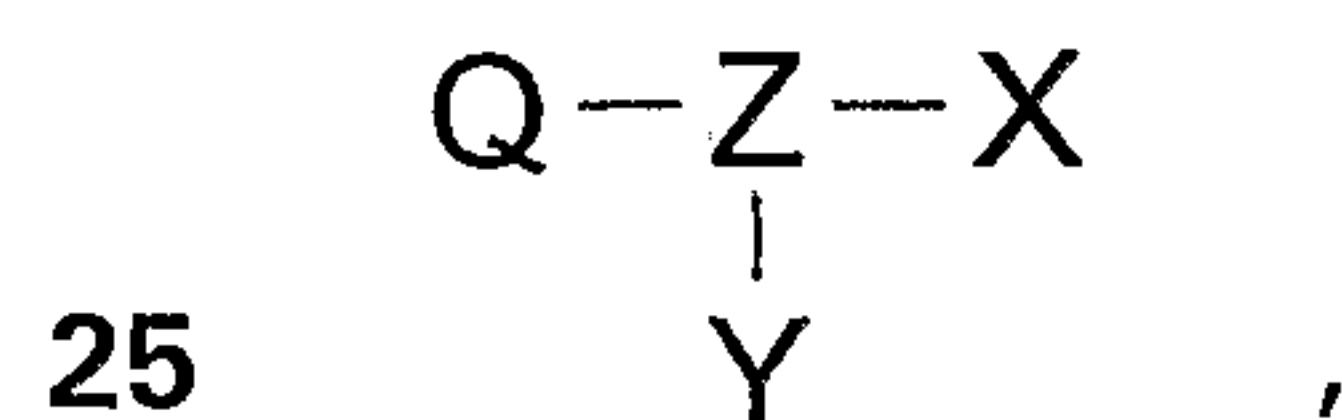
10           Z is a moiety that is cleavable prior to or during analysis of a biomolecule, including mass spectral analysis, without altering the structure of the biomolecule, including, but not limited to, a protein;

15           X is a functional group that interacts with and/or reacts with functionalities on the surface of a biomolecule, including, but not limited to, a protein, to form covalent bonds or bonds that are stable under conditions of mass spectrometric analysis, particularly MALDI analysis; and

            Y is a functional group that interacts with and/or reacts by imposing unique selectivity by introducing functionalities that interact noncovalently with target proteins.

### 20                      c.        Exemplary embodiment 3

            In another embodiment, the compounds for use in the methods provided herein have formula:



            where Q is a sorting function that is a compound, or one or more biomolecules (*e.g.*, a pharmaceutical drug preparation, a biomolecule, drug or other compound that immobilizes to the substrate and captures target biomolecules), which is(are) capable of specific noncovalent binding to a known compound to produce a tightly bound capture compound;

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Z is a moiety that is cleavable prior to or during analysis of a biomolecule, including mass spectral analysis, without altering the structure of the biomolecule, including, but not limited to, a protein;

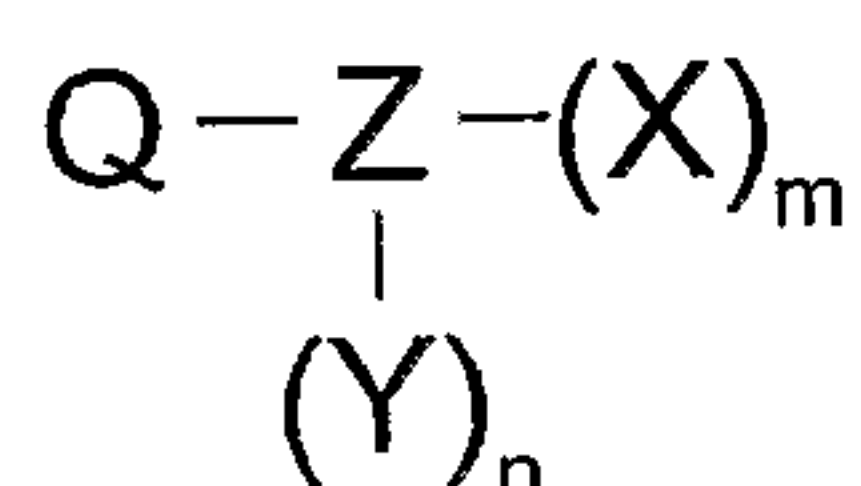
5 X is a functional group that interacts with and/or reacts with functionalities on the surface of a biomolecule, including, but not limited to, a protein, to form covalent bonds or bonds that are stable under conditions of mass spectrometric analysis, particularly MALDI analysis; and

10 Y is a functional group that interacts with and/or reacts by imposing unique selectivity by introducing functionalities that interact noncovalently with target proteins.

**d. Exemplary embodiment 4**

In another embodiment, the compounds for use in the methods provided herein have the formulae:

15



20 or  $\text{Q}-\text{Z}-(\text{X})_m$  or  $\text{Q}-\text{Z}-(\text{Y})_n$ ,

where Q, Z, X and Y are as defined above; m is an integer from 1 to 100, in one embodiment 1 to 10, in another embodiment 1 to 3, 4 or 5; and n in an integer from 1 to 100, in one embodiment 1 to 10, in another embodiment 1 to 3, 4 or 5.

25

**e. other Exemplary embodiments**

In other embodiments, X is a pharmaceutical drug. The compounds of these embodiments can be used in drug screening by capturing biomolecules, including but not limited to proteins, which bind to the pharmaceutical drug. Mutations in the biomolecules interfering  
30 with binding to the pharmaceutical drug are identified, thereby determining possible mechanisms of drug resistance. See, *e.g.*, Hessler

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*et al.* (November 9-11, 2001) *Ninth Foresight Conference on Molecular Nanotechnology* (Abstract)(<http://www.foresight.org/Conferences/-MNT9/Abstracts/Hessler/>).

#### f. Other embodiments

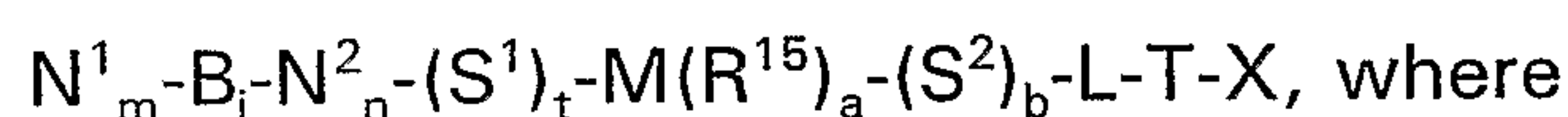
5 In certain embodiments, the compounds provided herein have the formula:



where  $N^1$ , B,  $N^2$ ,  $S^1$ , M,  $S^2$ , L, X, m, i, n, t, a and b are as defined above.

In further embodiments, the compounds for use in the methods provided

10 herein include a mass modifying tag and have the formula:



$N^1$ , B,  $N^2$ ,  $S^1$ , M,  $S^2$ , L, T, X, m, i, n, t, a and b are as defined above.

In other embodiments, including those where Z is not a cleavable linker, the compounds provided herein have the formula:

15  $N^1_m-B_i-N^2_n-(S^1)_t-M(R^{15})_a-(S^2)_b-X$ , where  $N^1$ , B,  $N^2$ ,  $S^1$ , M,  $S^2$ , X, m, i, n, t, a and b are as defined above.

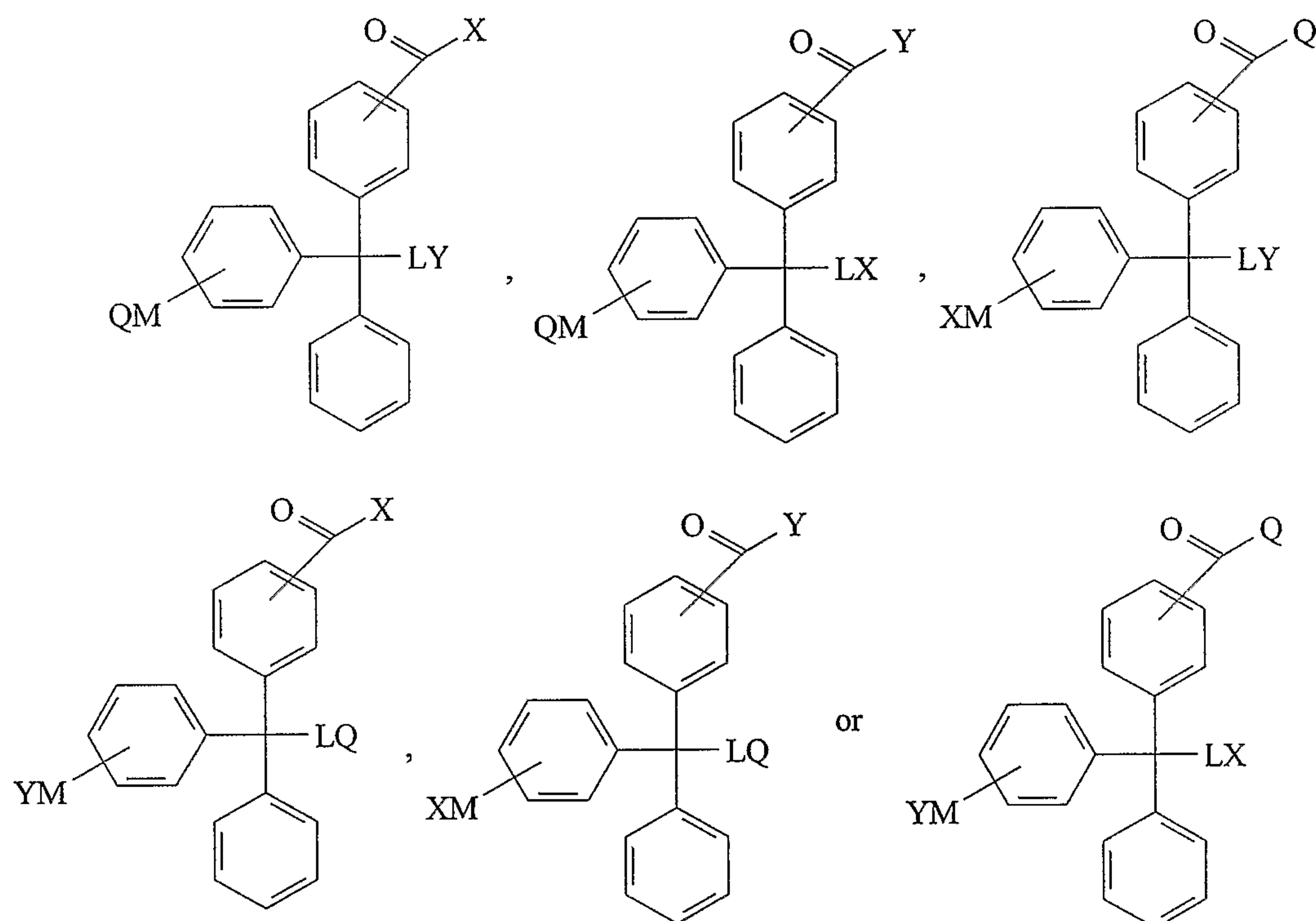
In another embodiment, the compounds for use in the methods provided herein include those of formulae:

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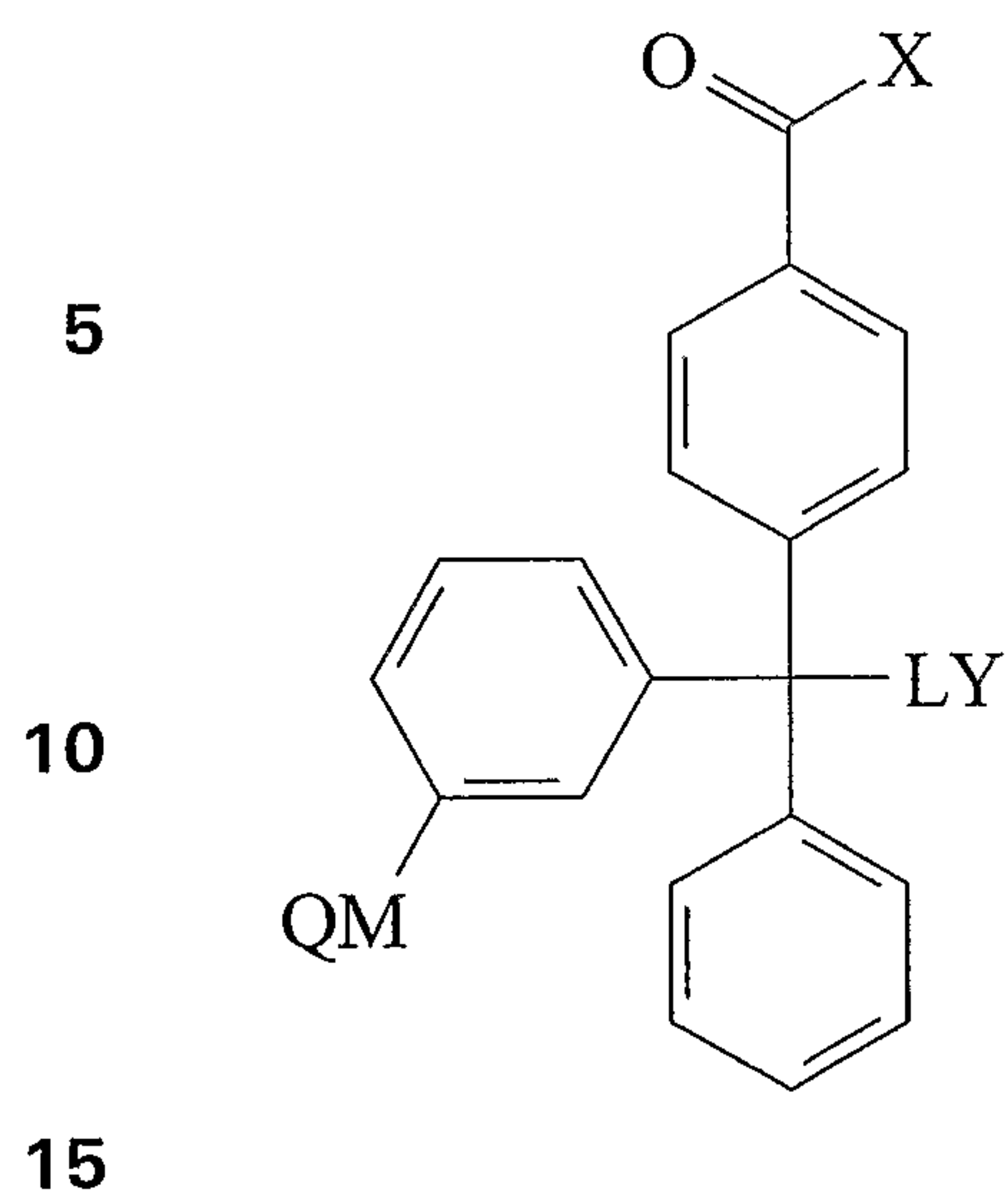
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where L and M are each independently O, S or NR<sup>3</sup>; X is a reactivity function, as described above; Y is a selectivity function, as described above; Q is a sorting function, as described above; and each R<sup>3</sup> is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, or substituted or unsubstituted heteroaralkyl.

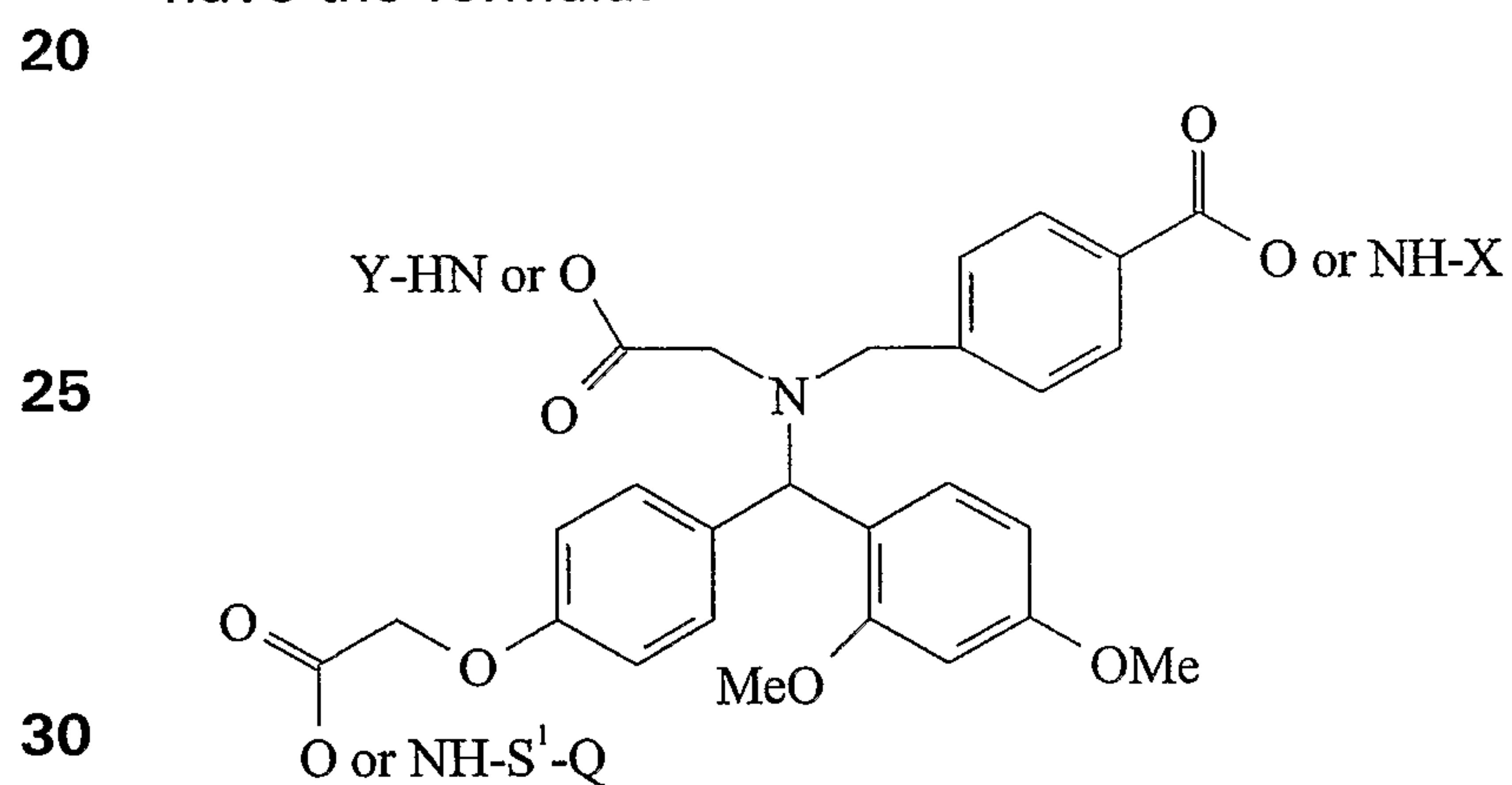
**10** In another embodiment, the capture compounds provided herein have the formula:

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where L, M, X, Y and Q are as defined above.

In another embodiment, the capture compounds provided herein have the formula:

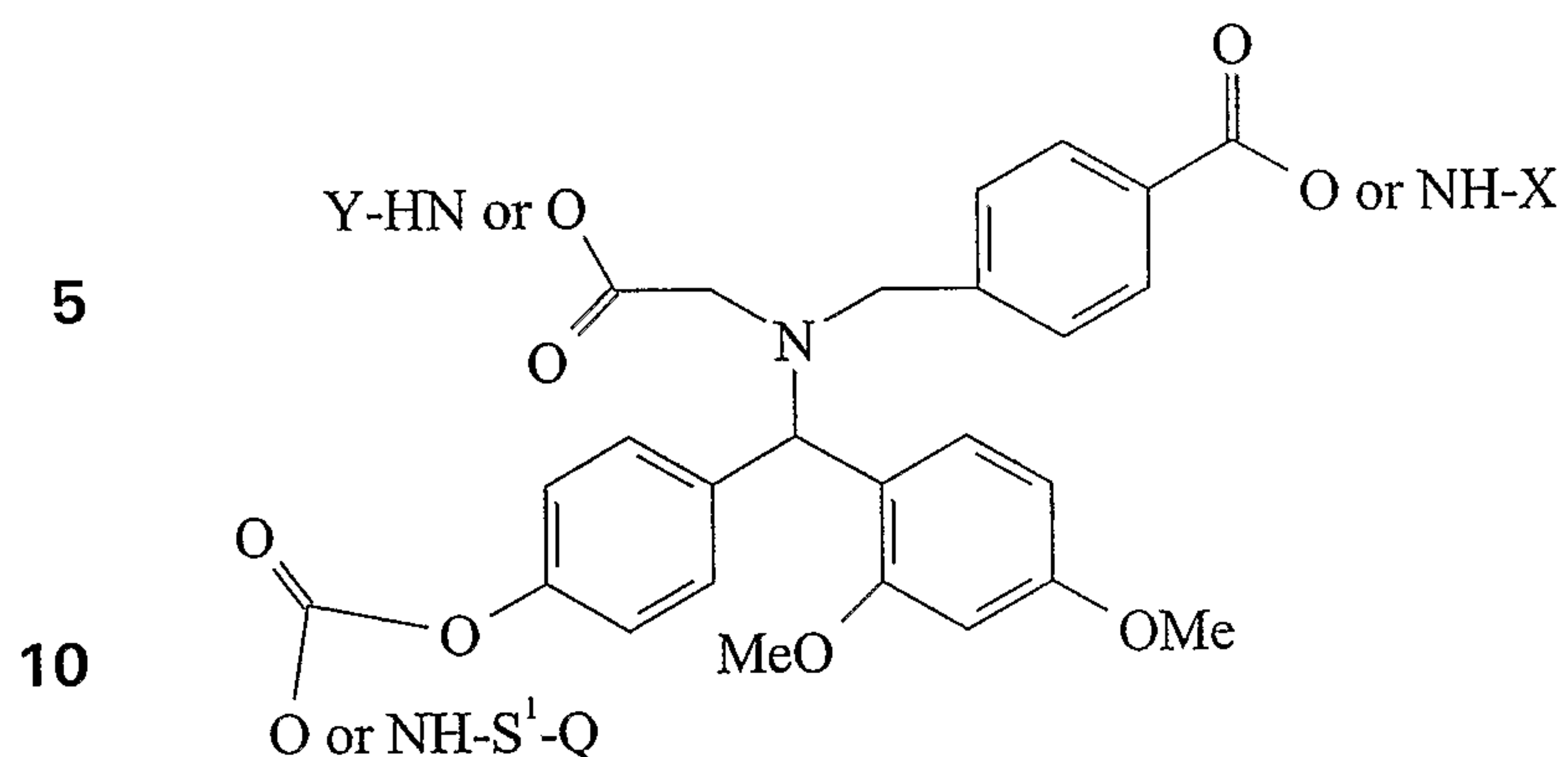


where X, Y, Q and S<sup>1</sup> are as defined above.

35 In another embodiment, the capture compounds provided herein have the formula:

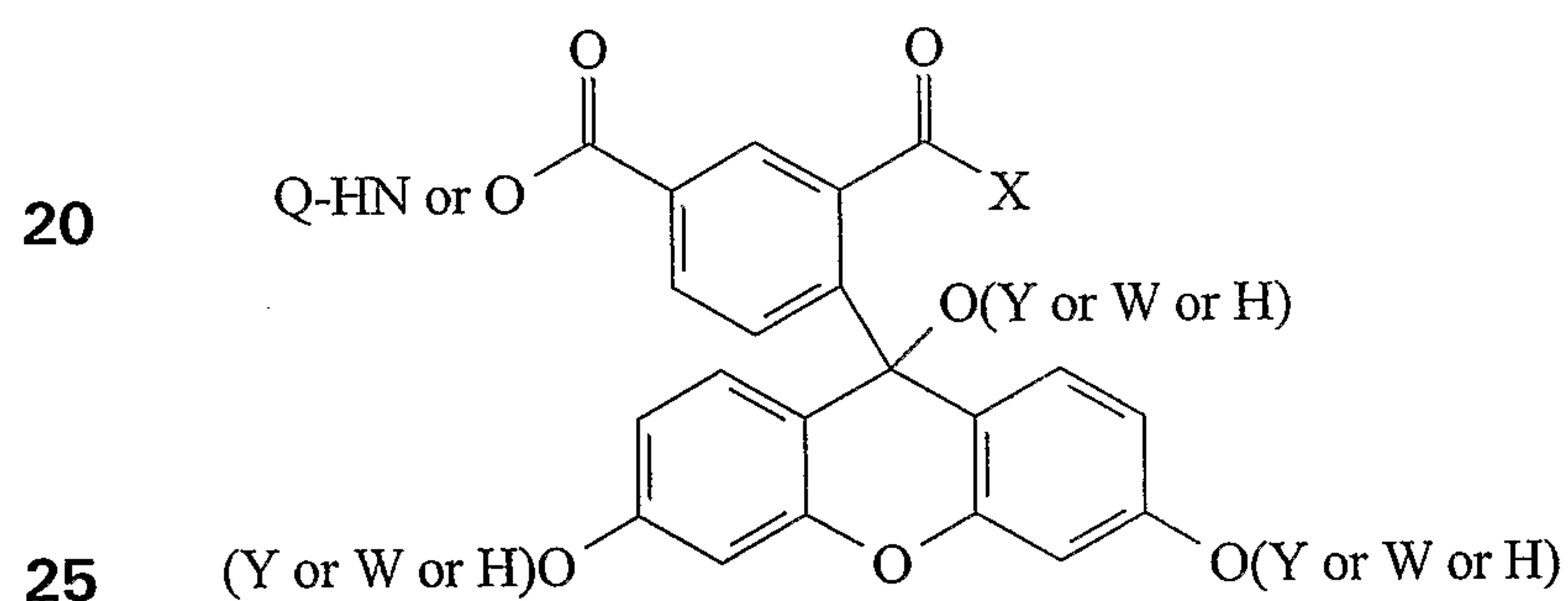
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where Q, Y, X and S<sup>1</sup> are as defined above.

15 In another embodiment, the capture compounds provided herein have the formula:



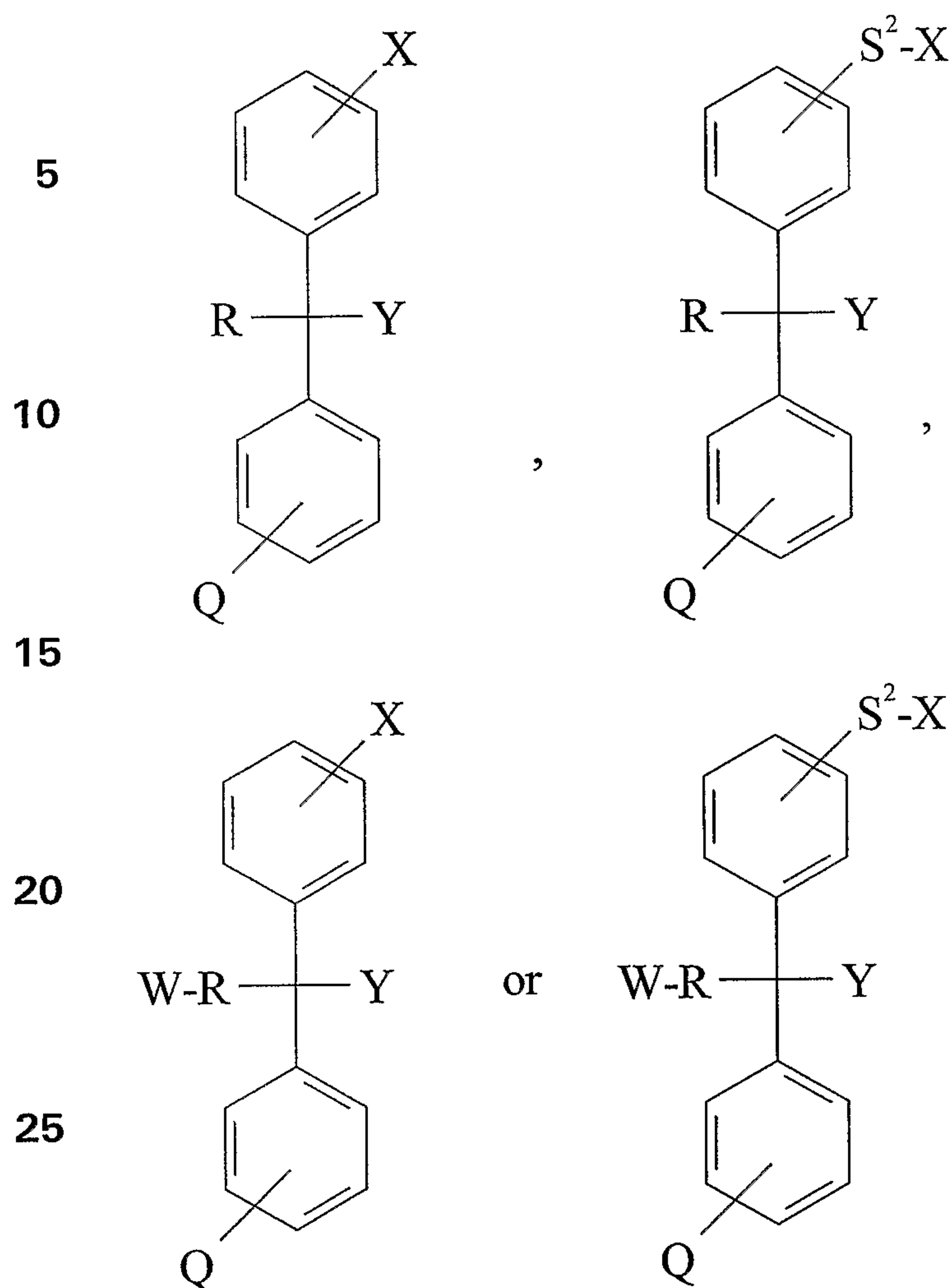
where X, Y, Q and W are as defined above.

30 In another embodiment, the capture compounds for use in the methods provided herein have the formulae:

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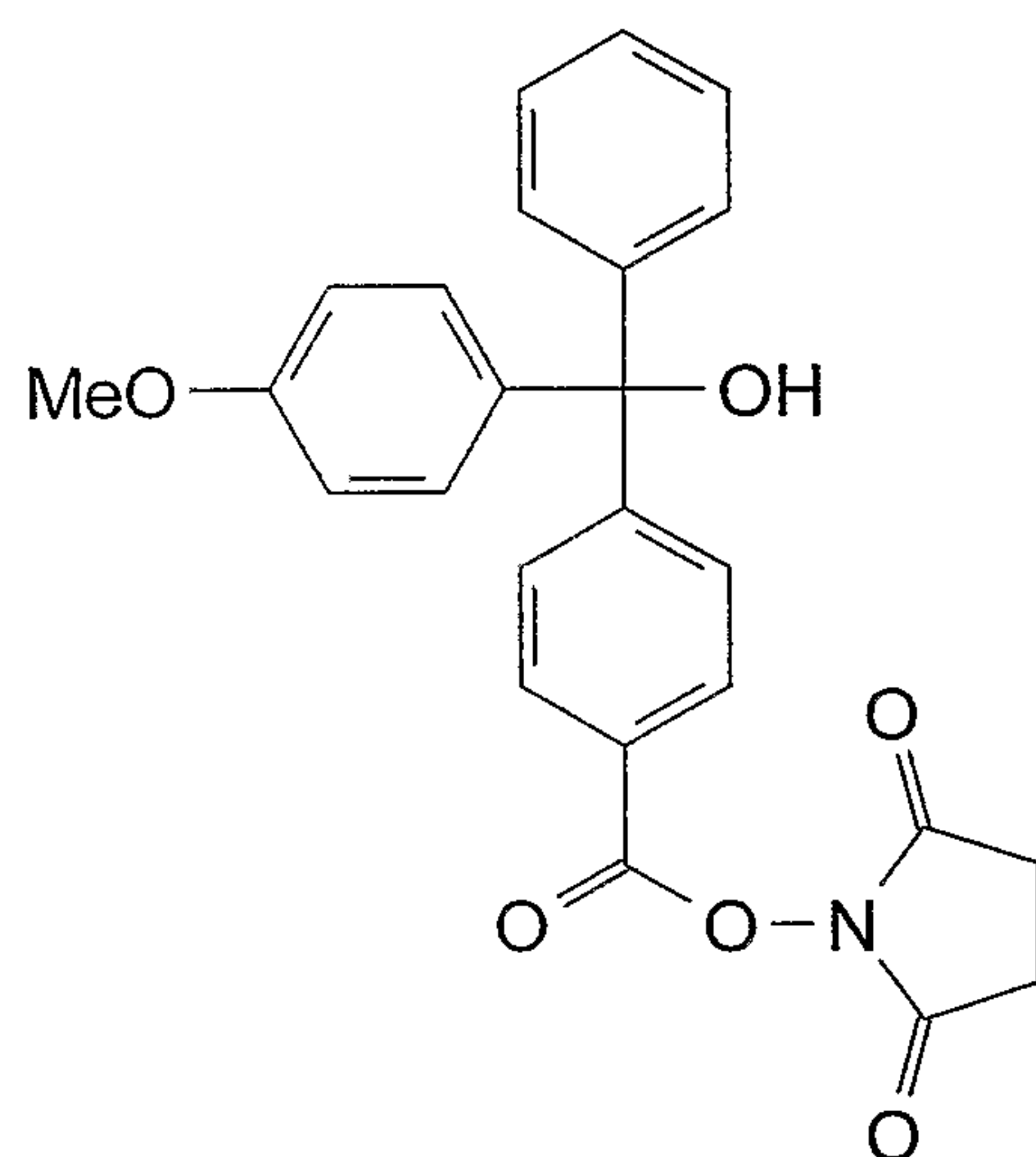
where X, Y, Q and W are selected as above; and R is substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl, or substituted or unsubstituted aralkyl. In another embodiment, R is selected from cyclohexyl, cyclohexyl-(CH<sub>2</sub>)<sub>n</sub>-, isopropyl, and phenyl-(CH<sub>2</sub>)<sub>n</sub>-, where n is 1, 2 or 3. As shown in the formulae above, R is optionally substituted with W.

In other embodiments, the compounds for use in the methods provided herein include:

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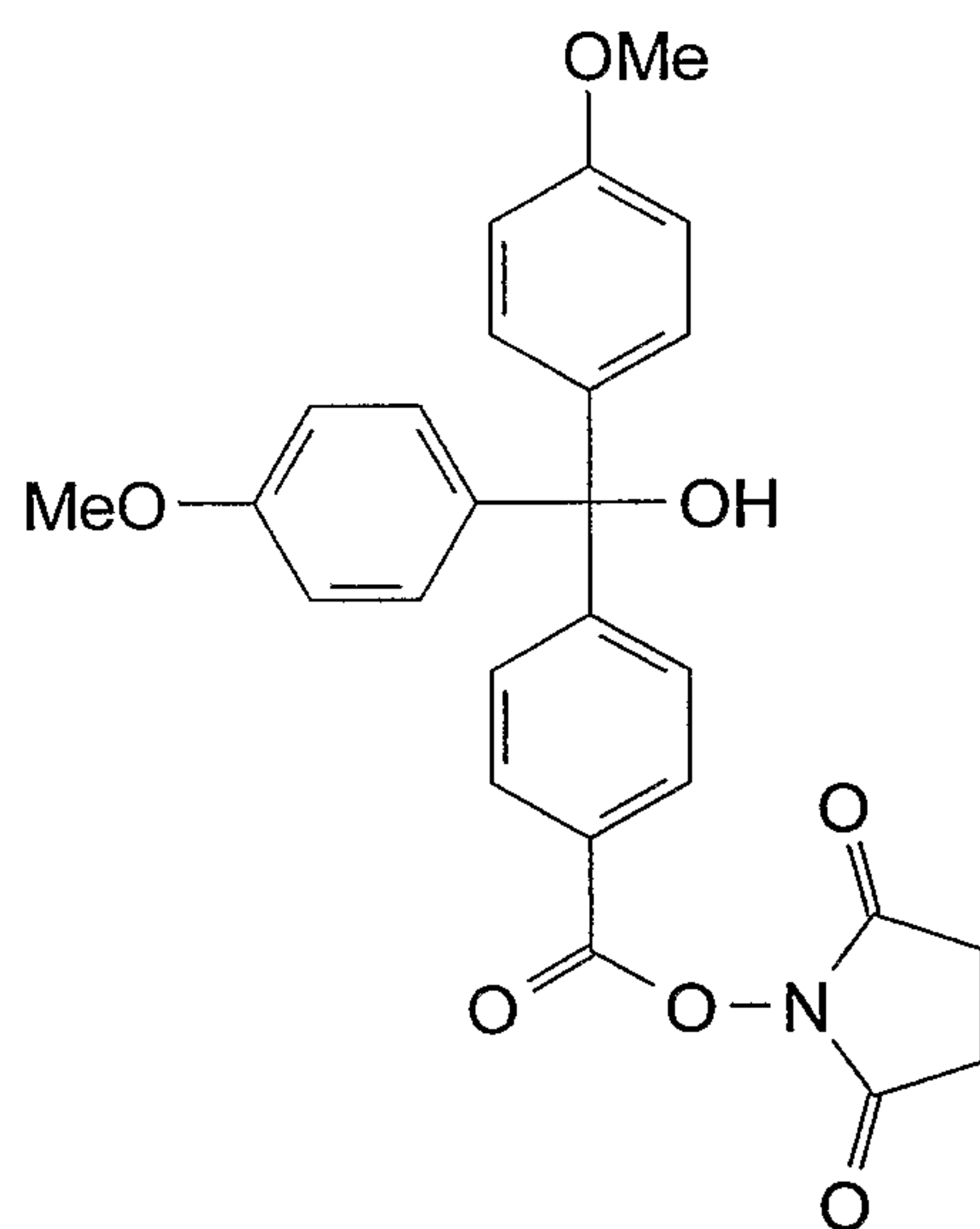
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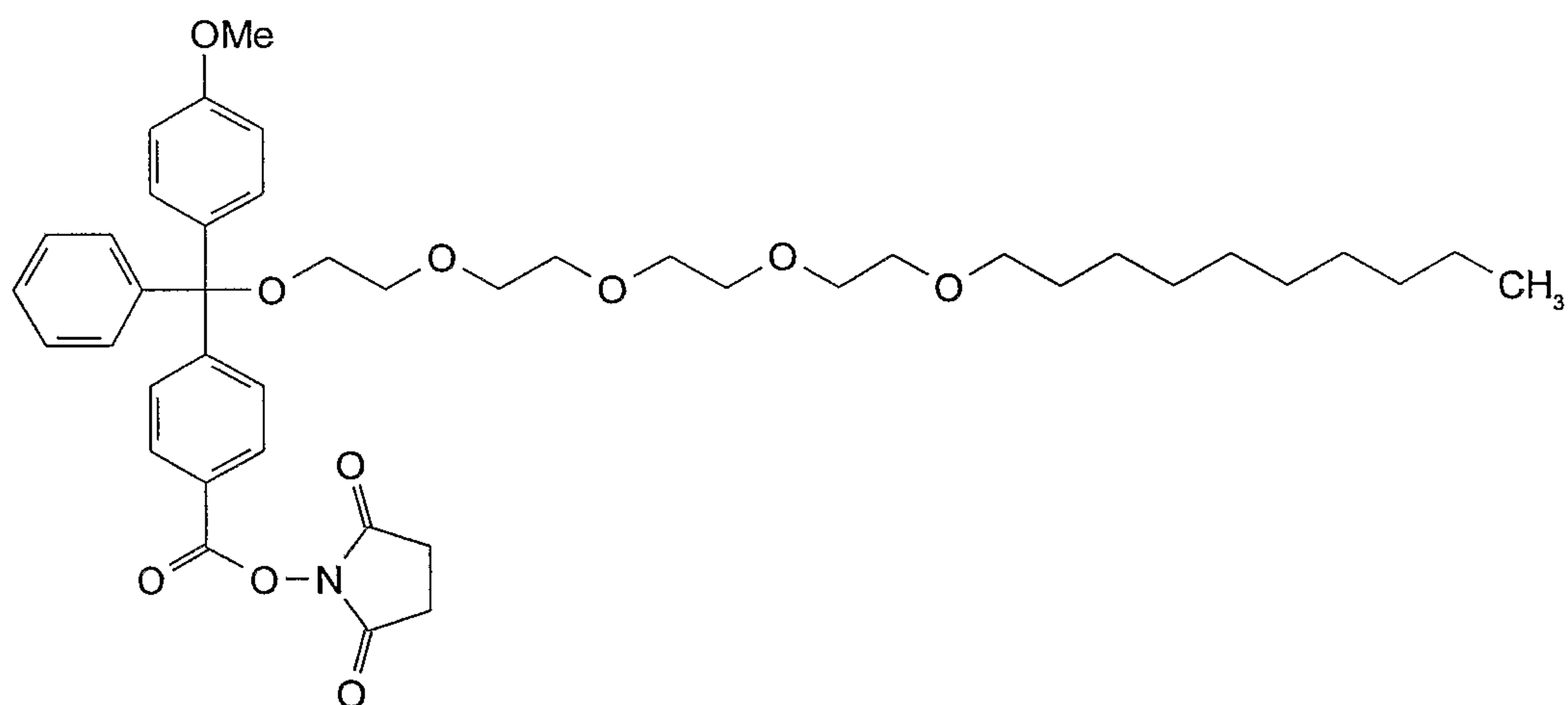
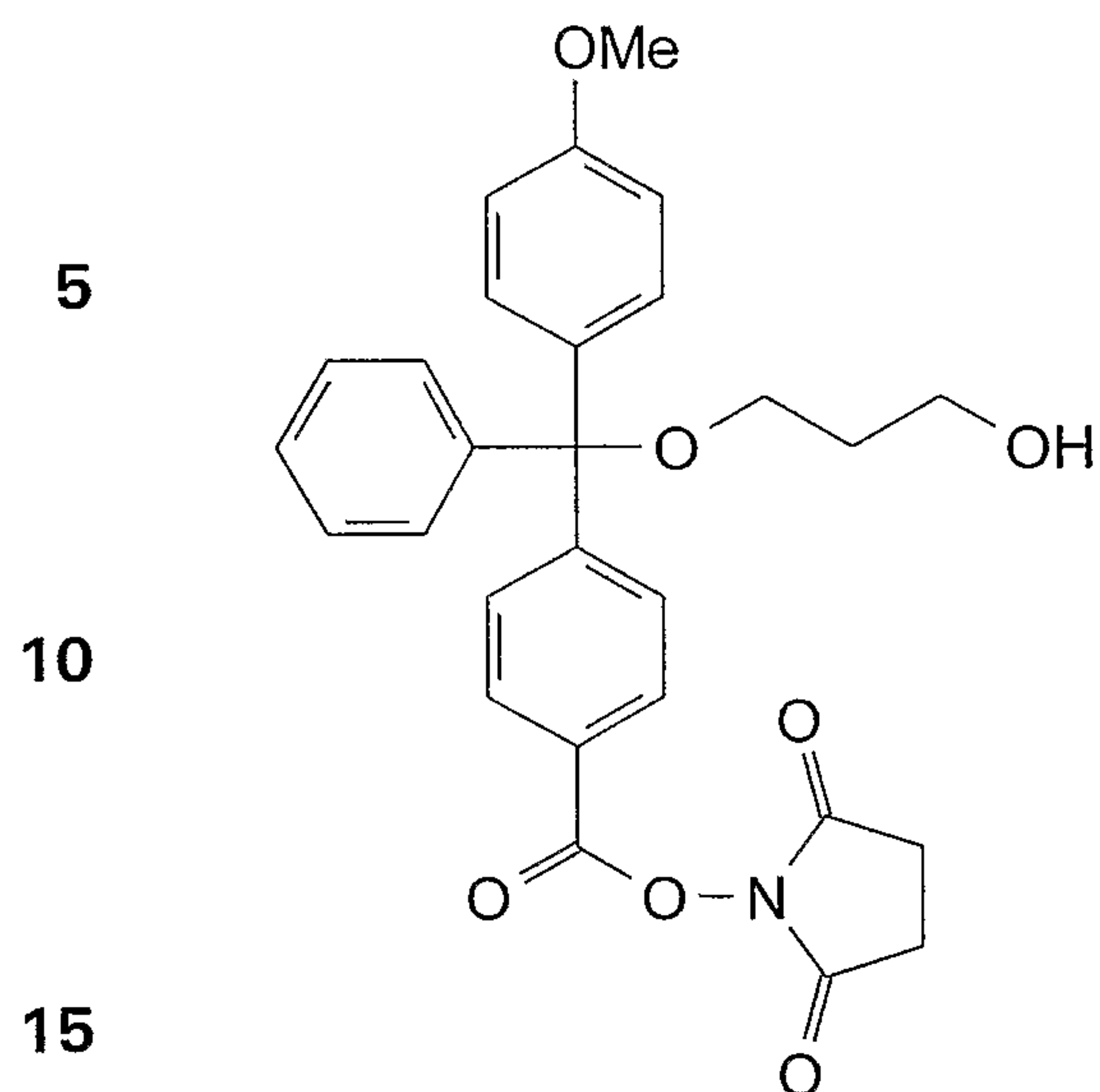
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Specific compounds within these embodiments are those resulting from all combinations of the groups listed above for the variables

20 contained in this formula and all can include Q groups. It is intended herein that each of these specific compounds is within the scope of the disclosure herein.

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#### D. Preparation of the Capture Compounds

The capture compounds are designed by assessing the target biomolecules and reaction conditions. For example, if the target biomolecules are proteins, X functions suitable to effect covalent or  
5 binding to proteins with high affinity are selected. Y is selected according to the complexity of the target mixture and the desired specificity of binding by X. Q is selected according the number of divisions of the mixture that are desired; and W is selected based upon the environment of the biomolecules that is probed. A variety of capture  
10 compounds are designed according to such criteria.

The capture compounds once designed can be synthesized by methods available to those of skill in the art. Preparation of exemplary capture compounds is described below. Any capture compound or similar  
15 capture compound can be synthesized according to a method discussed in general below or by minor modification of the methods by selecting appropriate starting materials or by methods known to those of skill in the art.

In general, the capture compounds can prepared starting with the central moiety Z. In certain embodiments, Z is  $-(S^1)_t-M(R^{15})_a-(S^2)_b-L-$ . In  
20 these embodiments, the capture compounds can be prepared starting with an appropriately substituted (*e.g.*, with one or more  $R^{15}$  groups) M group.  $M(R^{15})_a$  is optionally linked with  $S^1$  and/or  $S^2$ , followed by linkage to the cleavable linker L. Alternatively, the L group is optionally linked to  $S^2$ , followed by reaction with  $M(R^{15})_a$ , and optionally  $S^1$ . This Z group is  
25 then derivatized on its  $S^1$  (or  $M(R^{15})_a$ ) terminus to have a functionality for coupling with an oligonucleotide or oligonucleotide analog Q (*e.g.*, a phosphoramidite, H-phosphonate, or phosphoric triester group). The Q group will generally be N-protected on the bases to avoid competing reactions upon introduction of the X moiety. In one embodiment, the Z

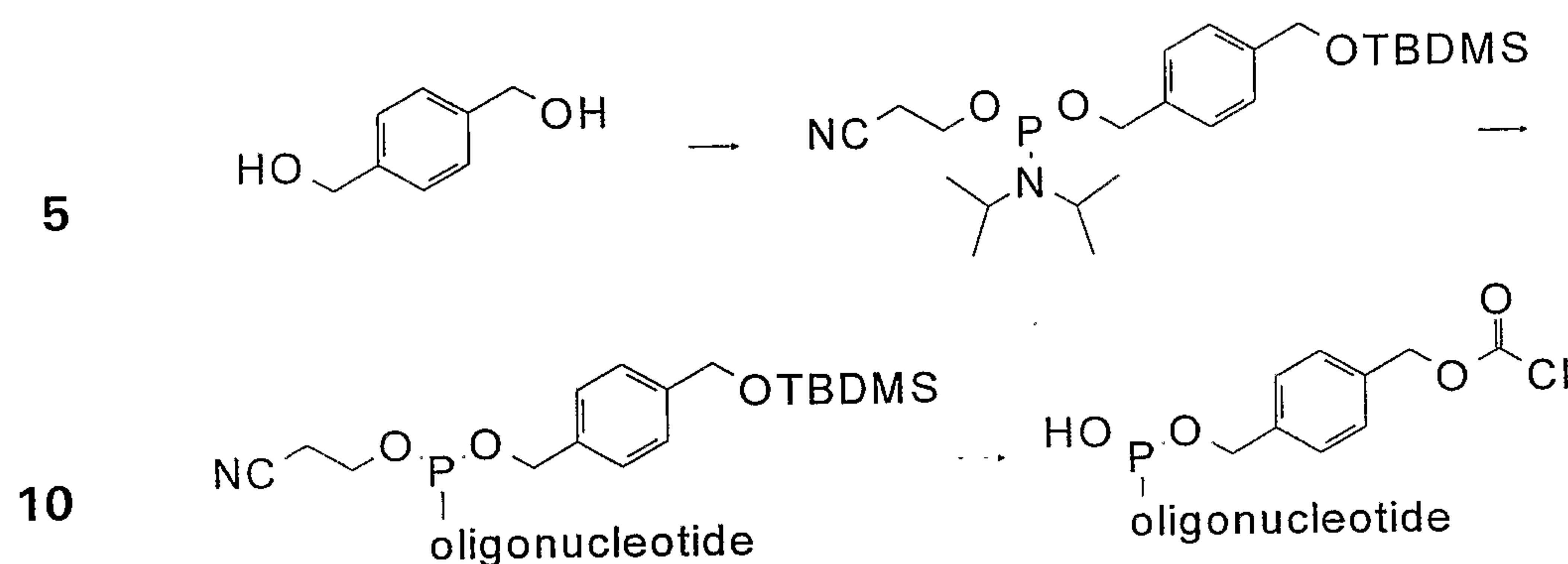
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group is reacted with a mixture of all possible permutations of an oligonucleotide or oligonucleotide Q (*e.g.*,  $4^i$  permutations where  $i$  is the number of nucleotides or nucleotide analogs in B). The resulting Q-Z capture compound or capture compounds is(are) then derivatized through  
5 the L terminus to possess an X group for reaction with a biomolecule, such as a protein. If desired, the N-protecting groups on the Q moiety are then removed. Alternatively, the N-protecting groups can be removed following reaction of the capture compound with a biomolecule, including a protein. In other embodiments, Q can be synthesized on Z, including  
10 embodiments where Z is an insoluble support or substrate, such as a bead. In a further embodiment, Q is presynthesized by standard solid state techniques, then linked to M. Alternatively, Q can be synthesized stepwise on the M moiety.

Provided below are examples of syntheses of the capture  
15 compounds provided herein containing alkaline-labile and photocleavable linkers. One of skill in the art can prepare other capture compounds disclosure by routine modification of the methods presented herein, or by other methods known to those of skill in the art.

For synthesis of a compound provided herein containing an  
20 alkaline-labile linker, 1,4-di(hydroxymethyl)benzene (*i.e.*, M) is mono-protected, *e.g.*, as the corresponding mono-*tert*-butyldimethylsilyl ether. The remaining free alcohol is derivatized as the corresponding 2-cyanoethyl-*N,N*-diisopropylphosphoramidite by reaction with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite. Reaction of this amidite with an  
25 oligonucleotide, (*i.e.*, Q), is followed by removal of the protecting group to provide the corresponding alcohol. Reaction with, *e.g.*, trichloromethyl chloroformate affords the illustrated chloroformate (*i.e.*, X).

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For the synthesis of a compound provided herein containing a

15 photocleavable linker, 2-nitro-5-hydroxybenzaldehyde (*i.e.*, a precursor of L) is reacted with, *e.g.*, 3-bromo-1-propanol to give the corresponding ether-alcohol. The alcohol is then protected, *e.g.*, as the corresponding *tert*-butyldimethylsilyl ether. Reaction of this compound with

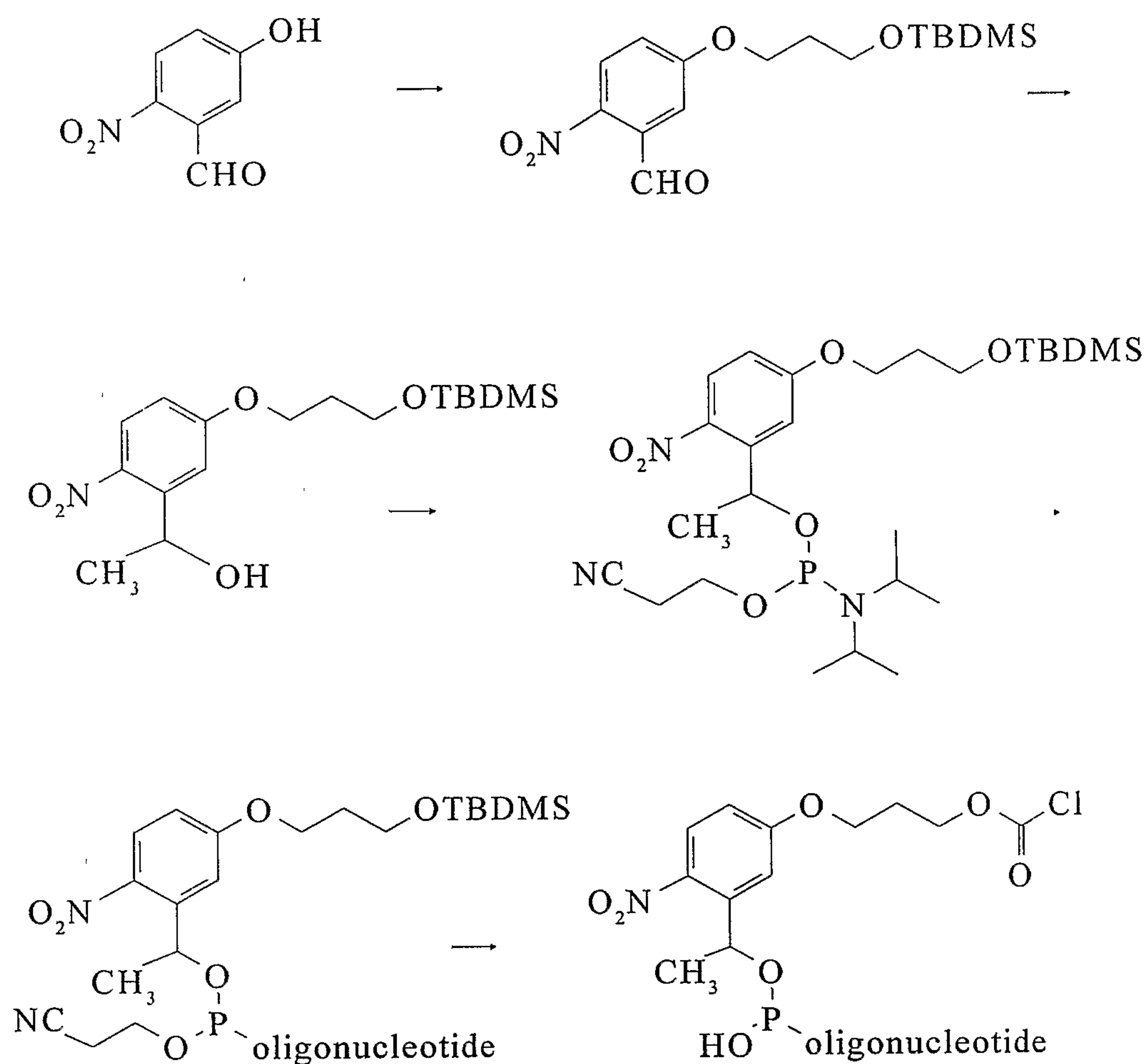
20 trimethylaluminum gives the corresponding benzyl alcohol, which is derivatized as its phosphoramidite using the procedure described above. The amidite is reacted with an oligonucleotide (*i.e.*, Q), followed by removal of the protecting group and derivatization of the resulting alcohol as the corresponding chloroformate (*i.e.*, X).

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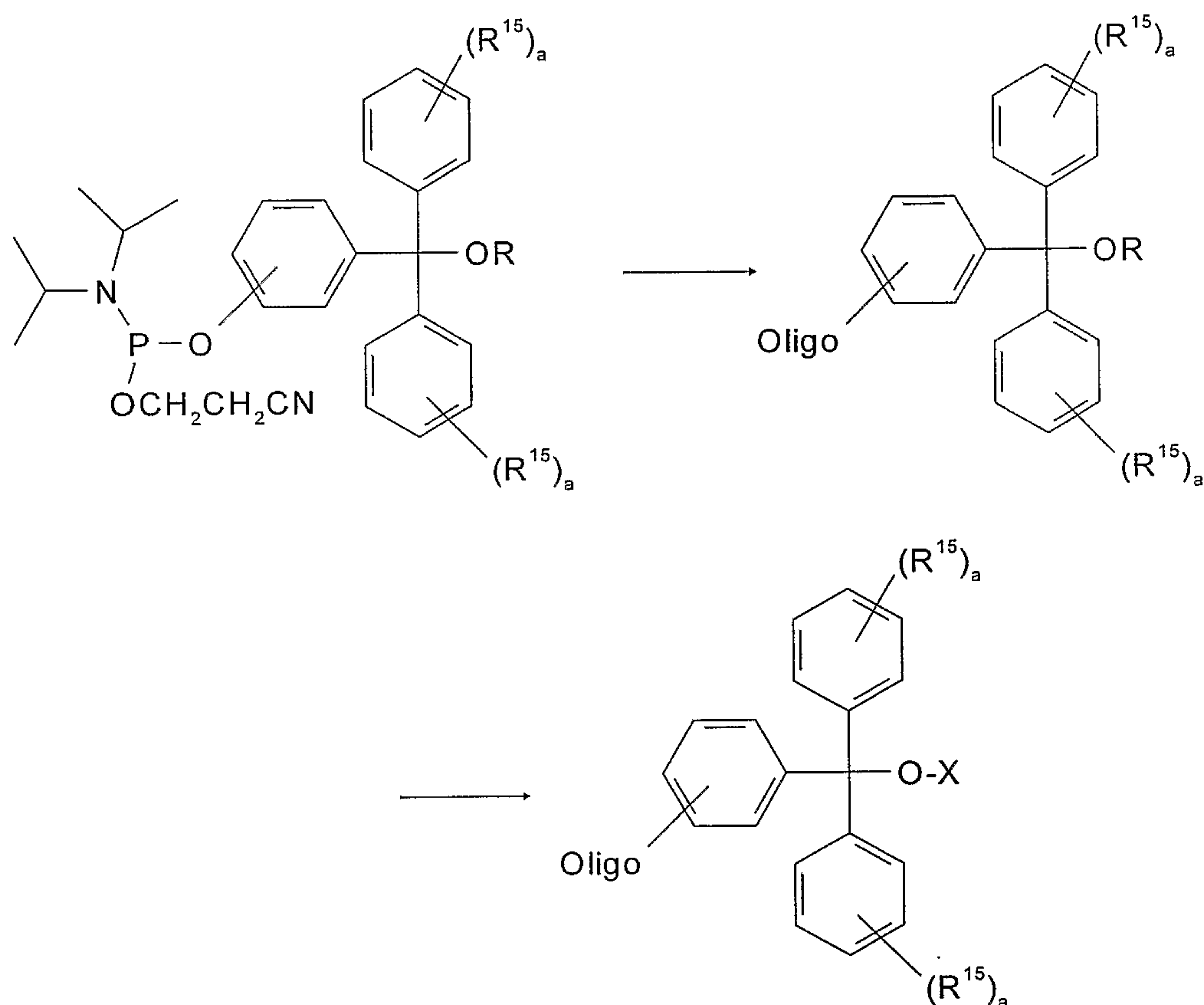
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For the synthesis of the compounds provided herein containing an acid labile linker, *e.g.*, a heterobifunctional trityl ether, the requisite phosphoramidite trityl ether is reacted with the oligonucleotide or oligonucleotide analog Q, followed by deprotection of the trityl ether and

5 capture of a biomolecule, *e.g.*, a protein, on the alcohol via a reactive derivative of the alcohol (X), as described above.

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The above syntheses are exemplary only. One of skill in the art will be able to modify the above syntheses in a routine manner to synthesize other compounds within the scope of the instant disclosure. Syntheses of capture compounds as provided herein are within the skill of the skilled artisan.

#### E. Methods of Use of the Compounds

The capture compounds provided herein can be used for the analysis, quantification, purification and/or identification of the components of biomolecule mixtures, including, but not limited to, protein mixtures. They can be used to screen libraries of small molecules to identify drug candidates, and they can be used to assess biomolecule-biomolecule interactions and to identify biomolecule complexes and

intermediates, such as those in biochemical pathways and other biological intermediates.

To initiate an analytical process, mixtures of biomolecules are obtained or prepared. They can then be pre-purified or partially purified  
5 as needed, according to standard procedures. Biomolecules are isolated from samples using standard methods. Figure 20a depicts an exemplary capture assay in which capture compounds are bound to biomolecules and analyzed by MALDI-TOF MS. Example 9 and Figures 20b-f show results of exemplary assays using a variety of capture compounds and  
10 known proteins.

#### 1. General methods

The collections provided herein have a wide variety of applications, including reducing the complexity of mixtures of molecules, particularly biomolecules, by contacting the collection with the mixtures  
15 to permit covalent binding of molecules in the mixtures. The capture compounds can be arrayed by virtue of the sorting function either before, during or after the contacting. Following contacting and arraying the loci of the array each contain a subset of the molecules in the mixture. The array can then be analyzed, such as by using mass  
20 spectrometry.

For example, proteins are isolated from biological fluids and/or tissues by cell lysis followed, for example, by either precipitation methods (*e.g.*, ammonium sulfate) or enzymatic degradation of the nucleic acids and carbohydrates (if necessary) and the low molecular weight material is  
25 removed by molecular sieving. Proteins also can be obtained from expression libraries. Aliquots of the protein mixture are reacted with the collections of capture compounds, generally members of the collection have different functionalities, such as different reactivity and/or selectivity, to separate the mixture into separate protein families

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according to the selected reactivity of X or the reactivity function plus the selectivity function. The diversity (number of different) selected for the sorting function Q depends on the complexity of the target mixture of biomolecules, such as proteins. Hence, for example, where there are sets  
5 of compounds differing in X and Y, solubility function and Q is an oligonucleotide, B is selected of an appropriate length to provide for sufficient number loci in the resulting array so that ultimately each "spot" on the array has about 5 to 50 or so biomolecules bound to a particular capture compound. In general, although not necessarily, all capture  
10 compounds with a particular "Q" are the same, so that each "spot" on the resulting array contains the same capture compounds. There, however, are embodiments, in which a plurality of different capture compounds can have the same Q functionality.

As noted, an array encompasses not only 2-D arrays on solid  
15 supports but any collection that is addressable or in which members are identifiable, such as by tagging with colored beads or RF tags or chemical tags or symbologies on beads. "Spots" are loci on the array, collections where capture compounds are sorted according to their "Q" function are separated.

20 In certain embodiments, the analysis is conducted using the smallest possible number of reactions necessary to completely analyze the mixture. Thus, in these embodiments, selection of the diversity of Q and of the number of X and X/Y groups of different reactivity will be a function of the complexity of the biomolecule mixture to be analyzed.  
25 Minimization of the diversity of B and the number of X and/or X/Y groups allows for complete analysis of the mixture with minimal complexity.

The separation of proteins from a complex mixture is achieved by virtue of the compound-protein products bound to different members of the collection. The supernatant, which contains the capture compound-

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protein products, is contacted with support bound or otherwise labeled or addressed recipient molecules, such as oligonucleotides on a support and allowed to bind, such as by hybridization to an array of complementary oligonucleotides. In one embodiment, a flat solid support that carries at  
5 spatially distinct locations, an array of oligonucleotides or oligonucleotide analogs that is complementary to the selected  $N^1_m-B_i-N^2_n$  oligonucleotide or oligonucleotide analog, is hybridized to the capture compound-biomolecule products.

In embodiments where Z is an insoluble support or substrate, such  
10 as a bead, separation of the compound-protein products into an addressable array can be achieved by sorting into an array of microwell or microtiter plates, or other microcontainer arrays or by labeling with an identifiable tag. The microwell or microtiter plates, or microcontainers, can include single-stranded oligonucleotides or oligonucleotide analogs that  
15 are complementary to the oligonucleotide or oligonucleotide analog Q.

After reaction or complexation of the compounds with the proteins, any excess compounds can be removed by adding a reagent designed to act as a "capturing agent." For example, a biotinylated small molecule, which has a functionality identical or similar to that that reacted with the  
20 selected X, is allowed to react with any excess compound. Exposure of this mixture to streptavidin bound to a magnetic bead, allows for removal of the excess of the compounds.

Hybridization of the compound-protein products to a complementary sequence is effected according to standard conditions  
25 (*e.g.*, in the presence of chaotropic salts to balance  $T_m$  values of the various hybrids). Any non-hybridized material can be washed off and the hybridized material analyzed.

In further embodiments, the methods herein use mixtures of the compounds provided herein that have permuted Q groups to achieve

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sorting of the biomolecules following reaction with the compounds.

These mixtures of compounds, in certain embodiments, have subsets

(*e.g.*, 64 or 256 or 1024) of different X reagents out of the  $4^i$

permutations in Q, where i is the number of nucleotides or analogs

5 thereof contained in the B moiety of Q (*e.g.*, 65,536 permutations for  $i = 8$ ). Reaction of the subsets separately with an aliquot of the biomolecule mixture to be analyzed results in conjugate mixtures that can be aligned with, *e.g.*, a microtiter plate format (*e.g.*, 96, 384 1536, etc.). Analysis using these subsets of compound mixtures provides further sorting of the  
10 biomolecules prior to analysis.

In other embodiments, selective pooling of the products of different X moiety-containing reagents (*e.g.*, amino- and thiol-reactive X groups; antibody and amino-reactive X groups; antibody and lectin X groups, etc.) can be performed for combined analysis on a single assay (*e.g.*, on a  
15 single chip).

Figure 1 depicts an exemplary method for separation and analysis of a complex mixture of proteins by use of MALDI-TOF mass spectrometry. Exposure of a compound as described herein, to a mixture of biomolecules, including, but not limited to, proteins (P1 to P4), affords  
20 a compound-protein array (NA = oligonucleotide moiety or oligonucleotide analog moiety, L = cleavable linker, P = protein). Separation of the array is effected by hybridization of the Q portion of the array to a complementary sequence attached to a support, such as an oligonucleotide chip. The proteins (P1 to P4) are then analyzed by  
25 MALDI-TOF mass spectrometry.

When the complexity of a mixture of biomolecules, including, but not limited to, proteins, is low, affinity chromatographic or affinity filtration methods can be applied to separate the compound-protein products from the protein mixture. If the proteins to be analyzed were

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fluorescently labeled prior to (or after) reaction with the compound but prior to hybridization, these labeled proteins also can be detected on the array. In this way the positions that carry a hybrid can be detected prior to scanning over the array with MALDI-TOF mass spectrometry and the  
5 time to analyze the array minimized. Mass spectrometers of various kinds can be applied to analyze the proteins (*e.g.*, linear or with reflection, with or without delayed extraction, with TOF, Q-TOFs or Fourier Transform analyzer with lasers of different wavelengths and xy sample stages).

Mass spectrometry formats for use herein, include, but are not  
10 limited to, matrix assisted laser desorption ionization (MALDI), continuous or pulsed electrospray (ES) ionization, ionspray, thermospray, or massive cluster impact mass spectrometry and a detection format such as linear time-of-flight (TOF), reflectron time-of-flight, single quadrupole, multiple quadrupole, single magnetic sector, multiple magnetic sector, Fourier  
15 transform, ion cyclotron resonance (ICR), ion trap, and combinations thereof such as MALDI-TOF spectrometry. For example, for ES, the samples, dissolved in water or in a volatile buffer, are injected either continuously or discontinuously into an atmospheric pressure ionization interface (API) and then mass analyzed by a quadrupole. The generation  
20 of multiple ion peaks that can be obtained using ES mass spectrometry can increase the accuracy of the mass determination. Even more detailed information on the specific structure can be obtained using an MS/MS quadrupole configuration.

Methods for performing MALDI are known to those of skill in the  
25 art. Numerous methods for improving resolution are also known. For example, resolution in MALDI TOF mass spectrometry can be improved by reducing the number of high energy collisions during ion extraction (see, *e.g.*, Juhasz *et al.* (1996) *Analysis, Anal. Chem.* 68:941-946, see also, *e.g.*, U.S. Patent No. 5,777,325, U.S. Patent No. 5,742,049, U.S.

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Patent No. 5,654,545, U.S. Patent No. 5,641,959, U.S. Patent No. 5,654,545, U.S. Patent No. 5,760,393 and U.S. Patent No. 5,760,393 for descriptions of MALDI and delayed extraction protocols).

Conditioning of molecules to be analyzed or of the capture-compound  
5 bound biomolecules prior to analysis also can be employed.

In MALDI mass spectrometry (MALDI-MS), various mass analyzers can be used, *e.g.*, magnetic sector/magnetic deflection instruments in single or triple  
quadrupole mode (MS/MS), Fourier transform and time-of-flight (TOF),  
10 including orthogonal time-of-flight (O-TOF), configurations as is known in the art of mass spectrometry. For the desorption/ionization process, numerous matrix/laser combinations can be used. Ion-trap and reflectron configurations also can be employed.

MALDI-MS requires the biomolecule to be incorporated into a  
15 matrix. It has been performed on polypeptides and on nucleic acids mixed in a solid (*i.e.*, crystalline) matrix. The matrix is selected so that it absorbs the laser radiation. In these methods, a laser, such as a UV or IR laser, is used to strike the biomolecule/matrix mixture, which is crystallized on a probe tip or other suitable support, thereby effecting  
20 desorption and ionization of the biomolecule. In addition, MALDI-MS has been performed on polypeptides, glycerol, and other liquids as a matrix.

A complex protein mixture can be selectively dissected, and in taking all data together, completely analyzed through the use of compounds with different functionalities X. The proteins present in a  
25 mixture of biological origin can be detected because all proteins have reactive functionalities present on their surfaces. If at each position on the compound-protein array, there is the same protein cleavable under the same conditions as L or is added without covalent attachment to the solid support and serving as an internal molecular weight standard, the relative

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amount of each protein (or peptide if the protein array was enzymatically digested) can be determined. This process allows for the detection of changes in expressed proteins when comparing tissues from healthy and disease individuals, or when comparing the same tissue under different physiological conditions (*e.g.*, time dependent studies). The process also allows for the detection of changes in expressed proteins when comparing different sections of tissues (*e.g.*, tumors), which can be obtained, *e.g.*, by laser bioposy.

Protein-protein interactions and protein-small molecule (*e.g.*, drug) interactions can be studied by contacting the compound-protein array with a mixture of the molecules of interest. In this case, a compound will be used that has no cleavable linkage L, or that has a linkage L that is stable under MALDI-TOF MS conditions. Subsequent scanning of the array with the mass spectrometer demonstrates that hybridized proteins of the protein array have effectively interacted with the protein or small molecule mixtures of interest.

Analysis using the well known 2-hybrid methodology is also possible and can be detected via mass spectrometry. See, *e.g.*, U.S. Patent Nos. 5,512,473, 5,580,721, 5,580,736, 5,955,280, 5,695,941. See also, Brent *et al.* (1996) *Nucleic Acids Res.* 24(17):3341-3347.

In the above embodiments, including those where Z contains a cleavable linkage, the compounds can contain a mass modifying tag. In these embodiments, the mass modifying tag is used to analyze the differences in structure (*e.g.*, side chain modification such as phosphorylation or dephosphorylation) and/or expression levels of biomolecules, including proteins. In one embodiment, two compounds (or two sets of compounds having identical permuted B moieties) are used that only differ in the presence or absence of a mass modifying tag (or have two mass tags with appropriate mass differences). One compound

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(or one set of compounds) is (are) reacted with "healthy" tissue and the mass modified compound(s) are reacted with the "disease" tissue under otherwise identical conditions. The two reactions are pooled and analyzed in a duplex mode. The mass differences will elucidate those  
5 proteins that are altered structurally or expressed in different quantity in the disease tissue. Three or more mass modifying tags can be used in separate reactions and pooled for multiplex analysis to follow the differences during different stages of disease development (*i.e.*, mass modifying tag 1 at time point 1, mass modifying tag 2 at time point 2  
10 etc.), or, alternatively, to analyze different tissue sections of a disease tissue such as a tumor sample.

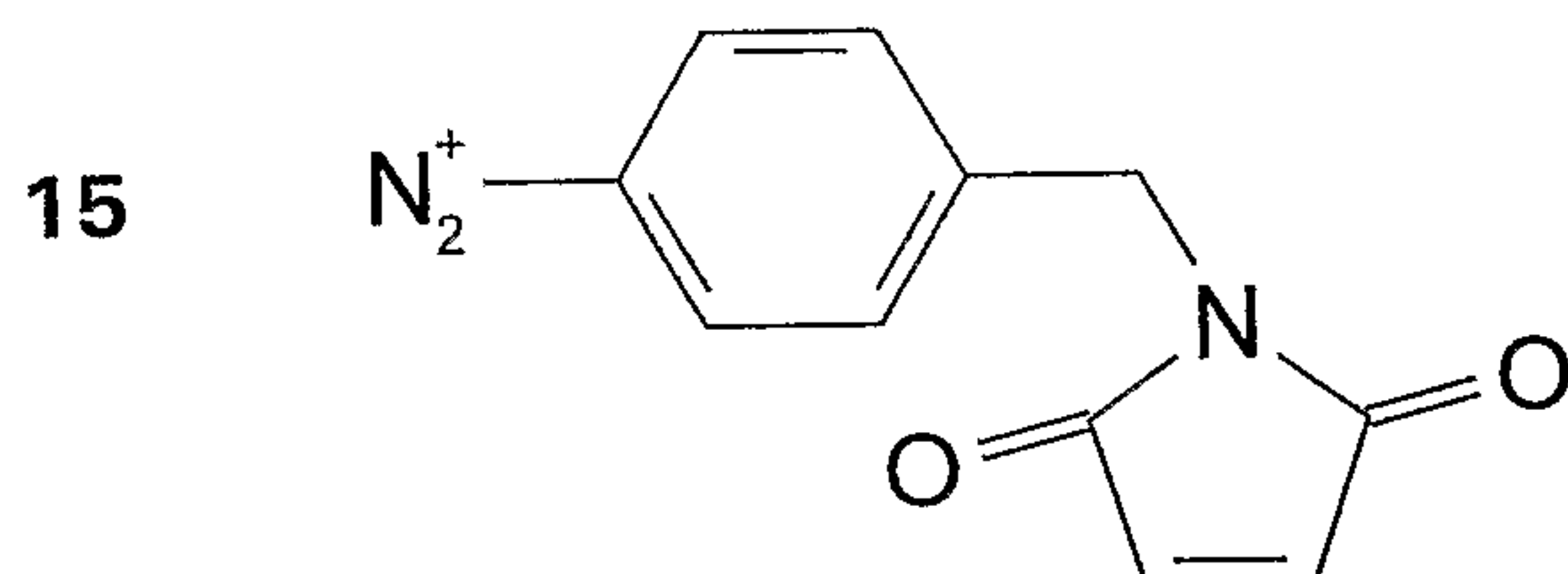
Selectivity in the reaction of the compounds provided herein with a biomolecule, such as a protein mixture also can be achieved by performing the reactions under kinetic control and by withdrawing  
15 aliquots at different time intervals. Alternatively, different parallel reactions can be performed (for example, all differing in the B moiety of the Q group) and either performed with different stoichiometric ratios or stopped at different time intervals and analyzed separately.

In embodiments where the capture compounds provided herein  
20 possess a luminescent or colorimetric group, the immobilized compound-biomolecule conjugate can be viewed on the insoluble support prior to analysis. Viewing the conjugate provides information about where the conjugate has hybridized (such as for subsequent MALDI-TOF mass spectrometric analysis). In certain embodiments, with selected reagents  
25 the quantity of a given protein from separate experiments (*e.g.*, healthy vs. disease, time point 1 vs. time point 2, etc.) can be determined by using dyes that can be spectrophotometrically differentiated.

In other embodiments, the methods are performed by tagging the biomolecules to be analyzed, including but not limited to proteins, with

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more than one, in one embodiment three to five, of the compounds provided herein. Such compounds possess functionality designed to target smaller chemical features of the biomolecules rather than a macromolecular feature. See, *e.g.*, Figure 3. Such smaller chemical features include, but are not limited to,  $\text{NH}_2$ , SH, SS (after capping SH, SS can be targeted by, *e.g.*, gold), and OH. In one non-limiting example, the phenolic OH of tyrosine is selectively captured using a diazo compound, such as an aryldiazonium salt. In this embodiment, the reaction can be performed in water. For example, a functionalized diazonium salt could be used where the functionality allows for subsequent capture of a compound provided herein, thereby providing a oligonucleotide-labelled biomolecule. One such functionalized diazonium salt is:



A biomolecule modified with this reagent is then labelled with an oligonucleotide possessing a diene residue. It is appreciated by those of skill in the art that many reagent couples other than dienophile/diene can be used in these embodiments. In the case of dienophile/diene, the reaction of the dienophile with the diene can be performed in the presence of many other functional groups, including N-hydroxysuccinimido-activated oligonucleotides reacting with an  $\text{NH}_2$  group. Thus, these two labelling specific reactions can be performed in one reaction. See, *e.g.*, Figure 5.

Subsequently, the multiply-tagged biomolecules are hybridized on an array of antisense oligonucleotides, in one embodiment a chip

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containing an array of antisense oligonucleotides. Such multiply-tagged biomolecules can be sorted with greater selectivity than singly tagged biomolecules. See, *e.g.*, Figure 4.

In embodiments where the compounds for use in the methods provided herein are insoluble or poorly soluble in water or aqueous buffers, organic solvents are added to the buffers to improve solubility. In one embodiment, the ratio of buffer:organic solvent is such that denaturation of the biomolecule does not occur. In another embodiment, the organic solvents used include, but are not limited to, acetonitrile, formamide and pyridine. In another embodiment, the ratio of buffer:organic solvent is about 4:1. To determine if an organic co-solvent is needed, the rate of reaction of the compounds provided herein with a water-soluble amine, such as 5'-aminothymidine, is measured. For example, the following reaction is performed in a variety of solvent mixtures well known to those of skill in the art to determine optimal conditions for subsequent biomolecule tagging and analysis:

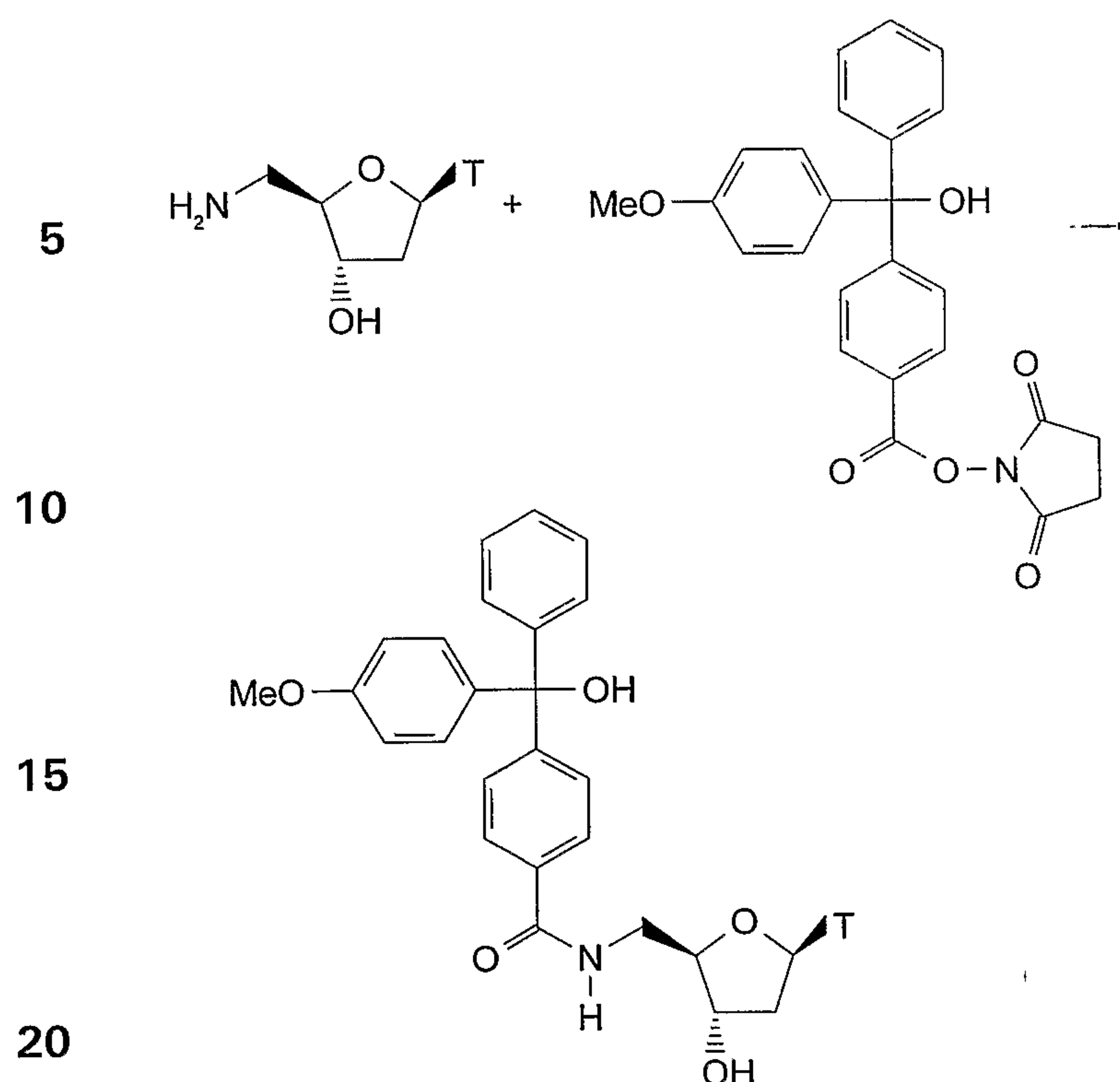
20

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## 2. Phenotype analyses

The collections of capture permit a top down holistic approach to analysis of the proteome and other biomolecules. As noted, the collections and methods of use provide an unbiased way to analyze biomolecules, since the methods do not necessarily assess specific classes of targets, but rather detect or identify changes in the samples. The changes identified include structural changes that are related to the primary sequences and modifications, including post-translational modifications. In addition, since the capture compounds can include a solubility function they can be designed for reaction in hydrophobic conditions, thereby permitting analysis of membrane-bound and membrane-associated molecules, particularly proteins.

Problems with proteome analysis arise from genetic variation that is not related to a target phenotype, proteome variation due to

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differences, such as gender, age, metabolic state, the complex mixtures of cells in target tissues and variations from cell cycle stage. Thus, to identify or detect changes, such as disease-related changes, among the biomolecule components of tissues and cells, homogeneity of the sample  
5 can be important. To provide homogeneity, cells, with different phenotypes, such as diseased versus healthy, from the same individual are compared. As a result, differences in patterns of biomolecules can be attributed to the differences in the phenotype rather than from differences among individuals. Hence, samples can be obtained from a  
10 single individual and cells with different phenotypes, such as healthy versus diseased and responders versus non-responders, are separated. In addition, the cells can be synchronized or frozen into a metabolic state to further reduce background differences.

Thus, the collections of capture compounds can be used to identify  
15 phenotype-specific proteins or modifications thereof or other phenotype-specific biomolecules and patterns thereof. This can be achieved by comparing biomolecule samples from cells or tissues with one phenotype to the equivalent cells to biomolecule samples from cells or tissues with another phenotype. Phenotypes in cells from the same individual and cell  
20 type are compared. In particular, primary cells, primary cell culture and/or synchronized cells are compared. The patterns of binding of biomolecules from the cells to capture compound members of the collection can be identified and used as a signature or profile of a disease or healthy state or other phenotypes. The particular bound biomolecule,  
25 such as a protein, also can be identified and new disease-associated markers, such as particular proteins or structures thereof, can be identified. Example 6 provides an exemplary embodiment in which cells are separated. See also Figure 19.

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Phenotypes for comparison include, but are not limited to:

- 1) samples from diseased versus healthy cells or tissues to identify proteins or other biomolecules associated with disease or that are markers for disease;
- 5        2) samples from drug responders and non responders (*i.e.* on 20-30% of malignant melanoma patients respond to alpha interferon and others to do not) to identify biomolecules indicative of response;
- 3) samples from cells or tissues with a toxicity profile to drugs or environmental conditions to identify biomolecules associated with the  
10 response or a marker of the response; and
- 4) samples from cells or tissues exposed to any condition or exhibiting any phenotype in order to identify biomolecules, such as proteins, associated with the response or phenotype or that are a marker therefor.
- 15        Generally the samples for each phenotype are obtained from the same organism, such as from the same mammal so that the cells are essentially matched and any variation should reflect variation due to the phenotype and not the source of the cells. Samples can be obtained from primary cells (or tissues). In all instances, the samples can be obtained  
20 from the same individual either before exposure or treatment or from healthy non-diseased tissue in order to permit identification of phenotype-associated biomolecules.

Cells can be separated by any suitable method that permits identification of a particular phenotype and then separation of the cells  
25 based thereon. Any separation method, such as, for example, panning or negative panning (where unwanted cells are captured and the wanted cells remain in the supernatant) where the live cells are recovered can be used. These methods include, but are not limited to:

- 1) flow cytometry;

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2) specific capture;

3) negative panning in which unwanted cells are captured and the targeted cells remain in the supernatant and live cells are recovered for analysis; and

5           4) Laser Capture Microdissection (LCM) (Arcturus, Inc Mountain View, CA).

Thus sorting criteria include, but are not limited to, membrane potential, ion flux, enzymatic activity, cell surface markers, disease markers, and other such criteria that permit separation of cells from an  
10 individual based on phenotype.

**a) Exemplary separation methods**

**1) Laser Capture Microdissection**

Laser Capture Microdissection (LCM) (Arcturus, Inc Mountain View, CA) uses a microscope platform combined with a low-energy IR  
15 laser to activate a plastic capture film onto selected cells of interest. The cells are then gently lifted from the surrounding tissue. This approach precludes any absorption of laser radiation by microdissected cells or surrounding tissue, thus ensuring the integrity of RNA, DNA, and protein prepared from the microdissected samples for downstream  
20 analysis.

**2) Flow cytometry for separation**

Flow cytometry is a method, somewhat analogous to fluorescent microscopy, in which measurements are performed on particles (cells) in liquid suspension, which flow one at a time through a focused laser beam  
25 at rates up to several thousand particles per second. Light scattered and fluorescence emitted by the particles (cells) is collected, filtered, digitized and sent to a computer for analysis. Typically flow cytometry measures the binding of a fluorochrome-labeled probe to cells and the comparison of the resultant fluorescence to the background fluorescence of

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unstained cells. Cells can be separated using a version of flow cytometry, flow sorting, in which the particles (cells) are separated and recovered from suspension based upon properties measured in flow. Cells that are recovered via flow sorting are viable and can be collected under  
5 sterile conditions. Typically, recovered subpopulations are in excess of 99.5% pure (see Figures 19a and 19b).

Flow cytometry allows cells to be distinguished using various parameters, including physical and/or chemical characteristics associated with cells or properties of cell-associated reagents or probes, any of  
10 which are measured by instrument sensors. Separation: Live v. Dead Forward and side scatter are used for preliminary identification and gating of cell populations. Scatter parameters are used to exclude debris, dead cells, and unwanted aggregates. In a peripheral blood or bone marrow sample, lymphocyte, monocyte and granulocyte populations can  
15 be defined, and separately gated and analyzed, on the basis of forward and side scatter. Cells that are recovered via flow sorting are viable and can be collected under sterile conditions. Typically recovered subpopulations are in excess of 99.5% pure.

Common cell sorting experiments usually involve  
20 immunofluorescence assays, *i.e.*, staining of cells with antibodies conjugated to fluorescent dyes in order to detect antigens. In addition, sorting can be performed using GFP-reporter constructs in order to isolate pure populations of cells expressing a given gene/construct.

#### a. Fluorescence

25 Fluorescent parameter measurement permits investigation of cell structures and functions based upon direct staining, reactions with fluorochrome labeled probes (*e.g.*, antibodies), or expression of fluorescent proteins. Fluorescence signals can be measured as single or multiple parameters corresponding to different laser excitation and

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fluorescence emission wavelengths. When different fluorochromes are used simultaneously, signal spillover can occur between fluorescence channels. This is corrected through compensation. Certain combinations of fluorochromes cannot be used simultaneously; those of skill in the art  
5 can identify such combinations.

**b. Immunofluorescence**

Immunofluorescence involves the staining of cells with antibodies conjugated to fluorescent dyes such as FITC (fluorescein), PE (phycoerythrin), APC (allophycocyanin), and PE-based tandem  
10 conjugates (R670, CyChrome and others.). Cell surface antigens are the usual targets of this assay, but antibodies can be directed at antigens or cytokines in the cytoplasm as well.

DNA staining is used primarily for cell cycle profiling, or as one method for measuring apoptosis. Propidium iodide (PI), the most  
15 commonly used DNA stain, cannot enter live cells and can therefore be used for viability assays. For cell cycle or apoptosis assays using PI, cells must first be fixed in order for staining to take place (see protocol). The relative quantity of PI-DNA staining corresponds to the proportion of cells in G0/G1, S, and G2/M phases, with lesser amounts of staining indicating  
20 apoptotic/necrotic cells. PI staining can be performed simultaneously with certain fluorochromes, such as FITC and GFP, in assays to further characterize apoptosis or gene expression.

Gene Expression and Transfection can be measured indirectly by using a reporter gene in the construct. Green Fluorescent Protein-type  
25 constructs (EGFP, red and blue fluorescent proteins) and  $\beta$ -galactosidase, for example, can be used to quantify populations of those cells expressing the gene/construct. Mutants of GFP are now available that can be excited at common frequencies, but emit fluorescence at different wavelengths. This allows for measurement of co-transfection, as well as

simultaneous detection of gene and antibody expression. Appropriate negative (background) controls for experiments involving GFP-type constructs should be included. Controls include, for example, the same cell type, using the gene insert minus the GFP-type construct.

### **5                      3)        Metabolic Studies and other studies**

Annexin-V can be labeled with various fluorochromes in order to identify cells in early stages of apoptosis. CFSE binds to cell membranes and is equally distributed when cells divide. The number of divisions cells undergo in a period of time can then be counted. CFSE can be used in conjunction with certain fluorochromes for immunofluorescence. Calcium flux can be measured using Indo-1 markers. This can be combined with immunofluorescent staining. Intercellular conjugation assays can be performed using combinations of dyes such as calcein or hydroethidine.

### b) Synchronizing cell cycles

15 Once sorted or separated cells are obtained they can be cultured,  
and, can be synchronized or frozen into a particular metabolic state. This  
enhances the ability to identify phenotype-specific biomolecules. Such  
cells can be separated by the above methods, including by flow  
cytometry. Further, cells in the same cell cycle, same metabolic state or  
20 other synchronized state can be separated into groups using flow  
cytometry (see, Figure 19c).

Cell cycles can be synchronized or frozen by a variety of methods, including but are not limited to, cell chelation of critical ions, such as by removal of magnesium, zinc, manganese, cobal and/or other ions that perform specific functions by EDTA or otherchelators (see, *e.g.*,  
25 EXAMPLES). Other methods include controlling various metabolic or biochemical pathways. Figure 18 depicts exemplary points of regulation of metabolic control mechanisms for cell synchronization. Examples of synchronizing or “freezing” Metabolic Control for synchronizing cells,

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include, but are not limited to, the following:

1) control of gene expression;

2) regulation of enzyme reactions;

3) negative control: Feedback inhibition or End product repression

5 and enzyme induction are mechanisms of negative control that lead to a decrease in the transcription of proteins;

4) positive control: catabolite repression is considered a form of positive control because it affects an increase in transcription of proteins.

10 5) Control of individual proteins translation:

a) oligonucleotides that hybridize to the 5' cap site have inhibit protein synthesis by inhibiting the initial interaction between the mRNA and the ribosome 40S sub-unit;

15 b) oligonucleotides that hybridize to the 5' UTR up to, and including, the translation initiation codon inhibit the scanning of the 40S (or 30S) subunit or assembly of the full ribosome (80S for eukaryotes or 70S for bacterial systems);

5) control of post translational modification:

20 6) control of allosteric enzymes, where the active site binds to the substrate of the enzyme and converts it to a product. The allosteric site is occupied by some small molecule that is not a substrate. If the protein is an enzyme, when the allosteric site is occupied, the enzyme is inactive, *i.e.*, the effector molecule decreases the activity of the enzyme. Some multicomponent allosteric enzymes have several sites occupied by various  
25 effector molecules that modulate enzyme activity over a range of conditions.

### 3. Analysis of low abundance proteins

Important disease-associated markers and targets could be low abundance proteins, that might not be detected by mass spectrometry. To ensure detection, a first capture compound display experiment can be performed. The resulting array of captured proteins is reacted with a non-selective dye, such as a fluorescent dye, that will light up or render visible more proteins on the array. The dye can provide a semi-quantitative estimate of the amount of a protein. The number of different proteins detected by the dye can be determined and then compared the number detected by mass spectrometric analysis. If there are more proteins detected using the dye, the experiments can be repeated using a higher starting number of cells so that low abundance proteins can be detected and identified by the mass spectrometric analysis.

For example, housekeeping proteins, such as actin and other such proteins, are present in high abundance and can mask low abundance proteins. Capture compounds or other purification compound selected or designed to capture or remove the high abundance proteins or biomolecules from a mixture before using a collection to assess the components of the mixture. Once the high abundance proteins are removed, low abundance proteins have an effectively higher concentration and can be detected. These methods, thus, have two steps: a first step to capture high abundance components of biomolecule mixtures, such as the actins. For example, a cell lysate can be contacted with capture molecules that include a reactivity group such as biotin or other general reactivity function linked to a sorting group to remove such high abundance proteins, and then use a suitable collection of capture compounds to identify lower abundance compounds remaining in the lysate.

Also, as discussed above, capture compounds can be

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designed, such as by appropriate selection of W, to interact with intact organelles before disrupting them in cells that have been gently lysed or otherwise treated to permit access to organelles and internal membranes. Then the captured organelles can be disrupted, such as one which can  
5 include an artificial membrane, such as a lipid bilayer or micelle coating, to capture the organelle proteins and other biomolecules in an environment that retains their three-dimensional structure. These captured proteins can be analyzed. This permits the capture compounds to interact with the captured proteins and other biomolecules in thier  
10 native tertiary structure.

#### 4. Monitoring protein conformation as an indicator of disease

The collections and/or members thereof can be used to detect or distinguish specific conformers of proteins. Hence, for example, if a particular conformation of a protein is associated with a disease (or  
15 healthy state) the collections or members thereof can detect one conformer or distinguish conformers based upon a pattern of binding to the capture compounds in a collection. Thus, the collections and/or members thereof can be used to detect conformationally altered protein diseases (or diseases of protein aggregation), where a disease-associated  
20 protein or polypeptide has a disease-associated conformation. The methods and collections provided herein permit detection of a conformer associated with a disease to be detected. These diseases include, but are not limited to, amyloid diseases and neurodegenerative diseases. Other diseases and associated proteins that exhibit two or more different  
25 conformations in which at least one conformation is associated with disease include those set forth in the following Table:

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|    | Disease                                                                                                                | Insoluble protein                                                                                              |
|----|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
|    | Alzheimer's Disease (AD)                                                                                               | APP, A $\beta$ , $\alpha$ 1-antichymotrypsin, tau, non-A $\beta$ component, presenellin 1, presenellin 2, apoE |
| 5  | Prion diseases, including but are not limited to, Creutzfeldt-Jakob disease, scrapie, bovine spongiform encephalopathy | PrP <sup>Sc</sup>                                                                                              |
|    | amyotrophic lateral sclerosis (ALS)                                                                                    | superoxide dismutase (SOD) and neurofilament                                                                   |
|    | Pick's Disease                                                                                                         | Pick body                                                                                                      |
|    | Parkinson's disease                                                                                                    | $\alpha$ -synuclein in Lewy bodies                                                                             |
| 10 | Frontotemporal dementia                                                                                                | tau in fibrils                                                                                                 |
|    | Diabetes Type II                                                                                                       | amylin                                                                                                         |
|    | Multiple myeloma                                                                                                       | IgGL-chain                                                                                                     |
|    | Plasma cell dyscrasias                                                                                                 |                                                                                                                |
|    | Familial amyloidotic polynueuropathy                                                                                   | Transthyretin                                                                                                  |
| 15 | Medullary carcinoma of thyroid                                                                                         | Procalcitonin                                                                                                  |
|    | Chronic renal failure                                                                                                  | $\beta_2$ -microglobulin                                                                                       |
|    | Congestive heart failure                                                                                               | Atrial natriuretic factor                                                                                      |
|    | Senile Cardiac and systemic amyloidosis                                                                                | transthyretin                                                                                                  |
| 20 | Chronic inflammation                                                                                                   | Serum Amyloid A                                                                                                |
|    | Atherosclerosis                                                                                                        | ApoAI                                                                                                          |
|    | Familial amyloidosis                                                                                                   | Gelsolin                                                                                                       |
|    | Huntington's disease                                                                                                   | Huntington                                                                                                     |

25           The collections can be contacted with a mixture of the conformers and the members that bind or retain each form can be identified, and a pattern thus associated with each conformer. Alternatively, those that bind to only one conformer, such as the conformer associated with disease can be identified, and sub-collections of one or more of such

30   capture compounds can be used as a diagnostic reagent for the disease.

**5. Small molecule identification and biomolecule-biomolecule interaction investigation**

Biomolecules, such as proteins, are sorted using a covalent or noncovalent interaction with immobilized capture compounds.

- 5 Collections, such as arrays of capture compounds bound to biomolecules, such as from cell lysates, then can be used to screen libraries or other mixtures of drug candidates or to further screen mixtures of biomolecules to see what binds to the bound biomolecules. The capture biomolecule-biomolecule complexes or biomolecule-drug candidate complexes can be
- 10 analyzed to identify biochemical pathways and also to identify targets with the candidate drug.

For example, protein-protein or protein-biomolecule interactions are exposed to test compounds, typically small molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules or

15 dsRNA, antibodies, fragments of antibodies, recombinant and sythetic antibodies and fragments thereof and other such compounds that can serve as drug candidates or lead compounds. Bound small molecules are identified by mass spectrometry or other analytical methods.

**F. Systems**

- 20 In further embodiments, the compounds and the methods described herein are designed to be placed into an integrated system that standardizes and automates the following process steps:

- Isolation of biomolecules from a biological source, including isolation of the proteins from cell lysates (lysis, enzymatic digestion, precipitation, washing)
- 25 • Optionally, removal of low molecular weight materials
- Optionally, aliquoting the biomolecule mixture, such as a protein mixture
- Reaction of the biomolecule mixture, such as a protein

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mixture, with compounds of different chemical reactivity (X) and sequence diversity (B) provided herein; this step can be performed in parallel using aliquots of the biomolecule mixture

- 5       • Optionally, removal of excess compound
- Hybridization of the compound-biomolecule conjugate, such as a compound-protein conjugate, to single stranded oligonucleotides or oligonucleotide analogs that are complementary to the Q moiety of the compound; the single
- 10       stranded oligonucleotides or oligonucleotide analogs are optionally presented in an array format and are optionally immobilized on an insoluble support
- Optionally, subsequent chemical or enzymatic treatment of the protein array
- 15       • Analysis of the biomolecule array, including, but not limited to, the steps of (i) deposition of matrix, and (ii) spot-by-spot MALDI-TOF mass spectrometry using an array mass spectrometer (with or without internal, *e.g.*, on-chip molecular weight standard for calibration and quantitation).

20       The system includes the collections provided herein, optionally arrays of such collections, software for control of the processes of sample preparation and instrumental analysis and for analysis of the resulting data, and instrumentation, such as a mass spectrometer, for analysis of the biomolecules. The system includes other devices, such as

25       liquid chromatographic devices, so that a protein mixture is at least partially separated. The eluate is collected in a continuous series of aliquots into, *e.g.*, microtiter plates, and each aliquot reacted with a capture compound provided.

In multiplex reactions, aliquots in each well can simultaneously

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react with one or more of the capture compounds provided herein that, for example each differ in X (*i.e.*, amino, thiol, lectin specific functionality) with each having a specific and differentiating selectivity moiety Y and in the Q group. Chromatography can be done in aqueous or  
5 in organic medium. The resulting reaction mixtures are pooled and analyzed directly. Alternatively, subsequent secondary reactions or molecular interaction studies are performed prior to analysis, including mass spectrometric analysis.

The systems provided herein can contain an assembly line, such as  
10 pipetting robots on xy stages and reagent supply/washing modules that are linked with a central separation device and a terminal mass spectrometer for analysis and data interpretation. The systems can be programmed to perform process steps including (see, *e.g.*, FIG. 2), for example:

- 15 1) Cell cultures (or tissue samples) are provided in microtiter plates (MTPs) with 1, 2...i wells. To each well, solutions are added for lysis of cells, thereby liberating the proteins. In some embodiments, appropriate washing steps are included, as well as addition of enzymes to digest nucleic acids and other non-protein components. In further embodiments,  
20 instead of regular MTPs, MTPs with filter plates in the bottom of wells are used. Cell debris is removed either by filtration or centrifugation. A conditioning solution for the appropriate separation process is added and the material from each well separately loaded onto the separation device.
- 25 2) Separation utilizes different separation principles such as charge, molecular sizing, adsorption, ion-exchange, and molecular exclusion principles. Depending on the sample size, suitable appropriate dimensions are utilized, such as microbore high performance liquid chromatography (HPLC).

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In certain embodiments, a continuous flow process is used and the effluent is continuously aliquotted into MTP 1,2...n.

- 3) Reaction with Proteome Reagents. Each MTP in turn is transferred to a Proteome Reagent Station harboring 1, 2...m reagents differing only in the oligonucleotide sequence part (*i.e.*, Q) or/and in the chemical nature of the functionality reacting with the proteins (*i.e.*, X). If there are more than one MTP coming from one tissue sample then reagent 1 is added to the same well of the respective MTPs 1, 2...n, *i.e.*, in well A1, reagent 2 in well A2, etc. In embodiments where the MTPs have 96 wells ( $i = 1-96$ ), 96 different Proteome Reagents (*i.e.*, 96 different compounds provided herien,  $m = 1-96$ ) are supplied through 96 different nozzles from the Proteome Reagent Station to prevent cross-contamination.

- 4) Pooling: Excess Proteome Reagent is deactivated, aliquots from each well belonging to one and the same tissue samples are pooled, and the remaining material is stored at conditions that preserve the structure (and if necessary conformation) of the proteins intact, thereby serving as master MTPs for subsequent experiments.

- 5) Excess Proteome Reagent is removed in the pooled sample using, *e.g.*, the biotin/streptavidin system with magnetic beads, then the supernatant is concentrated and conditioned for hybridization.

- 6) Transfer to an Oligonucleotide Chip. After a washing step to remove non-hybridized and other low molecular weight material, a matrix is added. Alternatively, before matrix addition, a digestion with, *e.g.*, trypsin or/and chymotrypsin

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is performed. After washing out the enzyme and the digestion products, the matrix is added.

- 5 7) Transfer of chip to mass spectrometer. In one embodiment, MALDI-TOF mass spectrometry is performed. Other mass spectrometric configurations suitable for protein analysis also can be applied. The mass spectrometer has an xy stage and thereby rasters over each position on the spot for analysis. The Proteome Reagent can be designed so that most of the reagent part (including the part hybridizing with the
- 10 oligonucleotide chip array) is cleaved either before or during mass spectrometry and therefore will be detected in the low molecular weight area of the spectrum and will be well separated from the peptide (in case of enzymatic digestion) or protein molecular weight signals in the mass
- 15 spectrum.

- 20 8) Finally, the molecular weight signals can be processed for noise reduction, background subtraction and other such processing steps. The data obtained can be archived and interpreted. The molecular weight values of the proteins (or the peptides obtained after enzymatic digestion) are associated with the human DNA sequence information and the derived protein sequence information from the protein coding regions. An interaction with available databases will reveal whether the proteins and their functions are already
- 25 known. If the function is unknown, the protein can be expressed from the known DNA sequence in sufficient scale using standard methods to elucidate its function and subsequent location in a biochemical pathway, where it plays its metabolic role in a healthy individual or in the

disease pathway for an individual with disease.

Since the master plates containing aliquots from the different proteins within a given tissue sample have been stored and are available, subsequent experiments then can be performed in a now-preselected way, *e.g.*, the proteins are displayed on the chip surface for protein-protein (biomolecule) interaction studies for target validation or/and to study the interaction with combinatorial libraries of small molecules for drug candidate selection.

#### G. Bioinformatics

10 The raw data generated from the analysis, such as mass spectrometry analysis, of the compound-protein species is processed by background subtraction, noise reduction, molecular weight calibration and peak refinement (*e.g.*, peak integration). The molecular weight values of the cleaved proteins or the digestion products are interpreted and compared with existing protein databases to determine whether the protein in question is known, and if so, what modifications are present (glycosylated or not glycosylated, phosphorylated or not phosphorylated, etc.). The different sets of experiments belonging to one set of compounds are composed, compared and interpreted. For example, one set of experiments uses a set of compounds with one X moiety and different Q moieties. This set of experiments provides data for a portion of the proteome, since not all proteins in the proteome will react with a given X moiety. Superposition of the data from this set of experiments with data from other sets of experiments with different X moieties provides data for the complete proteome.

Sets of experiments comparing tissues of healthy and disease individuals or from different physiological or developmental stages (*e.g.*, tumor progression, dependence of drug treatments to monitor results of therapy, immune response to virus or bacteria infection) or different

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tissue areas (*e.g.*, of a tumor) are investigated, and the final data archived.

The following examples are included for illustrative purposes only  
5 and are not intended to limit the scope of the invention.

Commercial grade solvents and reagents were used without purification unless otherwise specified, and were purchased from the following vendors: Anhydrous THF (Aldrich), CH<sub>2</sub>Cl<sub>2</sub> (Aldrich, Acros, EM Science), CHCl<sub>3</sub> (Aldrich, Mallinckrodt), Hexanes (Acros, EM science),  
10 Ethyl acetate (Aldrich, Acros), Acetone (Aldrich, EM science), Methyl alcohol (Aldrich), Diethyl ether (Fisher scientific). 4-Bromobenzoic acid (Aldrich), 2-amino-2-methyl-1-propanol (Acros), 1,3-dicyclo carbodiimide (Aldrich), N-hydroxysuccinimide (Aldrich), Maleimide (Aldrich), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Acros), Thionyl  
15 chloride (Aldrich), Pyridine (Aldrich), Magnesium turnings (Acros), 4-(Diphenylhydroxymethyl)benzoic acid (Fluka), Sodium ethoxide (Acros), Potassium carbonate, Sodium iodide, Carbon tetrachloride, methyl iodide, RED-Al (Aldrich), anhydrous Na<sub>2</sub>SO<sub>4</sub> (Acros), Acetic acid (EM science), Sodium hydroxide (Acros), Molecular sieves A<sup>o</sup><sub>4</sub> (Aldrich), and Acetyl  
20 chloride (Aldrich). <sup>1</sup>H NMR spectral data were obtained from a 500 MHz NMR spectrophotometer using CDCl<sub>3</sub> as a solvent. Mass spectral data were analyzed using the electrospray method.

#### EXAMPLE 1

Examples for N<sup>1</sup><sub>m</sub>-B<sub>i</sub>-N<sup>2</sup><sub>n</sub>

25 a. N<sup>1</sup> and N<sup>2</sup> as identical tetramers, B as a trimer

N<sup>1</sup> = N<sup>2</sup>, m = n = 4, i = 3, B = 64 sequence permutations

GTGC ATG GTGC

AAG

ACG

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AGG

TTG

CTG

GTG

5

...

...

...

GGG

b.  $N^1$  and  $N^2$  as non-identical tetramers, B as a tetramer

10

 $N^1 \neq N^2$ ,  $m = n = 4$ ,  $i = 4$ ,  $B = 256$  sequence permutations

GTCC ATCG CTAC

AACG

ACCG

AGCG

15

....

....

....

GGGG

c.  $N^1$  as a heptamer,  $N^2$  as an octamer, B as an octamer

20

 $N^1 \neq N^2$ ,  $m = 7$ ,  $n = 8$ ,  $i = 8$ ,  $B = 65,536$  sequence

permutations.

GCTGCCC

ATTCGTAC

GCCTGCCC

N<sup>1</sup>BN<sup>2</sup>

25

**EXAMPLE 2****Separation of proteins on a DNA array** $N^1_m$ -B<sub>i</sub>- $N^2_n$ -(S<sup>1</sup>)<sub>t</sub>-M(R<sup>15</sup>)<sub>a</sub>-(S<sup>2</sup>)<sub>b</sub>-L-X-Protein where B is a trimer; $m = n = 4$ ,  $i = 3$ ,  $t = b = 1$ ; underlined sequences are  $N^1$  and  $N^2$ 

30

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5 / CTGC ATG GTGC - S<sub>1</sub> - M(R<sup>15</sup>)<sub>a</sub> - S<sub>2</sub> - L - X - Protein 1  
 / --CACG TAC CACG  
 /  
 / CTGC AAG GTGC - S<sub>1</sub> - M(R<sup>15</sup>)<sub>a</sub> - S<sub>2</sub> - L - X - Protein 2  
 / --CACG TTC CACG  
 /  
 / CTGC ACG GTGC - S<sub>1</sub> - M(R<sup>15</sup>)<sub>a</sub> - S<sub>2</sub> - L - X - Protein 3  
 / --CACG TGC CACG  
 /  
 10 / ...  
 / ...  
 / ...  
 / CTGC GGG GTGC - S<sub>1</sub> - M(R<sup>15</sup>)<sub>a</sub> - S<sub>2</sub> - L - X - Protein 64  
 / --CACG CCC CACG  
 /  
 15

### EXAMPLE 3

#### I. Preparation of protein mixtures from cells or via protein translation of a cDNA library prepared from cells or tissues

20 The protein mixtures can be selectively divided on the physical or biochemical separation techniques

##### 1. Preparation of limited complexity protein pools using cell culture or tissue

25 Proteins can be isolated from cell culture or tissues according to methods well known to those of skill in the art. The isolated proteins are purified using methods well known to those of skill in the art (*e.g.*, TPAE, differential protein precipitation (precipitation by salts, pH, and ionic polymers), differential protein crystallization bulk fractionation, electrophoresis (PAGE, isoelectric focusing, capillary), and  
 30 chromatography (immunoaffinity, HPLC, LC)). Individual column fractions containing protein mixtures of limited complexity are collected for use as antigen.

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**2. Preparation of limited complexity protein pools using cDNA expression libraries (Figure 6)**

**a. RNA Isolation**

**i. Isolation of Total RNA**

- 5** Cultured cells or tissues are homogenized in a denaturing solution containing 4 M guanidine thiocyanate. The homogenate is mixed sequentially with 2 M sodium acetate (pH 4), phenol, and finally chloroform/isoamyl alcohol or bromochloropropane. The resulting mixture is centrifuged, yielding an upper aqueous phase containing total RNA.
- 10** Following isopropanol precipitation, the RNA pellet is dissolved in denaturing solution (containing 4 M guanidine thiocyanate), precipitated with isopropanol, and washed with 75% ethanol.

**ii. Isolation of Cytoplasmic RNA**

- Cells are washed with ice-cold phosphate-buffered saline and kept
- 15** on ice for all subsequent manipulations. The pellet of harvested cells is resuspended in a lysis buffer containing the nonionic detergent Nonidet P-40. Lysis of the plasma membranes occurs almost immediately. The intact nuclei are removed by a brief micro centrifuge spin, and sodium dodecyl sulfate is added to the cytoplasmic supernatant to denature
- 20** protein. Protein is digested with protease and removed by extractions with phenol/chloroform and chloroform. The cytoplasmic RNA is recovered by ethanol precipitation.

**b. mRNA purification**

- Messenger RNA is purified from total or cytoplasmic RNA
- 25** preparation using standard procedures. Poly(A)<sup>+</sup> RNA can be separated from total RNA by oligo (dT) binding to the Poly(A) tail of the mRNA. Total RNA is denatured to expose the Poly(A) (polyadenylated) tails. Poly(A)-containing RNA is then bound to magnetic beads coated with oligo(dT) and spirited from the total or cytoplasmic RNA through

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magnetic forces. The mRNA population can be further enriched for the presence of full-length molecules through the selection of a 5'-cap containing mRNA species.

**c. cDNA synthesis**

5 Different types of primers can be used to synthesis full length or 5'-end containing cDNA libraries from the isolated mRNA.

- i. **Oligo (dT) primer, which will generate cDNAs for all mRNA species (Figure 7)**

10 An example of the production of an adapted oligo dT primed cDNA library is provided in Figure 7.

- 15 ii. **Functional protein motif specific degenerate oligonucleotide primers will generate a limited number of genes belonging to the same protein family or of functionally related proteins (Figure 8)**

An example of the production of an adapted sequence motif specific cDNA library is provided in Figure 8.

- iii. **Gene specific oligonucleotide will produce cDNA for only one mRNA species (Figure 9)**

20 The oligonucleotides used for the cDNA production can contain additional sequences, 1) protein tag specific sequences for easier purification of the recombinant proteins (6 x His), 2) restriction enzyme sites, 3) modified 5'-end for cDNA purification or DNA construction purposes (Figure 10).

25 The conversion of mRNA into double-stranded cDNA for insertion into a vector is carried out in two parts. First, intact mRNA hybridized to an oligonucleotide primer is copied by reverse transcriptase and the products isolated by phenol extraction and ethanol precipitation. The RNA in the RNA-DNA hybrid is removed with RNase H as *E. coli* DNA  
30 polymerase I fills in the gaps. The second-strand fragments thus produced are ligated by *E. coli* DNA ligase. Second-strand synthesis is

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completed, residual RNA degraded, and cDNA made blunt with RNase H, RNase A, T4 DNA polymerase, and *E. coli* DNA ligase.

**d. Adapter ligation**

Adapter molecules can be ligated to both ends of the blunt ended  
5 double stranded cDNA or to only one end of the cDNA. Site directed  
adapter ligation could be achieved through the use of 5' modified  
oligonucleotides (for example biotinylated, aminated) during cDNA  
synthesis that prevents adapter ligation to the 3' end of the cDNA. The  
resulting cDNA molecules contain a 5'-end cDNA library comprised of the  
10 5' non-translated region, the translational start codon AUG coding for a  
methionine, followed by the coding region of the gene or genes. The  
cDNA molecules are flanked by known DNA sequence on their 5'- and 3'-  
ends (Figures 14, 15 and 16).

**e. cDNA amplification**

15 PCR Primers to the known 5'- and 3'-end sequences or known  
internal sequences can be synthesized and used for the amplification of  
either the complete library or specific subpopulations of cDNA using an  
extended an 5'- or 3'- amplification primer in combination with the primer  
located on the opposite site of the cDNA molecules (Figure 11).

20 **f. Primer design for the amplification of gene sub-  
populations**

The sub-population primers contain two portions (Figure 12). The  
5'-part of the primer is complementary to the sequence of a known  
sequence, extending with its 3'-end into the unknown cDNA sequence.  
25 Since each nucleotide in the cDNA part of the library can have an  
adenosine, cytidine, guanosine or thymidine residue, 4 different  
nucleotides possibilities exist for each nucleotide position. Four different  
amplification primers can be synthesized, each containing the same  
known sequence and extending by one nucleotide into the cDNA area of

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the library. The 4 primers only differ at their most 3'-nucleotide, being either A, C, G or T. If we suppose that each nucleotide (A, C, G, T) is equally represented in a stretch of DNA, each one of the 4 amplification primers will amplify one quarter of the total genes represented in the cDNA library. Extending the amplification primer sequence further and increasing the number of amplification primers, the complexity of the amplification products can be further reduced. Extending the sequence by 2 nucleotides requires the synthesis of 16 different primers decreasing the complexity by 16 fold, 3 nucleotides require 64 different primers and nucleotide extension requires  $n^4$  different primers.

**g. PCR amplification**

PCR amplification entails mixing template DNA, two appropriate oligonucleotide primers (5'- and 3'-end primers located in the known added sequences directed in complementary orientation), *Taq* or other thermostable DNA polymerases, deoxyribonucleoside triphosphates (dNTPs), and a buffer. The PCR products are analyzed after cycling on DNA gels or through analysis on an ABI 377 using the genescan analysis software. These analysis methods allow the determination of the complexity of the amplified cDNA pool.

**h. Production of a protein expression library**

Each amplified cDNA library sub-population is cloned 5' to 3' in a bacterial (*E. coli*, etc.) or eukaryotic (Baculovirus, yeast, mammalian) protein expression system. The gene is introduced with its own translational initiation signal and a 6xHis tag in all 3 frames. For example: the cDNA is restricted with two different, rare-cutting restriction enzymes (5'-end BglII and 3'-end Not I) and cloned in the 5' to 3' orientation in the Baculovirus transfer vector pVL1393 under the direct control of the polyhedra promoter.

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**i. Protein expression**

Linearized Baculovirus DNA and recombinant transfer-vector DNA are cotransfected into susceptible Sf9 insect cells with calcium phosphate. For cotransfection, 10 ug of purified plasmid DNA is  
5 prepared. An initial recombinant Baculovirus stock is prepared and Sf9 cells are infected for recombinant protein production.

**j. Protein purification**

The expressed recombinant proteins contain an affinity tag (an example is a 6xHis tag). They are purified on Ni-NTA agarose.  
10 Approximately 1 to 2 mg of 6xHis recombinant fusion protein is routinely obtained per liter of insect cell culture.

**k. Purification Tag removal**

If the expression vector or the amplification primer was constructed with a proteolytic cleavage site for thrombin, the purification tag can be  
15 removed from the recombinant proteins after the protein affinity purification step.

**II. Antibody generation by immunization of different animals with individual protein mixtures****3. Preparation of Antibody protein capture reagents**

20 A purified protein preparation translated from a pool of cDNAs is injected intramuscularly, intradermally, or subcutaneously in the presence of adjuvant into an animal of the chosen species (rabbit). Booster immunizations are started 4 to 8 weeks after the priming immunization and continued at 2- to 3-week intervals. The polyclonal antiserum is  
25 purified using standards known to those skilled in the art.

The purified antibody batches can be used directly as protein capture reagents without modification. In this case the antibody batches from different animals have to be kept separate (each batch is one capture reagent).

**III. Antibody proteins are isolated and conjugated with nucleic acid sequences that correspond to the original antigen preparation resulting in the antibody capture reagents**

5 Generation of bi-functional capture/sorting molecules for sorting of the complex protein mixture on a solid phase.

The glycosylated C<sub>H</sub><sup>2</sup> domain of the polyclonal antibodies are conjugation to 5' modified oligonucleotides using standard conjugation methods. The resulting molecule has one protein capture moiety (antibody) and one nucleic acid moiety (oligonucleotide) (Figure 13).

10 The antibody batches after immunization of an animal with a reduced complexity protein pool are conjugated with the one oligonucleotide sequence. Antibodies produced from multiple immunization events with different protein pools are conjugated to an oligonucleotide with a different sequence (Figure 13).

15 **4. Capture of target proteins using reactivity, functionality and sorting by oligonucleotide hybridization**

Two different methods have been developed for making oligonucleotides bound to a solid support: they can be synthesised *in situ*, or presynthesised and attached to the support. In either case, it is possible  
20 to use the support-bound oligonucleotides in a hybridization reaction with oligonucleotides in the liquid phase to form duplexes; the excess of oligonucleotide in solution can then be washed away.

The support can take the form of particles, for example, glass spheres, or magnetic beads. In this case the reactions could be carried  
25 out in tubes; or in the wells of a microtitre plate. Methods for synthesising oligonucleotides and for attaching presynthesised oligonucleotides to these materials are known (see, *e.g.*, Stahl *et al.* (1988) *Nucleic Acids Research* 16(7):3025-3039).

**a. Preparation of amine-functionalized solid support**

30 Oligonucleotides of a defined sequence are synthesized on an amine-

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functionalized glass support. An amine function was attached at discrete locations on the glass slide using a solution of 700  $\mu$ l of  $\text{H}_2\text{N}(\text{CH}_2)_3\text{Si}(\text{OCH}_2\text{CH}_3)_3$  in 10 ml of 95% ethanol at room temperature for 3 hours. The treated support is washed once with methanol and then once with ethyl ether. The support was dried at room temperature and then baked at 110 °C for 15 hours. It was then washed with water, methanol and water, and then dried.

The glass slide was reacted for 30 minutes at room temperature with 250 mg (1 millimole) of phthallic anhydride in the presence of 2 ml of anhydrous pyridine and 61 mg of 4-dimethylaminopyridine.

The product was rinsed with methylene dichloride, ethyl alcohol and ether, and then dried. The products on the slide were reacted with 330 mg of dicyclohexylcarbodiimide (DCC) for 30 minutes at room temperature. The solution was decanted and replaced with a solution of 117 mg of 6-amino-1-hexanol in 2 ml of methylene dichloride and then left at room temperature for approximately 8 hours.

**b. Oligonucleotide synthesis on a solid support**

The amine-functionalized solid support was prepared for oligonucleotide synthesis by treatment with 400 mg of succinic anhydride and 244 mg of 4-dimethylaminopyridine in 3 ml of anhydrous pyridine for 18 hours at room temperature. The solid support was treated with 2 ml of DMF containing 3 millimoles (330 mg) of DCC and 3 millimoles (420 mg) of p-nitrophenol at room temperature overnight. The slide was washed with DMF,  $\text{CH}_3\text{CN}$ ,  $\text{CH}_2\text{Cl}_2$  and ethyl ether. A solution of 2 millimoles (234 mg) of  $\text{H}_2\text{N}(\text{CH}_2)_6\text{OH}$  in 2 ml of DMF was reacted with the slide overnight. The product of this reaction was a support,  $-\text{O}(\text{CH}_2)_3\text{NHCO}(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_5\text{CH}_2\text{OH}$ . The slide was washed with DMF,  $\text{CH}_3\text{CN}$ , methanol and ethyl ether.

The functionalized ester resulting from the preparation of the glass

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support was used for the synthesis of a oligonucleotide sequence. Each nucleoside residue was added as a phosphoramidite according to known procedures (see, *e.g.*, U.S. Patent Nos. 4,725,677 and 5,198,540, and RE34,069, see, also Caruthers *et al.* U.S. Patent No. 4,415,732).

5           **5. Protein analysis of the captured proteins and complex protein sample comparison**

The purified antibody batches can be either 1) directly attached to a solid surface, and incubated with protein samples, 2) incubated with the samples and subsequently bound to a solid support without using the  
10 capture compound or 3) the capture compound can be used to capture its corresponding protein in a sample and subsequently sort the captured proteins through specific nucleotide hybridization (Figure 14).

15           **IV. Antisense oligonucleotide capture reagents are immobilized in discrete and known locations on a solid surface to create an antibody capture array**

6.           **Preparation of capture array surface**

5'-aminated oligonucleotides are synthesized using phosphoramidate chemistry and attached to N-oxysuccinimide esters. The attached oligonucleotide sequences are complementary to the sorting  
20 oligonucleotides of the bi-functional antibody molecules (Figure 13). Proteins are captured through nucleic acid hybridization of their sorting oligonucleotide to the complementary sequence attached to the solid surface oligonucleotide.

25           **V. The antibody capture reagents are added to the total protein mixture (reactivity step). The reaction mixture is then added to the solid surface array under conditions that allow oligonucleotide hybridization (sorting step).**

7.           **Capture compound/protein capture and sorting**

The bi-functional antibodies are incubated with the protein  
30 sample under conditions that allow the antibodies to bind to their corresponding antigen. The bi-functional antibody molecule with the

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captured protein is added to the oligonucleotide prepared capture array. Under standard DNA annealing conditions that do not denature the antigen-antibody, binding the bi-functional antibody will hybridize with its nucleic acid moiety to the complementary oligonucleotide.

**5 VI. The captured protein is identified using MALDI mass spectrometry**

**8. Analysis of the capture proteins**

The attached proteins are analyzed using standard protein analysis methods, such as mass spectrometry.

**EXAMPLE 4**

**10 Synthesis of Trityl based Protein capture compounds (see Figure 15)**

**A. Synthesis of 2-(4-bromophenyl)-4,4-dimethyl-1,3-oxazoline,  
1**

To 4-Bromobenzoic acid (50 g, 0.25M) placed in a 500 mL round bottom flask fitted with a reflux condenser was added 150 mL of thionyl  
**15** chloride and refluxed for 8 hr. The excess thionyl chloride was removed under vacuum and the white solid obtained was dissolved in 100 ml of dry  $\text{CH}_2\text{Cl}_2$  and kept in an ice bath. To this ice cooled solution of bromo benzoylchloride was added drop wise 45 g of 2-amino-2-methylpropan-1-ol dissolved in another 100 mL of dry  $\text{CH}_2\text{Cl}_2$  with stirring for the period  
**20** of 1 h. The ice bath was removed and the reaction mixture was stirred at room temperature for over night. The precipitated white solid was filtered and washed several times with  $\text{CH}_2\text{Cl}_2$  (4x100 mL). The combined  $\text{CH}_2\text{Cl}_2$  was removed under rotaevaporator and the solid obtained was slowly dissolved in 150 mL of thionyl chloride and refluxed for 3 h. The excess  
**25** of  $\text{SOCl}_2$  was evaporated to one-sixth the volume and poured in to 500 mL of dry ether cooled in ice bath and kept in the refrigerator overnight. The ether was removed and the precipitated hydrochloride was dissolved in 500 mL of cold water. The aqueous solution was carefully neutralized using 20% KOH solution on cold condition (ice bath) and the brown oily

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residue separated was extracted with  $\text{CH}_2\text{Cl}_2$  (3x200 mL) and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Removal of the solvent gave 42 g (67%) of 2-(4-bromophenyl)-4,4-dimethyl-1,3-oxazoline as a yellow oil.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.36 (s, 6H), 4.08 (s, 2H), 7.52 (d, 2H), 7.79 (d, 2H). Mass: 254.3 ( $\text{M}^+$ ).

**B. Synthesis of phenyl-{3-[2-(tetrahydropyran-2-yloxy)-ethoxy]-phenyl}-methanone, 2**

1. **Method A:** In a 100 mL two neck round bottom flask placed with 550 mg (8 mM) of  $\text{NaOEt}$  in 20 mL of dry  $\text{DMF}$  was added 3-hydroxy benzophenone (1 g, 5 mM) under argon atmosphere. The reaction was stirred at room temperature for 10 min and added 2-bromoethoxy tetrahydropyran (1 g, 5 mM) dissolved in 5 mL of dry  $\text{DMF}$  by drop wise. The reaction mixture was heated, at  $60^\circ\text{C}$  for overnight, cooled and poured into ice water and extracted with  $\text{CH}_2\text{Cl}_2$  (2x50 mL). The combined solvent was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated. The crude residue obtained was purified by silica gel column chromatography using hexane/ $\text{EtOAc}$  (9:1) mixture as an eluent. Yield : 680mg (42%).

2. **Method B:** To the stirred mixture of 3-hydroxy benzophenone (1 g, 5 mM), anhydrous  $\text{K}_2\text{CO}_3$  (3g, 23 mM) and  $\text{NaI}$  (500 mg) in dry acetone (40mL) was added 2-bromoethoxytetrahydropyran (1g, 5 mM) dissolved in 10 mL of dry acetone and refluxed for 20 h. The precipitate was filtered and was washed with acetone (3x20 mL). The combined filtrate was evaporated and the yellowish residue obtained was purified by silica gel column chromatography using hexane/ $\text{EtOAc}$  (9:1) mixture as an eluent. Yield: 55- 60%.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.5-1.63 (m, 4H), 1.72 (m, 1H), 1.82 (m, 1H), 3.52 (m, 1H), 3.8-3.9 (m, 2H), 4.07 (m, 1H), 4.21 (m, 2H), 4.70(t, 1H), 7.15 (d, 1H), 7.37(m, 3H), (7.47 (t, 2H), 7.58(t,1H), 7.80(d,1H). Mass: 327.2( $\text{M}^+$ ), 349.3.

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(M + Na<sup>+</sup>).

C. Grignard reaction: Synthesis of 2-{4'-(3-(2-tetrahydropyran-2-yloxy)ethoxy)phenyl-4"-phenyl}}-4,4-dimethyl-1,3-oxazoline, 3

5 To a 100 mL two necked round-bottomed flask fitted with reflux condenser was placed activated Mg turnings (720 mg, 30 mM), a few crystals of I<sub>2</sub> and molecular sieves (A4) under argon. To this mixture 10 mL of THF was added. The mixture was heated to 50 °C and 2-(4-bromophenyl)-4,4-dimethyl-1,3-oxazoline (6.5g, 26 mM) dissolved in 15 mL of dry THF, a catalytic amount of CH<sub>3</sub>I, RED-Al and CCl<sub>4</sub> were added with stirring and refluxed for 3h. After that the reaction mixture was cooled to room temperature and added phenyl-{3-[2-(tetrahydropyran-2-yloxy)-ethoxy]-phenyl}-methanone (5.1 g, 15.6 mM) dissolved in 15 mL of dry THF and again refluxed for 3 h, cooled and 3mL of water added.

15 The solvent was removed under rovaevaporator and extracted with CHCl<sub>3</sub> (3x100 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The residue obtained on removal of the solvent was separated by silica gel column chromatography using hexane/EtOAc (7:3) as an eluent. Evaporation of the column fraction yielded 2-{4'-(3-(2-tetrahydropyran-2-yloxy)ethoxy)phenyl-4"-phenyl}}-4,4-dimethyl-1,3-oxazoline (3) as a yellow crystalline solid (1.4g, 18%). <sup>1</sup>H-NMR(500 MHz, CDCl<sub>3</sub>) δ ppm: 1.37 (s, 6H), 1.5-1.63 (m, 4H), 1.68 (m, 1H), 1.80(m, 1H), 2.85 (s, 1H, -OH), 3.49 (m, 1H), 3.75(m, 1H), 3.85(m, 1H), 3.97 (m, 1H), 4.09(m, 4H), 4.66 (t, 1H), 6.80(d, 1H), 6.84(d, 1H), 6.88(s,1H), 7.18-7.31(m, 6H), 7.34 (d, 2H), 7.87(d, 2H). Mass: 502.6 (M + 1), 524.5 (M + Na<sup>+</sup>)

D. 4,4-Dimethyl-2-[4-(phenyl-[2-(tetrahydro-pyran-2-yloxy)-ethoxy]-{3-[2-(tetrahydro-pyran-2-yloxy)-ethoxy]-phenyl}-methyl)-phenyl]-4,5-dihydrooxazole, 4

To the stirred mixture of 2-{4'-(3-(2-tetrahydropyran-2-yloxy)ethoxy)phenyl-4"-phenyl}}-4,4-dimethyl-1,3-oxazoline (3, 200 mg,

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0.4 mM) and NaH (100 mg, 4 mM) in 3 mL of dry DMF at r.t. was added 2-(2-bromoethoxy)tetrahydro-2H-pyran (500 mg, 2.4 mM) and the reaction was allowed to stir at r.t. for 2h. Then the reaction mixture was poured in to ice water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent gave 4 as a yellow oily residue in quantitative yield.

**E. 4-{(2-Hydroxy-ethoxy)-[3-(2-hydroxy-ethoxy)-phenyl]-phenyl-methyl}-benzoic acid, 5**

A solution of 4 (360 mg) in 3 mL of 80% aqueous acetic acid was heated at 75 °C for 12h. Then the solution was evaporated and the residue obtained was refluxed with 20% NaOH/EtOH (1:1, v/v, 3 mL) for 2 h. The solvent was removed and 10 mL of ice cooled water was added to the residue and the aqueous solution was acidified with 1N HCl. The precipitated yellow solid was filtered and washed several times with water and dried under high vacuum. Yield: 270 mg (100%, quantitative).

**F. 4-{(2-Hydroxy-ethoxy)-[3-(2-hydroxy-ethoxy)-phenyl]-phenyl-methyl}-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester, 6**

1. **Method A:** To a stirred solution of trityl acid 5 (110 mg, 0.26 mM) and N-hydroxy succinimide (80 mg, 0.7 mM) in dry 1,4-dioxane (2 mL) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 105 mg, .5 mM) dissolved in 2 mL of water. The reaction mixture was stirred for 12 h at r.t. and the extracted with CHCl<sub>3</sub> (3x10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solid obtained on evaporation of the solvent was purified by preparative TLC plate. Yield: 5 mg.

2. **Method B:** To a stirred solution of trityl acid 5 (12 mg, 0.03 mM) in dry THF (4 mL) was added dicyclohexyl carbodiimide (DDC, 10 mg, 0.05 mM). The reaction mixture was stirred for 30 min at r.t., N-hydroxysuccinimide (11.5 mg, 0.1 mM) and a catalytic amount of DMAP was added and allowed to stir for overnight. The solvent was removed under

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rotaevaporator and the solid obtained was dissolved in dry ether. The precipitated DCU was filtered and the solvent ether was evaporated. The crude solid obtained was purified by preparative TLC plate. Yield 7 mg (50%). <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ ppm : 2.90 (s, 4H), 3.92(t, 4H), 4.02 (t, 4H), 6.83( m, 2H), 7.25 (m, 3H), 7.34 (m, 4H), 7.50(d, 2H), 8.0(d, 2H).

**G. 4,4-Dimethyl-2-[4-(phenyl-(3-phenyl-propoxy)-{3-[2-(tetrahydro-pyran-2-yloxy)-ethoxy]-phenyl}-methyl)-phenyl]-4,5-dihydro-oxazole, 7**

10 To the stirred mixture of 2-{4'-(3-(2-tetrahydropyran-2-yloxy)ethoxy)phenyl-4"-phenyl)}-4,4-dimethyl-1,3-oxazoline (**3**, 300 mg, 0.6 mM) and NaH (100 mg, 4 mM) in 3 mL of dry DMF at r.t. was added 3-bromo-1-phenyl propane (250mg, 1.2 mM) and the reaction was allowed to stir at r.t. for 2h. Then the reaction mixture was poured into  
15 ice water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent gave **7** as a yellow color residue in quantitative yield.

**H. 4-[[3-(2-Hydroxy-ethoxy)-phenyl]-phenyl-(3-phenyl-propoxy)-methyl]-benzoic acid, 8**

20 A solution of **7** (550 mg) in 3 mL of 80% aqueous acetic acid was heated at 75 °C for overnight. Then the solution was evaporated and the residue obtained was refluxed with 20% NaOH/EtOH (1:1, v/v, 3 mL) for 2 h. The solvent was removed, 10 mL of ice cooled water was added to the residue and the aqueous solution acidified with 1N HCl, extracted  
25 with CH<sub>2</sub>Cl<sub>2</sub> (60 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent gave yellow solid Yield: 485 mg (quantitative).

**I. 4-[[3-(2-Hydroxy-ethoxy)-phenyl]-phenyl-(3-phenyl-propoxy)-methyl]-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester, 9**

To a stirred solution of trityl acid **8** (200 mg, 0.42 mM) in dry THF  
30 (6 mL) was added dicyclohexyl carbodiimide (DDC, 206mg, 1 mM). The

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reaction mixture was stirred for 30 min at r.t., and N-hydroxysuccinimide (70 mg, 0.6 mM) and a catalytic amount of DMAP was added and was allowed to stir for overnight. The solvent was removed under rotaevaporator and the solid obtained was dissolved in dry ether. The precipitated DCU was  
 5 filtered and the solvent ether was evaporated. The crude solid obtained was separated by silica column chromatography using  $\text{CH}_2\text{Cl}_2$ . Yield: about 120 mg.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm : 1.70 (m, 2H), 1.9 (t, 2H), 2.9 (s, 4H), 3.5(m, 2H), 3.9 (t, 2H), 4.0(t, 2H), 6.85( m, 4H), 7.25 (m, 4H), 7.32 (m, 5H), 7.51(m, 3H), 8.09(d, 2H).

10 J. 1-{4-[[3-(2-Hydroxy-ethoxy)-phenyl]-phenyl-(3-phenyl-propoxy)-methyl]-benzoyl}-pyrrole-2,5-dione, 10

To a stirred solution of trityl acid 8 (280 mg, 0.42 mM) in dry THF (6 mL) was added dicyclohexyl carbodiimide (DDC, 400mg, 1.95mM). The reaction mixture was stirred for 30 min at r.t., and maleimide (100  
 15 mg, 1.1 mM) and a catalytic amount of DMAP was added and allowed to stir for overnight. The solvent was removed under rotaevaporator and the solid obtained was dissolved in dry ether. The precipitated DCU was filtered and the solvent ether was evaporated. Part of the product was purified by preparative TLC. Yield: 12 mg.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm : 1.78  
 20 (m, 2H), 1.95 (m 2H), 2.9 (s, 4H), 3.51(m, 2H), 3.93(t, 2H), 4.02(t, 2H), 6.8( m, 5H), 7.25 (m, 5H), 7.29 (m, 5H), 7.37(m, 3H), 7.48(d, 2H) ,  
 Mass: 561.3 ( $\text{M}^+$ ).

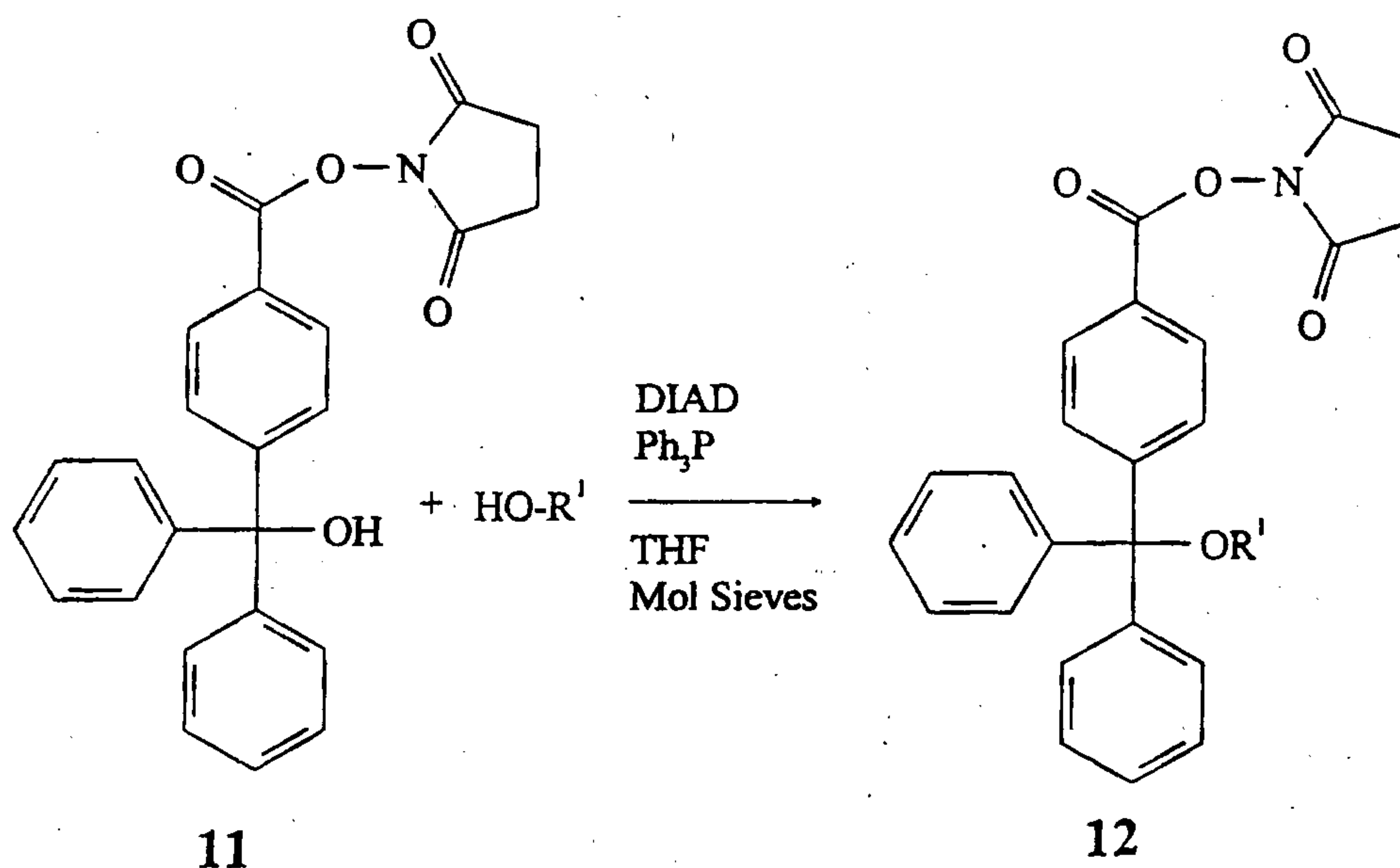
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**EXAMPLE 5**

This Example shows addition of a selectivity function onto a capture compound possessing a N-hydroxy succinimide ester reactivity function. Compounds with sorting can be prepared by using an

5 appropriate analog of compound 11 below.

**Procedure for Mitsunobu Reaction of Trityl Capture Reagents**

- 1.1 equivalents of triphenylphosphine are added to a reaction vial and dissolved in 1.0 ml THF. 1.1 equivalents of diisopropyl azidodicarboxylate are added to this solution and mixed for 5 minutes.
- 10 Add 1 equivalent of 11 and stir for 5 minutes. Add nucleophile ( $\text{R}_1\text{—OH}$ ) and stir overnight at 50 °C. Preparative TLC purified the products.

**EXAMPLE 6****Cell synchronization**

- H460 lung cancer and SW480 colon cancer cells were synchronized in
- 15 Go/G1 with simvastatin and lovastatin (HMG-CoA reductase inhibitors), which can enrich a cancer cell population in Go/G1. Cells arrested in

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G2/M phase were obtained by treatment with nocodazole.

#### Cell Culture and Reagents

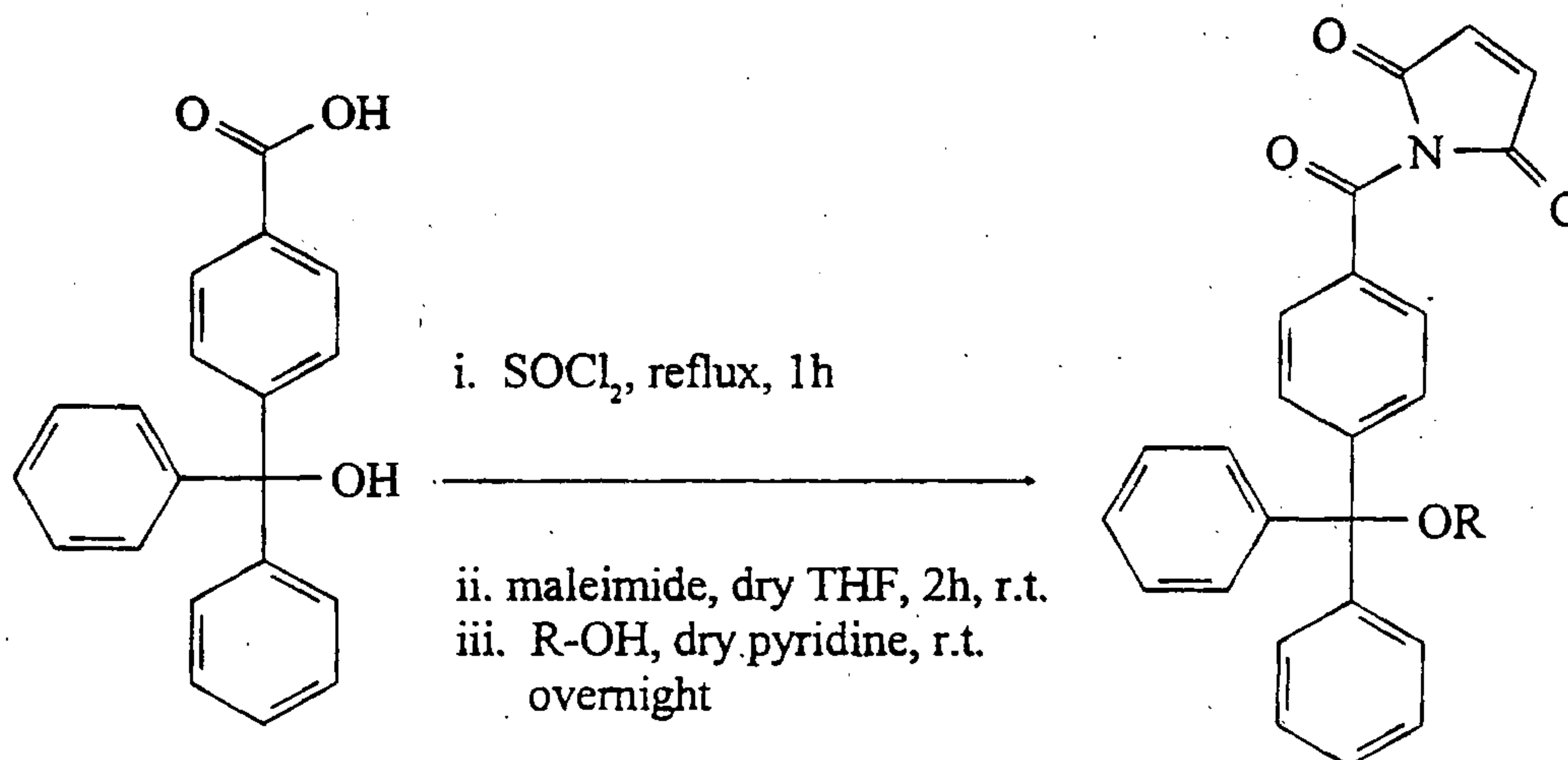
The SW480 cell line was cultured in Dulbecco's modified Eagle medium (DMEM), the H460 cell line (ATCC Manassas, VA) was cultured  
 5 in RPMI 1640, whereas the FK101 was cultured in serum-free medium (SFM) with 5% CO<sub>2</sub> at 37° C. The cell culture media were supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, penicillin(100U/ml)and streptomycin(100U/ml).

#### Synchronization of Cells

10 H460 and SW480 cells enriched in G<sub>1</sub> phase were obtained after incubation with serum-free medium for 48 hours, or treatment with U026, lovastatin or simvastatin. Cells in S phase were synchronized by incubating cells with medium containing no serum for 24 hours, followed by aphidicolin treatment (2ug/ml) for 20 hours and release of cells from  
 15 aphidicolin for 3 hours. Cells arrested in G2/M phase were obtained by treatment with nocodazole (0.4-0.8 mg/ml) for 16-20 hours.

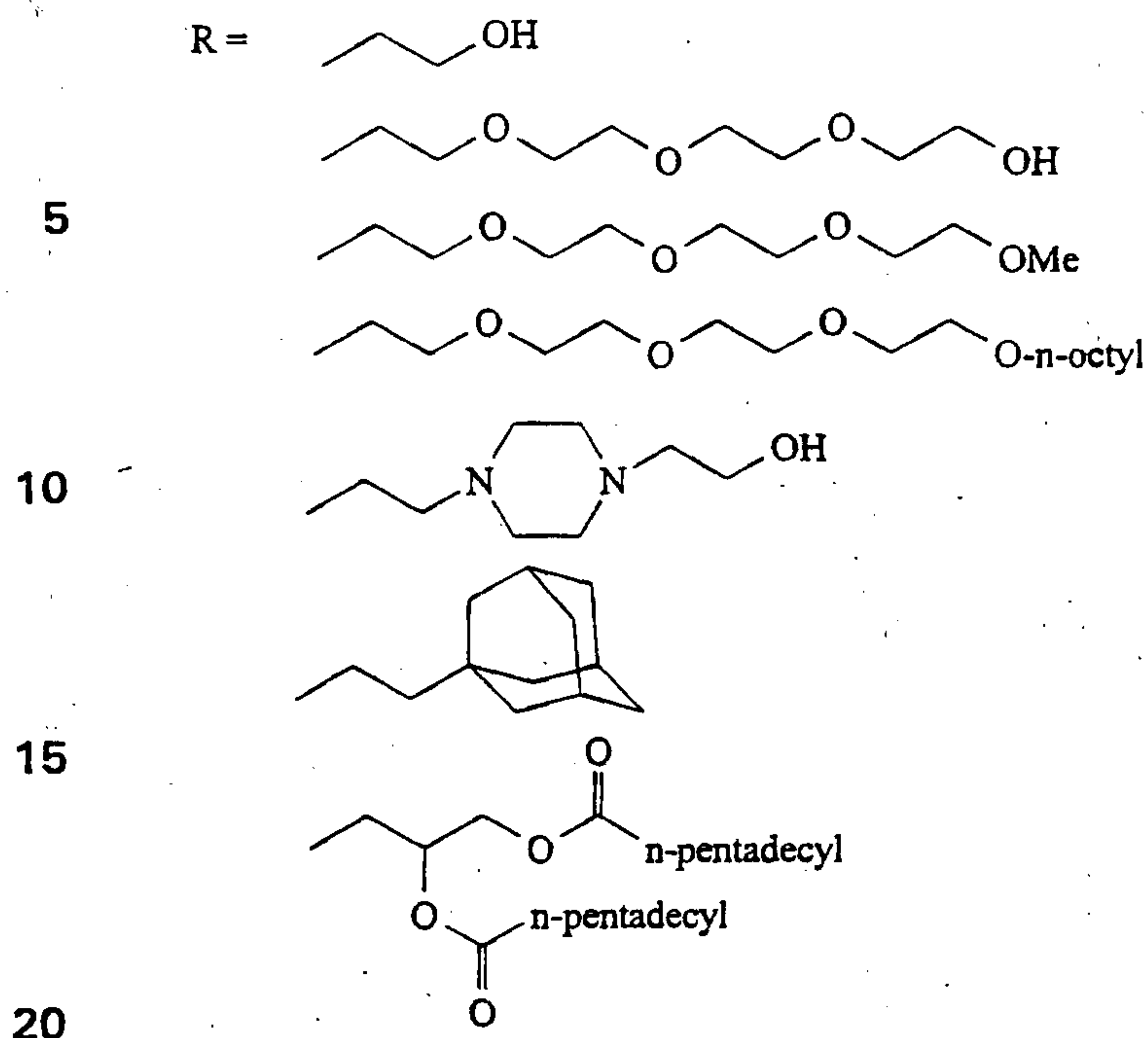
#### EXAMPLE 7

##### Synthesis of (4,4'-bisphenyl-hydroxymethyl)benzoyl maleimide derivatives



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General Procedure: A solution of 4-(diphenylhydroxymethyl)benzoic acid (0.04 mM) in 1 mL of  $\text{SOCl}_2$  was refluxed for 1 h and the excess  $\text{SOCl}_2$  was removed under high vacuum. To this yellow solid residue obtained

25 was added maleimide (0.045 mM) dissolved in dry freshly distilled THF (1 mL) and stirred at room temperature for 2h. The solvent was removed and added the corresponding alcohol (ROH, 2-5 fold excess) dissolved in dry pyridine (1mL) with stirring. After the reaction mixture stirred at room temperature for overnight the solution was extracted with  $\text{CH}_2\text{Cl}_2$  (5x3mL)

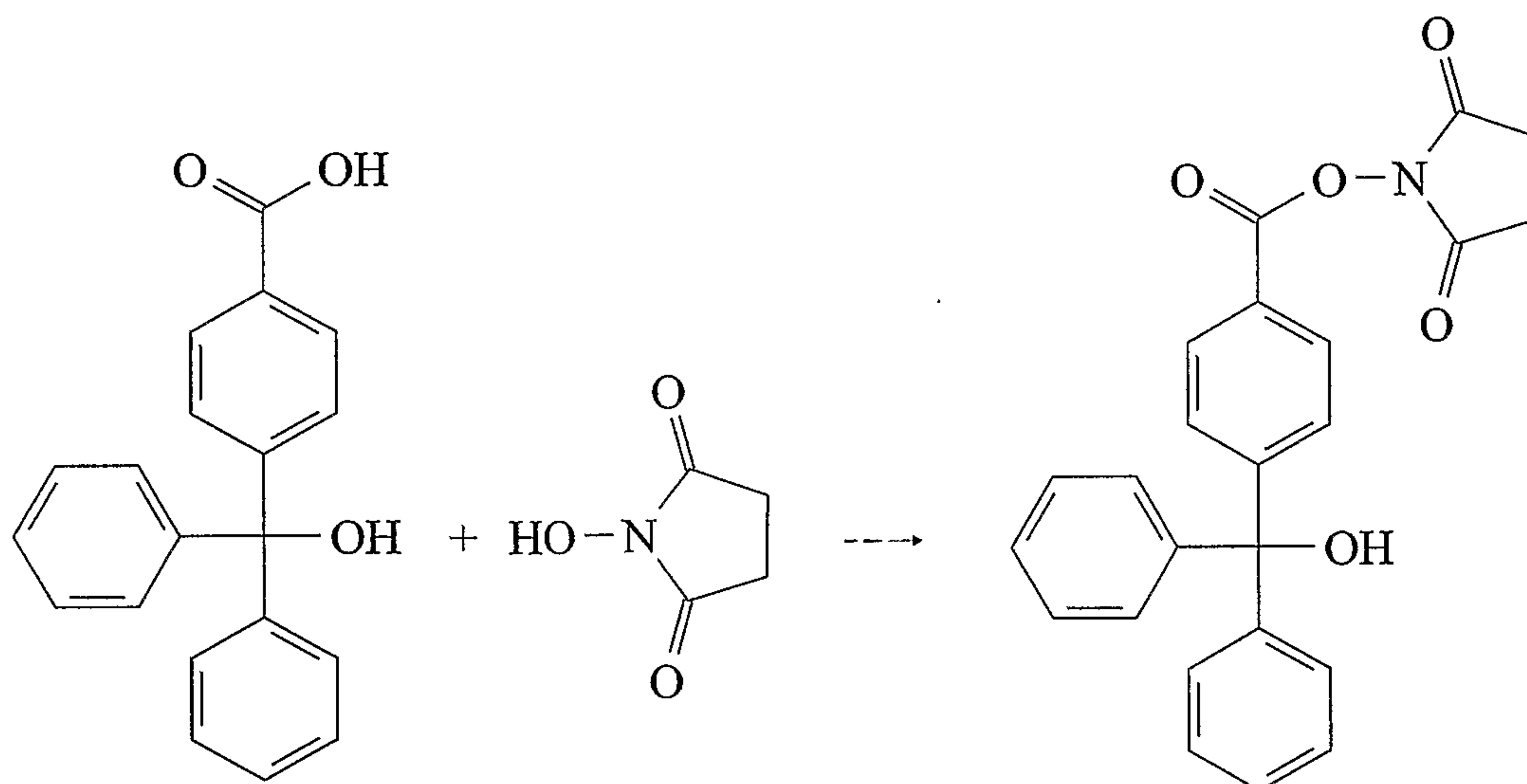
30 and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The residue obtained on evaporation of the solvent was separated by preparative TLC (Silica Gel, 500  $\mu\text{m}$  plate) and gave the product 1 in 50-60% yield. The trityl derivatives 1 were fully characterized by  $^1\text{H}$  NMR and mass spectral data.

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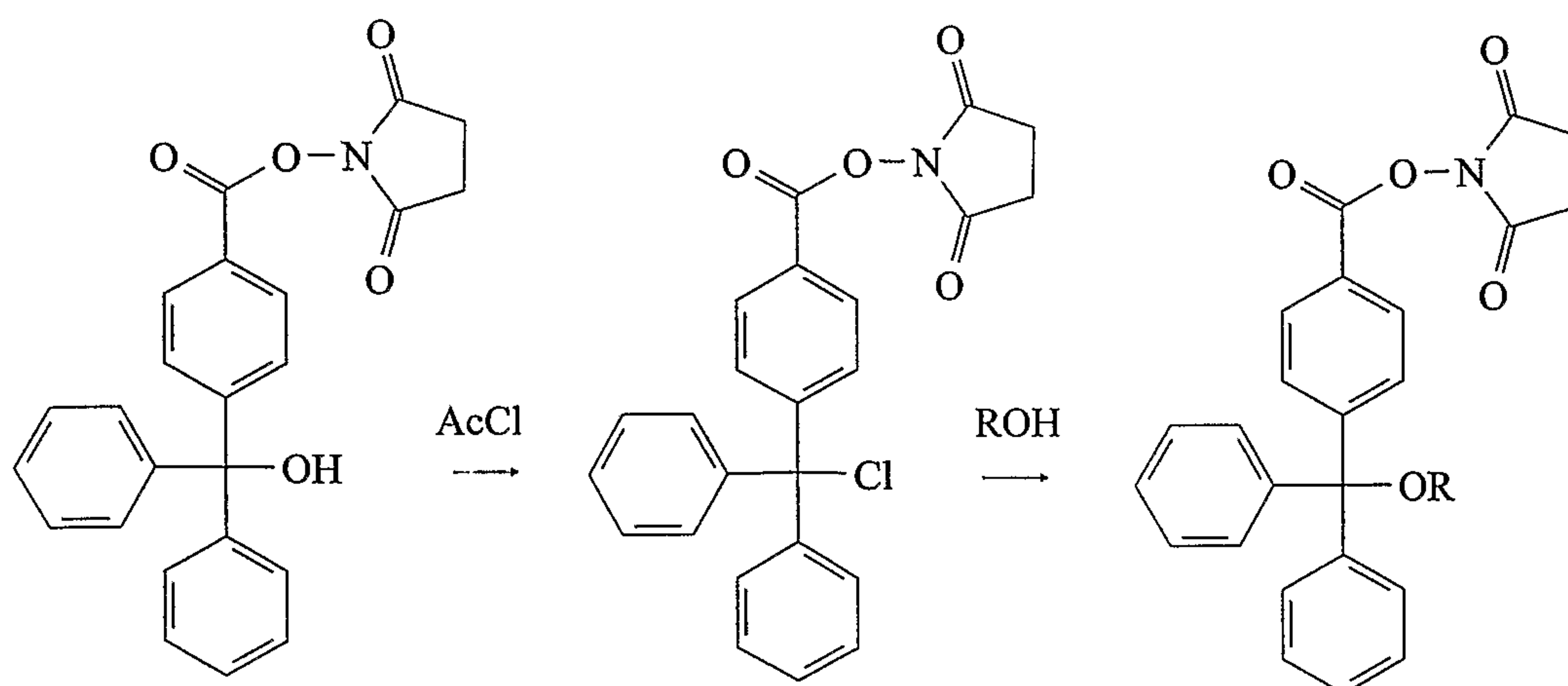
## EXAMPLE 8

## Succinimidyl Ester Trityl Capture Compound Synthesis

## Procedure 1



4-(Diphenylhydroxymethyl) benzoic acid was reacted with 2  
 5 equivalents of N-hydroxysuccinimide using 1.2 equivalents of Diisopropyl carbodiimide. The desired product was purified by Flash Silica chromatography and characterized by ESI mass spectrometry.

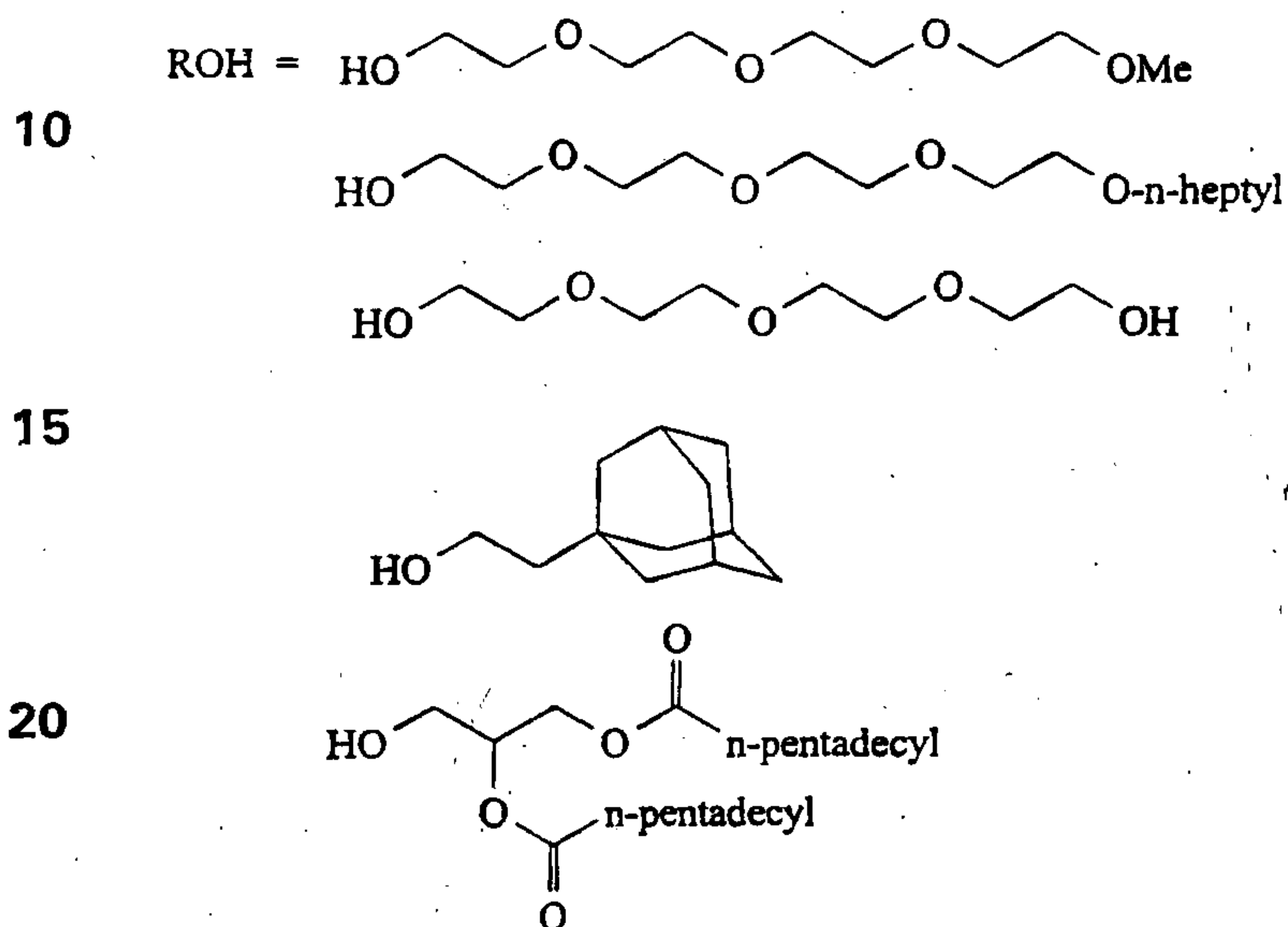


The 125  $\mu$ moles of product from above was added to 1.0 ml

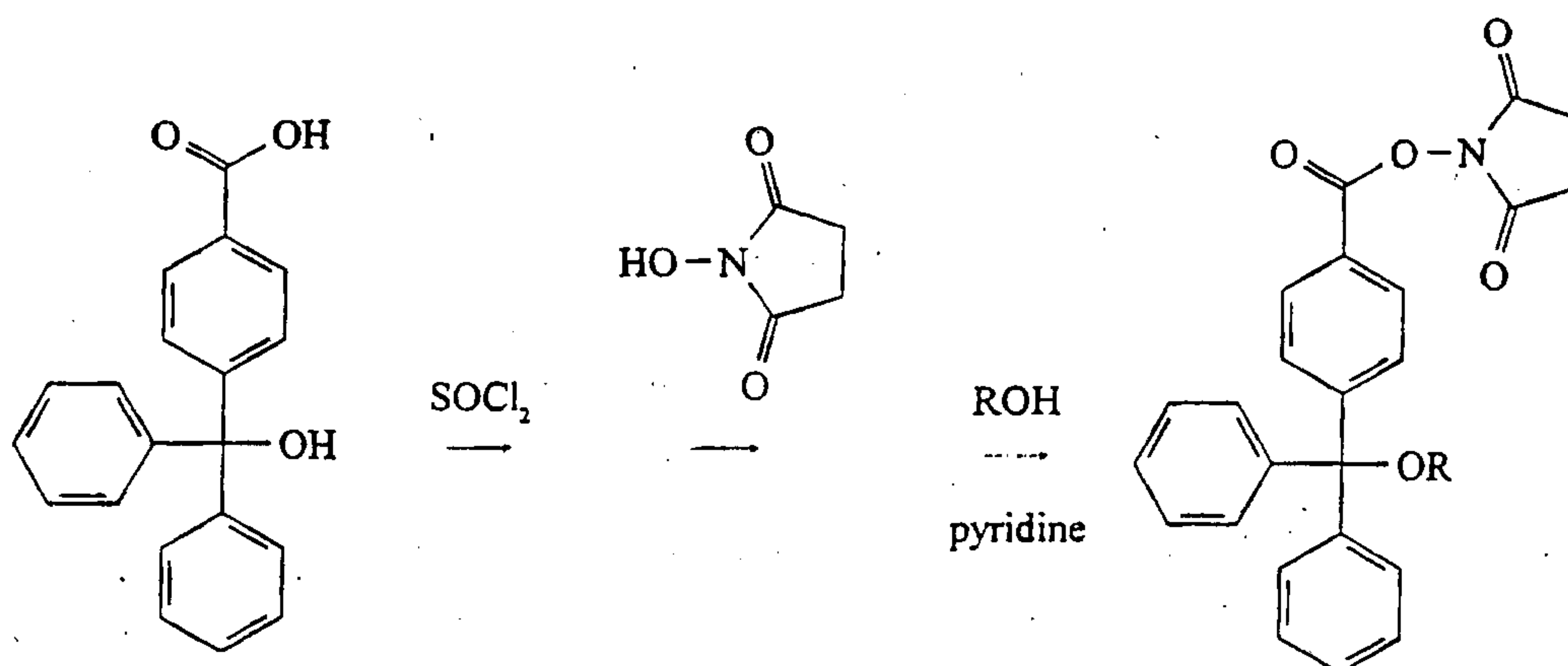
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Acetyl Chloride. This reaction mixture was stirred at room temperature for 1 hour and evaporated three times with toluene to remove excess acetyl chloride. Equal volumes of the reaction mixture were added to nucleophiles (see below) dissolved in 1.0 M Pyridine/THF. These reaction mixtures were mixed at 60°C for 2 hours. The resulting products were extracted from  $\text{CHCl}_3$  and 10% HOAc. Products were purified by Preparative TLC (Ether). MS and NMR characterize purified products.



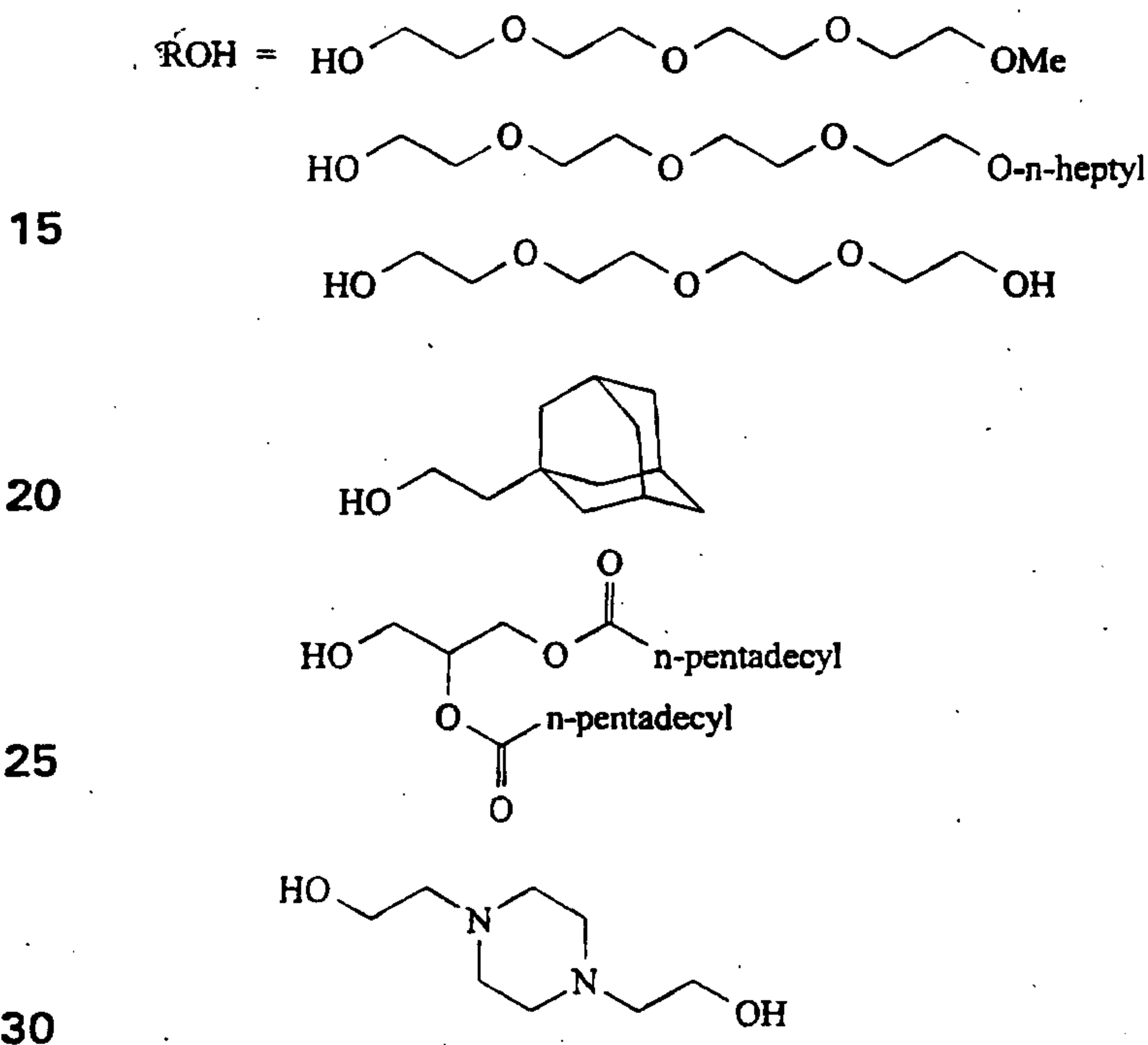
25

**Procedure 2**

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1.64 mmoles of 4-(Diphenylhydroxymethyl) benzoic acid was dissolved into 5 ml Thionyl Chloride. This reaction mixture is heated to 79°C and stirred for 75 minutes. The Thionyl Chloride is removed under N<sub>2</sub> (g) stream. 1.3 equivalents of N-hydroxysuccinimide dissolved in dry THF is added to this dried reaction mixture and stirred for 1 hour. The THF solvent is removed under N<sub>2</sub> (g) stream. The product is dissolved into dry Pyridine. Equal volumes of this solution are added to nucleophiles dissolved in Pyridine. (See below). The resulting products are extracted from CHCl<sub>3</sub> and 10% HOAc. Products are purified by Preparative TLC (Ether). MS and NMR characterize purified products.



### EXAMPLE 9

This example shows exemplary capture binding assays and the effects of selectivity functions on binding. This example shows that changing selectivity can alter reactivity of the capture compound thereby providing a means to probe biomolecule structures and to permit sorting or diversity.

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reduction using the collections. In this example, the core group of the capture compounds is a trityl group and the reactive group is succinimide, which interacts with a primary amine. Compound 1341 is a non-selective compound that has a reactivity group, but no selectivity group.

- 5 Compound 1343 (see Figure 20) is exemplary of such compound where the selectivity group is -OH. As the selectivity group changes there is a difference in reactivity on the target proteins (lysozyme, cytochrome C and ubiquitin).

#### Lysozyme

- 10 Three different capture compounds (designated HKC 1343, 1349, 1365; chemical structure of each compound is listed below the Compound name) were reacted individually with Lysozyme (Accession number P00698; Figure 20b). The capture experiments were analyzed using MALDI-TOF Mass Spectrometry. Binding was performed in 20 uL
- 15 sample volumes with a 5 uM Lysozyme concentrations in 25 mM HEPES buffer solution, pH 7.0. The trityl-based capture compounds were added to the protein solution at a 10 uM concentration. The binding reaction was incubated at room temperature for 30 minutes. The reaction was quenched using 1 uL of a 100 mM TRIZMA base solution.

- 20 The capture compound-protein binding mixture was prepared for mass spectrometry by mixing a 1 uL aliquot of a binding reaction with 1 uL of a 10mg/mL sinapinic acid in 30% aqueous acetonitrile. The sample was deposited as a 500 nL spot on the surface of the mass target plates and air-dried before mass spectrometric analysis. The results of the mass
- 25 spectrometry analysis, which are shown in Figure 20b, demonstrate that addition of selectivity groups to compounds permits alterations in the binding specificity of capture compounds.

#### Cytochrome C

Four different capture compounds (designated HKC 1341, 1343,

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1349, 1365; chemical structure of each compound is listed below the Compound name) were reacted individually with Cytochrome C (accession number: P00006, Figure 20c). The capture experiments were analyzed using MALDI-TOF Mass Spectrometry. Binding was performed in 20 uL sample volumes with a 5 uM Cytochrome C concentrations in 25 mM HEPES buffer solution, pH 7.0. The trityl-based capture compounds were added to the protein solution at a 10 uM concentration. The binding reaction was incubated at room temperature for 30 minutes. The reaction was quenched using 1 uL of a 100 mM TRIZMA base solution. The capture compound-protein binding mixture was prepared for mass spectrometry analysis by mixing a 1 uL aliquot of the binding reaction with 1 uL of 10mg/mL sinapinic acid in 30% aqueous acetonitrile. The sample was deposited as a 500 nL spot on the surface of mass target plates and subsequently air-dried before mass spectrometric analyses. The results of the mass spectrometry analysis, which shown in Figure 20c, demonstrate that addition of selectivity groups to compounds permits alterations in the binding specificity of capture compounds.

**HKC 1343**

One of the exemplary capture compounds (HKC 1343) was incubated with a mixture of three different proteins (Ubiquitin, [P02248], Cytochrome C [P00006] and Lysozyme [P00698]) (see, Figure 20d). The capture experiment was analyzed using MALDI-TOF Mass Spectrometry. The binding reactions were performed in a 20 uL sample volume with all three proteins at 5 uM concentrations in 25 mM HEPES buffer solution pH 7.0. The trityl-based capture compound was added to the protein solution at a 25 uM concentration. The binding reaction was incubated at room temperature for 30 minutes and the reaction quenched using 1 uL of a 100 mM TRIZMA base solution. The capture compound-protein binding mixture was prepared for mass spectrometry by mixing a

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1 uL aliquot of the binding reaction with 1 uL of 10mg/mL sinapinic acid in 30% aqueous acetonitrile. The sample was deposited as a 500 nL spot on the surface of mass target plates and air-dried before mass spectral analysis. The results of the mass spectrometry analysis, which are shown in Figure 20d, demonstrate that a plurality of compounds bound to a single capture agent that is selective can be identified by mass spectrometric analysis.

#### HKC 1365

Another of the exemplary capture compounds (HKC 1365) was incubated with a mixture of three different proteins (Ubiquitin [P02248], Cytochrome C [P00006] and Lysozyme [P00698]; see Figure 20d). The capture experiment was analyzed using MALDI-TOF Mass Spectrometry. The binding reactions were performed in a 20 uL sample volume with all three proteins at 5 uM concentrations in 25 mM HEPES buffer solution pH 7.0. The trityl-based capture compound was added to the protein solution at a 15 uM concentration. The binding reaction was incubated at room temperature for 30 minutes, and quenched using 1 uL of a 100 mM TRIZMA base solution. The capture compound-protein binding mixture was prepared for mass spectrometry by mixing a 1 uL aliquot of the binding reaction with 1 uL of a 10mg/mL sinapinic acid in 30% aqueous acetonitrile. The sample was deposited as a 500 nL spot on the surface of the mass target plates and air-dried before mass spectral analyses. The results of the mass spectrometry analysis, which are shown in Figure 20e, demonstrate that a plurality of compounds bound to a single capture agent that is selective can be identified by mass spectrometric analysis.

#### Reaction of cytochrome C with a non-specific compound

Figure 20f shows mass spectra for a time course reaction of cytochrome C with a non-specific compound (HKC 1341). The

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succinamide reactive group shows specificity and reactivity with the lysines of cytochrome c. The top spectrum shows no modification at time 0, the middle spectrum shows 1-9 modifications resulting from binding of HKC1341 after 30 minutes, and the bottom spectrum shows, after 24 hours, 17 and 18 modifications, which correspond to the number of lysines (18) in cytochrome c.

#### EXAMPLE 10

This example shows the selectivity of the capture compound reacting a mixture of capture compounds and a mixture of proteins

##### 10 Materials:

Reaction buffer: 25 mM HEPES, pH 7.0

Proteins: mixture of ubiquitin, cytochrome c and lysozyme (molar ratio is 1/5/6), the protein stock is made as 5 mg/ml (total proteins) in reaction buffer.

15 Capture compounds: HKC 1343 and HKC 1365, stock solution is 1 mM in acetonitrile.

##### Capturing reaction

A protein dilution (mixture) is prepared in the reaction buffer at the concentration of 0.5, 2.5 and 3  $\mu$ M, for ubiquitin, cytochrome c and lysozyme, respectively. 19.5  $\mu$ l is used for one capturing reaction. Each reaction is started by adding 0.5  $\mu$ l of 1 mM compound stock solution (final 25  $\mu$ M). The reaction mixture is incubated at room temperature for 30 min before the reaction is stopped by the addition of 5 mM TRIZMA.

25 Three different reactions are run. The first two tubes contain HKC 1343 and HKC 1365 individually, and a third one is started by adding compounds HKC 1343 and 1365 (final concentration 25  $\mu$ M for each compound). After the reaction, 1  $\mu$ l of each sample is mixed with equal volume of matrix and subjected to MALDI analysis. Statistic significance of the results is ensured by triplicate each reaction sample.

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Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

## SEQUENCE LISTING

<110> HK Pharmaceuticals, Inc.  
 Köster, Hubert  
 Siddiqi, Suhaib  
 Little, Daniel

<120> Capture Compounds, Collections Thereof  
 And Methods For Analyzing The Proteome And Complex  
 Compositions

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|-----|-----|-----|-----|-----|-----|-----|
| 1   |     | 5   |     | 10  |     | 15  |
| Val | Met | Thr | Ala | Ala | Ser | Cys |
|     |     |     | 20  |     |     |     |
|     |     |     |     |     |     | 25  |

<210> 34  
 <211> 30  
 <212> PRT  
 <213> Homo Sapien

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 34 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn      | Pro | Met | Tyr | Asn | Ala | Val | Ser | Asn | Ala | Asp | Leu | Met | Asp | Phe | Lys |
| 1        |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Asn      | Leu | Leu | Asp | His | Leu | Glu | Glu | Lys | Met | Pro | Leu | Glu | Asp |     |     |
|          |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

<210> 35  
 <211> 18  
 <212> PRT  
 <213> Homo Sapien

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 35 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys      | Asn | Leu | Ala | Val | Ala | Ala | Ala | Ser | His | Ile | Tyr | Gln | Asn | Gln | Phe |
| 1        |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Val      | Gln |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

<210> 36  
 <211> 35  
 <212> PRT  
 <213> Homo Sapien

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 36 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys      | Trp | Lys | Val | Phe | Lys | Lys | Ile | Glu | Lys | Met | Gly | Arg | Asn | Ile | Arg |
| 1        |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Asn      | Gly | Ile | Val | Lys | Ala | Gly | Pro | Ala | Ile | Ala | Val | Leu | Gly | Glu | Ala |
|          |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Lys      | Ala | Leu |     |     |     |     |     |     |     |     |     |     |     |     |     |
|          |     |     | 35  |     |     |     |     |     |     |     |     |     |     |     |     |

<210> 37  
 <211> 16  
 <212> PRT  
 <213> Homo Sapien

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 37 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser      | Gly | Ser | Ala | Lys | Val | Ala | Phe | Ser | Ala | Ile | Arg | Ser | Thr | Asn | His |
| 1        |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

<210> 38  
 <211> 37  
 <212> PRT  
 <213> Homo Sapien

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 38 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala      | Cys | Asp | Thr | Ala | Thr | Cys | Val | Thr | His | Arg | Leu | Ala | Gly | Leu | Leu |
| 1        |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

Ser Arg Ser Gly Gly Val Val Lys Asn Asn Phe Val Pro Thr Asn Val  
                   20                  25                  30  
 Gly Ser Lys Ala Phe  
           35

<210> 39  
 <211> 37  
 <212> PRT  
 <213> Homo Sapien

<400> 39  
 Ala Cys Asn Thr Ala Thr Cys Val Thr His Arg Leu Ala Gly Leu Leu  
   1                  5                  10                  15  
 Ser Arg Ser Gly Gly Met Val Lys Ser Asn Phe Val Pro Thr Asn Val  
                   20                  25                  30  
 Gly Ser Lys Ala Phe  
           35

<210> 40  
 <211> 17  
 <212> PRT  
 <213> Homo Sapien

<400> 40  
 Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys Glu Gly Gly  
   1                  5                  10                  15  
 Leu

<210> 41  
 <211> 29  
 <212> PRT  
 <213> Homo Sapien

<400> 41  
 Gln Glu Gly Ala Pro Pro Gln Gln Ser Ala Arg Arg Asp Arg Met Pro  
   1                  5                  10                  15  
 Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys  
                   20                  25

<210> 42  
 <211> 2  
 <212> PRT  
 <213> Homo Sapien

<400> 42  
 Trp Gly  
   1

<210> 43  
 <211> 30  
 <212> PRT  
 <213> Homo Sapien

<400> 43  
 Ala Cys Tyr Cys Arg Ile Pro Ala Cys Ile Ala Gly Glu Arg Arg Tyr

1 5 10 15  
Gly Thr Cys Ile Tyr Gln Gly Arg Leu Trp Ala Phe Cys Cys  
20 25 30

<210> 44  
<211> 29  
<212> PRT  
<213> Homo Sapien

<400> 44  
Cys Tyr Cys Arg Ile Pro Ala Cys Ile Ala Gly Glu Arg Arg Tyr Gly  
1 5 10 15  
Thr Cys Ile Tyr Gln Gly Arg Leu Trp Ala Phe Cys Cys  
20 25

<210> 45  
<211> 33  
<212> PRT  
<213> Homo Sapien

<400> 45  
Ala Leu Trp Lys Thr Met Leu Lys Lys Leu Gly Thr Met Ala Leu His  
1 5 10 15  
Ala Gly Lys Ala Ala Leu Gly Ala Ala Ala Asp Thr Ile Ser Gln Thr  
20 25 30  
Gln

<210> 46  
<211> 17  
<212> PRT  
<213> Homo Sapien

<400> 46  
Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn  
1 5 10 15  
Gln

<210> 47  
<211> 13  
<212> PRT  
<213> Homo Sapien

<400> 47  
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr  
1 5 10

<210> 48  
<211> 11  
<212> PRT  
<213> Homo Sapien

<220>

<221> AMIDATION

<222> 11

<221> MOD\_RES

<222> 1

<223> Xaa is pyroglutamic acid

<400> 48

Xaa Pro Ser Lys Asp Ala Phe Ile Gly Leu Met  
1 5 10

<210> 49

<211> 4

<212> PRT

<213> Homo Sapien

<400> 49

Tyr Pro Trp Phe  
1

<210> 50

<211> 4

<212> PRT

<213> Homo Sapien

<400> 50

Tyr Pro Phe Phe  
1

<210> 51

<211> 21

<212> PRT

<213> Homo Sapien

<400> 51

Cys Ser Cys Ser Ser Leu Met Asp Lys Glu Cys Val Tyr Phe Cys His  
1 5 10 15  
Leu Asp Ile Ile Trp  
20

<210> 52

<211> 39

<212> PRT

<213> Homo Sapien

<220>

<221> AMIDATION

<222> 39

<400> 52

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser  
20 25 30  
Ser Gly Ala Pro Pro Pro Ser  
35

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<210> 53  
<211> 17  
<212> PRT  
<213> Homo Sapien

<400> 53  
Ala Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly Gly Val  
1 5 10 15  
Arg

<210> 54  
<211> 15  
<212> PRT  
<213> Homo Sapien

<400> 54  
Asx Gln Gly Val Asn Asp Asn Glu Glu Gly Phe Phe Ser Ala Arg  
1 5 10 15

<210> 55  
<211> 8  
<212> PRT  
<213> Homo Sapien

<400> 55  
Glu Ile Leu Asp Val Pro Ser Thr  
1 5

<210> 56  
<211> 4  
<212> PRT  
<213> Homo Sapien

<400> 56  
Phe Met Arg Phe  
1

<210> 57  
<211> 30  
<212> PRT  
<213> Homo Sapien

<400> 57  
Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Pro His Ala Val  
1 5 10 15  
Gly Asn His Arg Ser Phe Ser Asp Lys Asn Gly Leu Thr Ser  
20 25 30

<210> 58  
<211> 20  
<212> PRT  
<213> Homo Sapien

<220>

<221> AMIDATION  
<222> 20

<400> 58  
Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Pro Gln Gln Phe  
1 5 10 15  
Phe Gly Leu Met  
20

<210> 59  
<211> 5  
<212> PRT  
<213> Homo Sapien

<400> 59  
Arg Leu Arg Phe Asp  
1 5

<210> 60  
<211> 17  
<212> PRT  
<213> Homo Sapien

<400> 60  
Glu Gly Pro Trp Leu Glu Glu Glu Glu Glu Ala Tyr Gly Trp Met Asp  
1 5 10 15  
Phe

<210> 61  
<211> 27  
<212> PRT  
<213> Homo Sapien

<400> 61  
Val Pro Leu Pro Ala Gly Gly Gly Thr Val Leu Thr Lys Met Tyr Pro  
1 5 10 15  
Arg Gly Asn His Trp Ala Val Gly His Leu Met  
20 25

<210> 62  
<211> 28  
<212> PRT  
<213> Homo Sapien

<400> 62  
Gly Ser Ser Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys  
1 5 10 15  
Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg  
20 25

<210> 63  
<211> 42  
<212> PRT  
<213> Homo Sapien

&lt;400&gt; 63

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Ala | Glu | Gly | Thr | Phe | Ile | Ser | Asp | Tyr | Ser | Ile | Ala | Met | Asp | Lys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ile | His | Gln | Gln | Asp | Phe | Val | Asn | Trp | Leu | Leu | Ala | Gln | Lys | Gly | Lys |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Lys | Asn | Asp | Trp | Lys | His | Asn | Ile | Thr | Gln |     |     |     |     |     |     |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     |     |     |     |     |

&lt;210&gt; 64

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 64

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Ser | Gln | Gly | Thr | Phe | Thr | Ser | Asp | Tyr | Ser | Lys | Tyr | Leu | Asp | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Arg | Arg | Ala | Gln | Asp | Phe | Val | Asp | Trp | Leu | Met | Asn | Thr |     |     |     |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     |     |     |     |

&lt;210&gt; 65

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 65

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Arg | Phe | Ala | Cys | Asp | Pro | Asp | Gly | Tyr | Asp | Asn | Tyr | Phe | His | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Val | Pro | Gly | Gly |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 66

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 66

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Gly | Ser | Trp | Cys | Gly | Leu | Met | His | Tyr | Asp | Asn | Ala | Trp | Leu | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Asn | Thr | Gln | Gly |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 67

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 67

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ser | Lys | Trp | Cys | Arg | Asp | Gly | Tyr | Tyr | Ala | Asn | Tyr | Pro | Gln | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Trp | Thr | Gln | Gly |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 68

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 68

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ser | Thr | Leu | Cys | Trp | Phe | Glu | Gly | Tyr | Asp | Asn | Thr | Phe | Pro | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Lys | Tyr | Phe | Arg |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 69

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 69

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Gln | Glu | Cys | Lys | Tyr | Leu | Tyr | Tyr | Asp | Asn | Asp | Tyr | Leu | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Lys | Asp | Asp | Gly |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 70

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 70

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Leu | Arg | Arg | Cys | Leu | Tyr | Gly | Pro | Tyr | Asp | Asn | Ala | Trp | Val | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Asn | Ile | His | Glu |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 71

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 71

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Phe | Trp | Cys | Thr | Tyr | Glu | Asp | Tyr | Ala | Asn | Glu | Trp | Pro | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Pro | Gly | Tyr | Ser |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 72

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 72

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Cys | Ala | Val | Cys | Asn | Glu | Glu | Leu | Tyr | Glu | Asn | Cys | Gly | Gly | Cys |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ser | Cys | Gly | Lys |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |     |

&lt;210&gt; 73

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;400&gt; 73

Arg Thr Ser Pro Cys Gly Tyr Ile Gly Tyr Asp Asn Ile Phe Glu Cys  
1 5 10 15  
Thr Tyr Leu Gly  
20

<210> 74  
<211> 20  
<212> PRT  
<213> Homo Sapien

<400> 74  
Thr Gly Glu Trp Cys Ala Gln Ser Val Tyr Ala Asn Tyr Asp Asn Cys  
1 5 10 15  
Lys Ser Ala Trp  
20

<210> 75  
<211> 20  
<212> PRT  
<213> Homo Sapien

<400> 75  
Asn Val Ser Arg Cys Thr Tyr Ile His Tyr Asp Asn Trp Ser Leu Cys  
1 5 10 15  
Gly Val Glu Val  
20

<210> 76  
<211> 20  
<212> PRT  
<213> Homo Sapien

<400> 76  
Gly Val Ser Asn Cys Val Phe Trp Gly Tyr Ala Asn Asp Trp Leu Cys  
1 5 10 15  
Ser Asp Tyr Ser  
20

<210> 77  
<211> 44  
<212> PRT  
<213> Homo Sapien

<400> 77  
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln  
1 5 10 15  
Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly  
20 25 30  
Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu  
35 40

<210> 78  
<211> 15  
<212> PRT  
<213> Homo Sapien

```
<400> 82
Asp Ser His Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu
 1          5          10          15
Lys His His Ser His Arg Gly Tyr
```

20

<210> 83  
 <211> 4  
 <212> PRT  
 <213> Homo Sapien

<220>

<221> ACETYLATION  
 <222> 1

<221> MOD\_RES  
 <222> 4  
 <223> Xaa is Aspartic acid-fluroacetylmethylketone

<400> 83  
 Tyr Val Ala Xaa  
 1

<210> 84  
 <211> 6  
 <212> PRT  
 <213> Homo Sapien

<400> 84  
 Val Glu Pro Ile Pro Tyr  
 1 5

<210> 85  
 <211> 21  
 <212> PRT  
 <213> Homo Sapien

<400> 85  
 Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu  
 1 5 10 15  
 Glu Asn Tyr Cys Asn  
 20

<210> 86  
 <211> 30  
 <212> PRT  
 <213> Homo Sapien

<400> 86  
 Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr  
 1 5 10 15  
 Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr  
 20 25 30

<210> 87  
 <211> 51  
 <212> PRT  
 <213> Homo Sapien

<400> 87  
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu  
1 5 10 15  
Glu Asn Tyr Cys Asn Phe Val Asn Gln His Leu Cys Gly Ser His Leu  
20 25 30  
Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr  
35 40 45  
Pro Lys Thr  
50

<210> 88  
<211> 9  
<212> PRT  
<213> Homo Sapien

<400> 88  
Ile Ala Arg Arg His Pro Tyr Phe Leu  
1 5

<210> 89  
<211> 5  
<212> PRT  
<213> Homo Sapien

<400> 89  
Tyr Gly Gly Phe Leu  
1 5

<210> 90  
<211> 9  
<212> PRT  
<213> Homo Sapien

<220>

<221> AMIDATION  
<222> 9

<221> MOD\_RES  
<222> 1  
<223> Xaa is pyroglutamic acid

<400> 90  
Xaa Gln Trp Ala Val Gly His Phe Met  
1 5

<210> 91  
<211> 14  
<212> PRT  
<213> Homo Sapien

<400> 91  
Arg Thr Lys Arg Ser Gly Ser Val Tyr Glu Pro Leu Lys Ile  
1 5 10

<210> 92

<211> 5  
<212> PRT  
<213> Homo Sapien

<400> 92  
Tyr Gly Gly Phe Met  
1 5

<210> 93  
<211> 9  
<212> PRT  
<213> Homo Sapien

<220>

<221> AMIDATION  
<222> 9

<400> 93  
Tyr Gly Gly Gly Phe Met Arg Arg Val  
1 5

<210> 94  
<211> 22  
<212> PRT  
<213> Homo Sapien

<400> 94  
Phe Val Pro Ile Phe Thr Tyr Gly Glu Leu Gln Arg Met Gln Glu Lys  
1 5 10 15  
Glu Arg Asn Lys Gly Gln  
20

<210> 95  
<211> 9  
<212> PRT  
<213> Homo Sapien

<400> 95  
Pro Met Ser Met Leu Arg Leu Asn His  
1 5

<210> 96  
<211> 13  
<212> PRT  
<213> Homo Sapien

<400> 96  
Ile Pro Lys Lys Arg Ala Ala Arg Ala Thr Ser Asn His  
1 5 10

<210> 97  
<211> 6  
<212> PRT  
<213> Homo Sapien

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&lt;400&gt; 97

Gly Ala Val Ser Thr Ala  
1 5

&lt;210&gt; 98

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 10

&lt;400&gt; 98

His Lys Thr Asp Ser Phe Val Gly Leu Met  
1 5 10

&lt;210&gt; 99

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 10

&lt;400&gt; 99

Asp Met His Asp Phe Phe Val Gly Leu Met  
1 5 10

&lt;210&gt; 100

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 10

&lt;400&gt; 100

Gly Asn Leu Trp Ala Thr Gly His Phe Met  
1 5 10

&lt;210&gt; 101

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 36

&lt;400&gt; 101

Tyr Pro Ser Lys Pro Asp Asn Pro Gly Glu Asp Ala Pro Ala Glu Asp  
 1 5 10 15  
 Met Ala Arg Tyr Tyr Ser Ala Lys Arg His Tyr Ile Asn Leu Ile Thr  
 20 25 30  
 Arg Gln Arg Tyr  
 35

<210> 102  
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 <212> PRT  
 <213> Homo Sapien

<220>

<221> MOD\_RES  
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 <223> Xaa is pyroglutamic acid

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 1 5 10

<210> 103  
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 Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Leu Ala Asn  
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 Gln

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 1 5 10 15  
 Glu Val Pro Glu Met Glu Lys Arg Tyr Gly Gly Phe Met Arg Phe  
 20 25 30

<210> 105  
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 <213> Homo Sapien

<400> 105  
 Glu Gln Lys Gln Leu Gln  
 1 5

<210> 106  
 <211> 33  
 <212> PRT

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&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 33

&lt;221&gt; MOD\_RES

&lt;222&gt; 1

&lt;223&gt; Xaa is pyroglutamic acid

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 1 5 10 15  
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 20 25 30  
 Leu

&lt;210&gt; 107

&lt;211&gt; 28

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

&lt;220&gt;

&lt;221&gt; AMIDATION

&lt;222&gt; 28

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 1 5 10 15  
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 20 25

&lt;210&gt; 108

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&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

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 1 5 10 15  
 Glu Pro Arg Arg Glu Val Cys Glu Leu Asn Pro Asp Cys Asp Glu Leu  
 20 25 30  
 Ala Asp His Ile Gly Phe Gln Glu Ala Tyr Arg Arg Phe Tyr Gly Pro  
 35 40 45  
 Val

&lt;210&gt; 109

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Homo Sapien

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 20 25 30  
 Arg Pro Arg Tyr Asn His  
 35

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<400> 114  
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 <211> 36

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<212> PRT  
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<220>

<221> AMIDATION  
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20 25 30  
Arg Gln Arg Tyr  
35

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<400> 116  
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<210> 117  
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<210> 121  
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 1 5

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 Ala Arg Leu Gln Arg Leu Leu Gln Gly Leu Val  
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Cys Leu Lys

<210> 126  
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<400> 126  
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1 5 10 15  
Cys Leu Lys

<210> 127  
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Pro Gln Cys Gly Lys Cys Arg Ile Cys Lys Asn Pro Glu Ser Asn Tyr  
1 5 10 15  
Cys Leu Lys

<210> 128  
<211> 19  
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<400> 128  
Pro Leu Cys Arg Lys Cys Lys Phe Cys Leu Ser Pro Leu Thr Asn Leu  
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Cys Gly Lys

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Gln Lys

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Pro Leu Ala Arg Thr Leu Ser Val Ala Gly Leu Pro Gly Lys Lys  
1 5 10 15

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1 5 10 15  
Thr Asp

<210> 133  
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<213> Homo Sapien

<400> 133  
Thr Phe Gly Ser Gly Glu Ala Asp Cys Gly Leu Arg Pro Leu Phe Glu  
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Lys Lys Ser Leu Glu Asp Lys Thr Glu Arg Glu Leu Leu Glu Ser Tyr

Ile Asp Gly Arg 20 25 30  
35

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<400> 134  
Arg Lys Asp Val Tyr  
1 5

<210> 135  
<211> 9  
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<400> 135  
Gln Ala Lys Ser Gln Gly Gly Ser Asn  
1 5

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<220>

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<400> 137  
Thr Lys Pro Arg  
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<211> 11  
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<222> 1

<223> Xaa is pyroglutamic acid

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1 5 10

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<400> 139

Asp Leu Trp Gln Lys  
1 5

<210> 140

<211> 40

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<213> Homo Sapien

<400> 140

Asp Asn Pro Ser Leu Ser Ile Asp Leu Thr Phe His Leu Leu Arg Thr  
1 5 10 15  
Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln  
20 25 30  
Asn Arg Ile Ile Phe Asp Ser Val  
35 40

<210> 141

<211> 16

<212> PRT

<213> Homo Sapien

<400> 141

Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu  
1 5 10 15

<210> 142

<211> 27

<212> PRT

<213> Homo Sapien

<400> 142

Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly Ser  
1 5 10 15  
Met Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr  
20 25

<210> 143

<211> 9

<212> PRT

<213> Homo Sapien

<400> 143

Cys Tyr Phe Gln Asn Cys Pro Arg Gly  
1 5

<210> 144  
<211> 9  
<212> PRT  
<213> Homo Sapien

<400> 144  
Cys Tyr Ile Gln Asn Cys Pro Arg Gly  
1 5

<210> 145  
<211> 28  
<212> PRT  
<213> Homo Sapien

<400> 145  
His Ser Asp Ala Val Phe Thr Asp Asn Tyr Thr Arg Leu Arg Lys Gln  
1 5 10 15  
Met Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Asn  
20 25

<210> 146  
<211> 25  
<212> PRT  
<213> Homo Sapien

<400> 146  
Met Leu Thr Lys Phe Glu Thr Lys Ser Ala Arg Val Lys Gly Leu Ser  
1 5 10 15  
Phe His Pro Lys Arg Pro Trp Ile Leu  
20 25

<210> 147  
<211> 3  
<212> PRT  
<213> Homo Sapien

<220>

<221> UNSURE  
<222> 2  
<223> Xaa is a variable

<400> 147  
Tyr Xaa Asn  
1

<210> 148  
<211> 9  
<212> PRT  
<213> Homo Sapien

<400> 148  
Phe Gln Phe His Phe His Trp Gly Ser

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1 5

<210> 149  
<211> 11  
<212> PRT  
<213> Homo Sapien

<400> 149  
Ile Ile Ile Gln Phe His Phe His Trp Gly Ser  
1 5 10

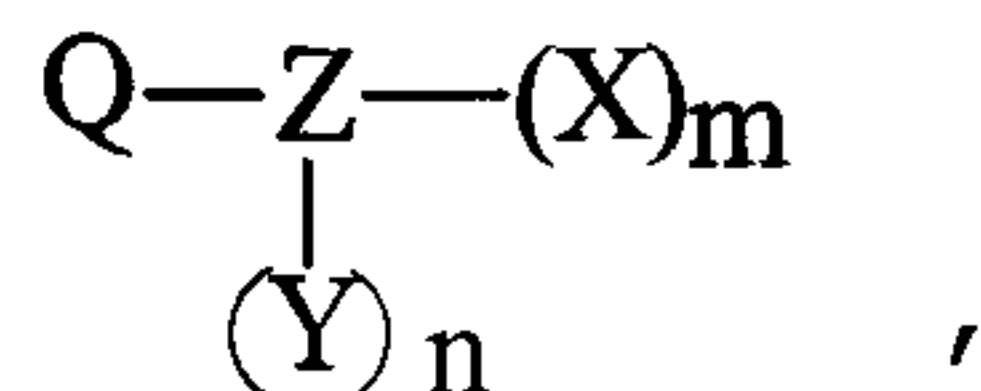
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CLAIMS:

1. A collection of capture compounds, comprising a plurality of different capture compounds, wherein each capture compound in the collection has the formula:

5



wherein:

m is an integer that is 1 to 100;

n is an integer from 1 to 100;

10 the collection includes at least ten different capture compounds;

moiety X is selected to covalently bind to biomolecules;

15 Y modulates one or more of the affinity, steric properties and electronic properties of the capture compound thereby increasing selectivity of the binding by X such that the capture compound binds to fewer biomolecules when the selectivity moiety Y is present than in its absence;

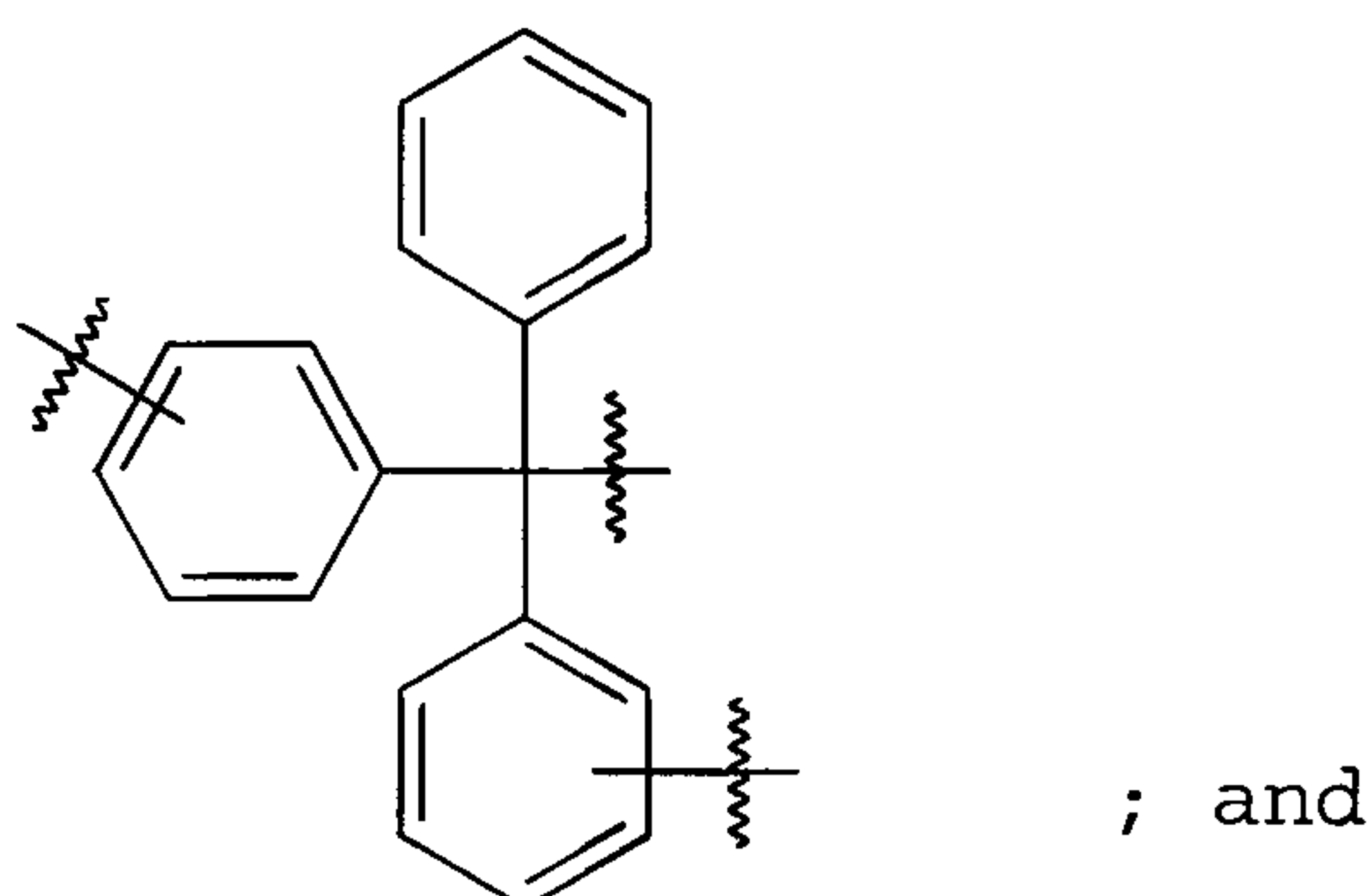
20 Q permits separation or immobilization of capture compounds in the collection; and

Z is a trityl derivative for presenting functional moieties X, Y and Q;

Z has the formula:

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5 the capture compounds are provided as a mixture in a composition or as an addressable array where each locus of the array contains the same compound or a mixture thereof.

2. The collection of claim 1, wherein Q permits separation by arraying of the capture compounds on a solid support by binding to the surface or a molecule thereon.

10

3. The collection of claim 1 or claim 2 that includes at least 100 different capture compounds.

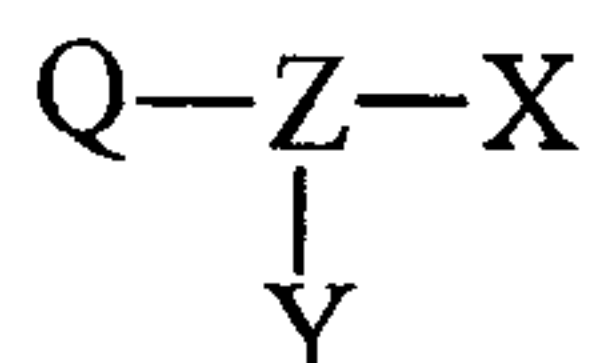
4. The collection of claim 1 or claim 2 that includes at least fifty different capture compounds.

15 5. The collection of claims 1-4, wherein Q is selected to immobilize capture compounds at addressable loci on a solid support.

6. A solid support, comprising the collection of compounds of any of claims 1-5, wherein capture compounds of the collection are arrayed at addressable loci on the support.

20

7. The collection of any of claims 1-5, wherein component capture compounds have the formula:



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8. The collection of claim 1, wherein:

Q is selected from among biotin, 6-His, BODIPY, an oligonucleotide, an adhesive peptide, a peptide nucleic acid (PNA) and a peptide.

9. The collection of claim 1, wherein

Q is an oligonucleotide or oligonucleotide analog that includes a single-stranded portion of sufficient length "j" to form a stable hybrid with a base-complementary single stranded nucleic acid molecule or analog.

10. The collection of any of claims 1-5 and 7-9, wherein Q has formula  $N^1_s-B_i-N^2_u$ , wherein:

$N^1$ , B and  $N^2$  are oligonucleotides or oligonucleotide analogs comprising s, i and u members, respectively;

B is a region of sequence permutations that contains at least two bases; and the sum of s, i and u is at least 5.

11. The collection of claim 10, wherein the sum of s, i and u is about 5 up to about 50.

12. The collection of claim 10 or claim 11, wherein each member of  $N^1$ , B and  $N^2$  is independently selected from among monomer building blocks of deoxyribonucleic acid, ribonucleic acid, peptide nucleic acid (PNA) and analogs thereof.

13. The collection of any of claims 1- 5 and 7-12, wherein Z is a photocleavable, acid cleavable, alkaline cleavable, oxidatively cleavable, or reductively cleavable group.

14. The collection of any of claims 1-5 and 7-13, wherein Z is cleavable prior to or during analysis of the biomolecule.

15. The collection of any of claims 1-5 and 7-14, wherein X is selected from the group consisting of an active ester, an active halo moiety, an amino acid side chain-specific functional group and a metal complex.

16. The collection of any of claims 1-5 and 7-15, wherein X is an  $\alpha$ -halo ether, an  $\alpha$ -halo carbonyl group, maleimido, a gold complex, a mercury complex, an epoxide or an isothiocyanate.

17. The collection of any of claims 1-5 and 7-15, wherein X is  $-C(=O)O-Ph-pNO_2$ ,  $-C(=O)O-C_6F_5$ ,  $-C(=O)-O-(N-succinimidyl)$ ,  $-OCH_2-I$ ,  $-OCH_2-Br$ ,  $-OCH_2-Cl$ ,  $-C(O)CH_2I$ ,  $-C(O)CH_2Br$  or  $-C(O)CH_2Cl$ .

18. The collection of any of claims 1-5 and 7-17, wherein member compounds of the collection comprise a mass modifying tag linked to Z.

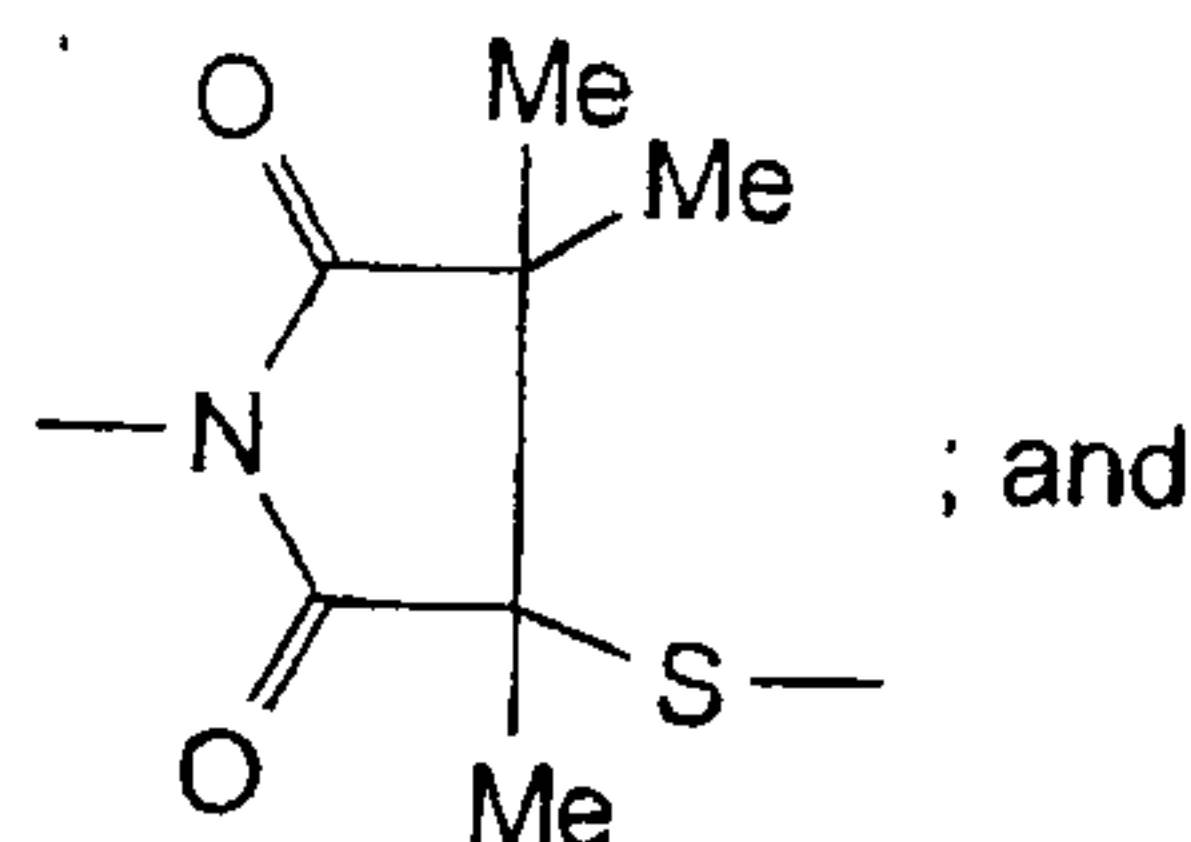
19. The collection of claim 18, wherein the mass modifying tag is selected from (i)-(vii) as follows:

(i) the mass modifying tag has the formula  $-X^1R^{10}-$ , wherein:

$X^1$  is a divalent group selected from  $-O-$ ,  $-O-C(O)-(CH_2)_y-C(O)O-$ ,  $-NH-C(O)-$ ,  $-C(O)-NH-$ ,  $-NH-C(O)-(CH_2)_y-C(O)O-$ ,  $-NH-C(S)-NH-$ ,  $-O-P(O-alkyl)-O-$ ,  $-O-SO_2-O-$ ,  $-O-C(O)-CH_2-S-$ ,  $-S-$ ,  $-NH-$  and

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$R^{10}$  is a divalent group selected from  $-(CH_2CH_2O)_z-CH_2CH_2O-$ ,  $-(CH_2CH_2O)_z-CH_2CH_2O$ -alkylene, alkylene, alkenylene, alkynylene, arylene, heteroarylene,  $-(CH_2)_z-CH_2-O-$ ,  $-(CH_2)_z-CH_2-O$ -alkylene,  $-(CH_2CH_2NH)_z-CH_2CH_2NH-$ ,  $-CH_2-CH(OH)-CH_2O-$ ,  $-Si(R^{12})(R^{13})-$ ,  $-CHF-$  and  $-CF_2-$ ; where  $y$  is an integer from 1 to 20;  $z$  is an integer from 0 to 200;  $R^{11}$  is the side chain of a naturally occurring  $\alpha$ -amino acid; and  $R^{12}$  and  $R^{13}$  are each independently selected from alkyl, aryl and aralkyl;

- (ii) the mass modifying tag is  $-S-S-$ ;
- (iii) the mass modifying tag is  $-S-$ ;
- (iv) the mass modifying tag is  $-(NH-(CH_2)_y-NH-C(O)-(CH_2)_y-C(O))_z-NH-(CH_2)_y-NH-C(O)-(CH_2)_y-C(O)O-$ , where  $y$  and  $z$  are selected as in (i);
- (v) the mass modifying tag is  $-(NH-(CH_2)_y-C(O))_z-NH-(CH_2)_y-C(O)O-$ , where  $y$  and  $z$  are selected as in (i);
- (vi) the mass modifying tag is  $-(NH-CH(R^{11})-C(O))_z-NH-CH(R^{11})-C(O)O-$ , where  $R^{11}$  and  $z$  are selected as in (i); or
- (vii) the mass modifying tag is  $-(O-(CH_2)_y-C(O))_z-NH-(CH_2)_y-C(O)O-$ , where  $y$  and  $z$  are selected as in (i).

20. The collection of any of claims 1-5 and 7-19, further comprising biomolecules covalently bound to the X moiety of one or more capture compounds in the collection.

21. The collection of claim 20, wherein the biomolecules comprise proteins.

22. The collection of any of claims 1-5 and 7-21, wherein the capture compounds further comprise a solubility group W that influences the solubility properties of the capture compound.

23. The collection of any of claims 1-5, 7-14, and 18-22, wherein the selectivity function Y is selected from those set forth in Figures 17a-17hhhh and/or the reactivity function X is selected from those set forth in Figures 16a-b.

24. The collection of claim 1, wherein Y is selected from among a receptor ligand, an enzyme substrate, an enzyme inhibitor, a transition state analog, a glue peptide, a peptide mimetic and a statin.

25. The collection of claim 1, wherein Y is a peptide ligand selected from among the peptide ligands set forth herein as SEQ ID NO:1 through SEQ ID NO:149.

26. The collection of claim 1, wherein X is a photoreactive group.

27. The collection of claim 1, wherein each capture compound in the collection comprises the same X moiety but differs in the Y moiety.

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28. The collection of claim 1, wherein the biomolecules comprise proteins.
29. A composition, comprising a collection of any of claims 1-5 and 10-19, wherein each Q is an oligonucleotide, and the capture compounds are hybridized to a plurality of oligonucleotides or analogs thereof that are complementary to each Q.
30. The composition of claim 29, wherein the oligonucleotides or analog thereof that are complementary to Q are immobilized on a solid support as an array.
31. A method for analysis of biomolecules, comprising:
  - a) contacting a sample composition comprising a biomolecule with a collection of capture compounds of any of claims 1-5 and 7-28, to form capture compound-biomolecule complexes; and
  - b) identifying or detecting bound biomolecules.
32. The method of claim 31, wherein capture compounds in the collection further comprise a solubility group W that influences the solubility properties of the capture compound.
33. The method of claim 31 or claim 32, wherein the biomolecules are proteins.
34. The method of any of claims 31-33, wherein:
 

the capture compounds are in an addressable array; and

each locus in the array contains a different capture compound.
35. The method of any of claims 31-33, wherein identification comprises mass spectrometric analysis of the bound biomolecules.
36. The method of any of claims 31-35, further comprising:
 

chemical or enzymatic treatment of the biomolecule-capture compound complexes to remove or cleave portions thereof prior to mass spectrometric analysis.
37. The method of any of claims 31-36, wherein the biomolecules bound to the capture compounds are treated with a protease prior to mass spectrometric analysis.
38. The method of any of claims 31-37, wherein each capture compound in the collection comprises the same X moiety but differs in the Y moiety.
39. The method of any of claims 31-38, wherein each capture compound in the collection comprises different Y and Q moieties.
40. The method of any of claims 31-37 and 39, wherein each capture compound in the collection comprises different X and Y moieties.
41. The method of any of claims 31-40, wherein:
 

each sorting function Q is an oligonucleotide that includes a single-stranded portion of length "j" for hybridizing to a complementary oligonucleotide, wherein j is at least 5 bases; and the method further comprises:

hybridizing the capture compounds to a set of complementary oligonucleotides, which attached to a solid support, wherein the hybridizing is effected before or after the contacting step, thereby immobilizing the capture compounds or capture compound biomolecule complexes on the solid support.
42. The method of claim 41, wherein the single-stranded oligonucleotides or oligonucleotide analogs that are complementary to the Q moiety on the capture compounds are immobilized on a solid support.

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43. The method of claim 41 or claim 42, wherein the single stranded oligonucleotides or oligonucleotide analogs that are complementary to the Q moiety comprise an addressable array.

44. The method of any of claims 31-43, wherein the collection comprises at least 10, 50, 100, 500 or 1000 different capture compounds.

45. The method of any of claims 31-44, wherein the contacting step is performed in an aqueous medium and the biomolecules are hydrophilic.

46. The method of any of claims 31-44, wherein the contacting step is performed in a hydrophobic medium and the biomolecules are hydrophobic.

47. The method of any of claim 31-46, wherein identification or detection is effected by mass spectrometric analysis of the biomolecules bound to capture compounds.

48. The method of claim 47, wherein the mass spectrometric format is matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

49. The method of any of claims 31-48, wherein:  
the sorting function Q permits arraying the compounds on a solid support; and  
the method further comprises arraying the capture compounds on a solid support before, during or after the contacting step, wherein:  
the resulting biomolecule-capture compound complexes are at discrete spots on a solid support.

50. The method of claim 49, wherein mass spectrometric analysis of the bound biomolecules comprises:

- (i) addition of matrix to the biomolecule-capture compound complexes; and
- (ii) spot-by-spot matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

51. The method of any of claims 35-50, wherein mass spectrometric analysis of the bound biomolecules, comprises:

- (i) addition of matrix to the biomolecules bound to capture compounds; and
- (ii) matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry of biomolecules bound to capture compounds.

52. The method of any of claims 31-51, wherein the sample composition comprising a biomolecule is a cell lysate.

53. The method of claim 52, wherein the cells from which the lysate is produced are synchronized or frozen in a metabolic state.

54. The method of any of claims 31-53, wherein the analysis is orthogonal time of flight (O-TOF) mass spectrometry.

55. The method of any of claims 31-53, wherein the analysis is electrospray (ES) mass spectrometry.

56. The method of claim 31, wherein:  
the biomolecule is a protein; and  
the contacting step is performed under conditions whereby the interactions of the moiety Y of the capture compounds with protein in the composition are kinetically controlled.

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57. A method for separating protein conformers, comprising:  
contacting a sample composition comprising a biomolecule with a collection of capture compounds of any of claims 1-5 and 7-28;  
separating the members of the collection; and  
identifying the bound proteins from the mixture, whereby each conformer has different binding specificity for members of the collection.
58. The method of claim 57, wherein identification is effected by mass spectrometry.
59. The method of claim 57 or claim 58, wherein at least one conformer is associated with a disease.
60. A method for reducing diversity of a complex mixture of biomolecules, comprising:  
contacting the mixture with a collection of capture compounds of any of claims 1-5 and 7-28, to form capture compounds with bound biomolecules; and after contacting,  
separating capture compounds with bound biomolecules.
61. A method for identification of phenotype-specific biomolecules, comprising:  
sorting cells from a single subject according to a predetermined phenotype to produce at least two separated sets of cells;  
contacting mixtures of biomolecules from each set of sorted cells with a collection of capture compounds of any of claims 1-5 and 7-28; and  
comparing the binding patterns of biomolecules from each set bound to capture compounds to identify biomolecules that differ for each set, thereby identifying phenotype-specific biomolecules.
62. The method of claim 61, wherein the cells are synchronized or frozen in a metabolic state before sorting and/or after sorting.
63. The method of claim 61 or claim 62, wherein the biomolecules comprise proteins.
64. The method of any of claims 61-63, wherein the bound biomolecules are identified by mass spectrometry.
65. The method of any of claims 61-64, wherein the phenotypes are diseased and healthy phenotypes.
66. The method of claim 65, wherein the diseased phenotype is a tumor and the healthy phenotype is non-tumor.
67. A system for analysis of mixtures of biomolecules, comprising:  
a collection of claim 1;  
a computer programmed with instructions for controlling and directing analysis of biomolecules using the collections;  
a mass spectrometer; and

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software for analysis of data produced by the mass spectrometer.

68. The system of claim 67 that is an automated system.

69. The system of claim 67 or claim 68, further comprising a liquid chromatographic device.

70. A method of analysis of biomolecules, comprising:

(a) reacting a first mixture of biomolecules with the collection of capture compounds of claim 1 to form a mixture of capture compound-bound biomolecules, wherein the capture compounds comprising the first mixture include a first mass modifying tag;

(b) reacting a second mixture of biomolecules with the collection of capture compounds of any of claim 1-5 and 7-28, to form a second mixture of capture compound-bound biomolecules, wherein the compounds comprising the second mixture of capture compound-bound molecules have either (i) no mass modifying tag; or (ii) a second mass modifying tag;

(c) pooling the products of steps (a) and (b) to produce a mixture thereof;

(d) sorting the differently tagged capture compound-bound biomolecules in the mixture of step c) according to Q moiety to produce an array of sorted complexes; and

(e) analyzing the complexes at each locus.

71. A method for analyzing biomolecule interactions, comprising:

a) contacting a mixture of biomolecules with a collection of capture compounds of any of claims 1-5 and 7-28, to form capture compound-bound biomolecules, wherein the complexes are stable to matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry conditions;

b) contacting the capture compound-bound biomolecules with a mixture containing compounds selected from the group consisting of mixtures of biomolecules and small molecules, wherein compounds in the mixture bind to biomolecules bound to capture compounds;

c) before or after step b) immobilizing the capture compounds on a solid support via the Q group of each set of capture compounds;

d) analyzing the bound compounds by mass spectrometry.

72. The method of claim 71, wherein the small molecules are candidate drugs and are selected from the group consisting of small organic molecules, peptides, peptide mimetics, antibodies, fragments of antibodies and recombinant or synthetic antibodies and fragments thereof; and

the method is a method for identifying candidate drugs that bind to biomolecules.

73. The method of claim 71 or claim 72, wherein the capture compound-biomolecule complexes are contacted in step a) with a mixture of biomolecules to identify components of biomolecule complexes or biochemical pathways.

74. The method of any of claim 71-73, wherein the biomolecules are proteins.

75. The method of claim 71, wherein:

the mixtures of biomolecules comprise protein mixtures; and

the method is for determining biomolecule-protein interactions.

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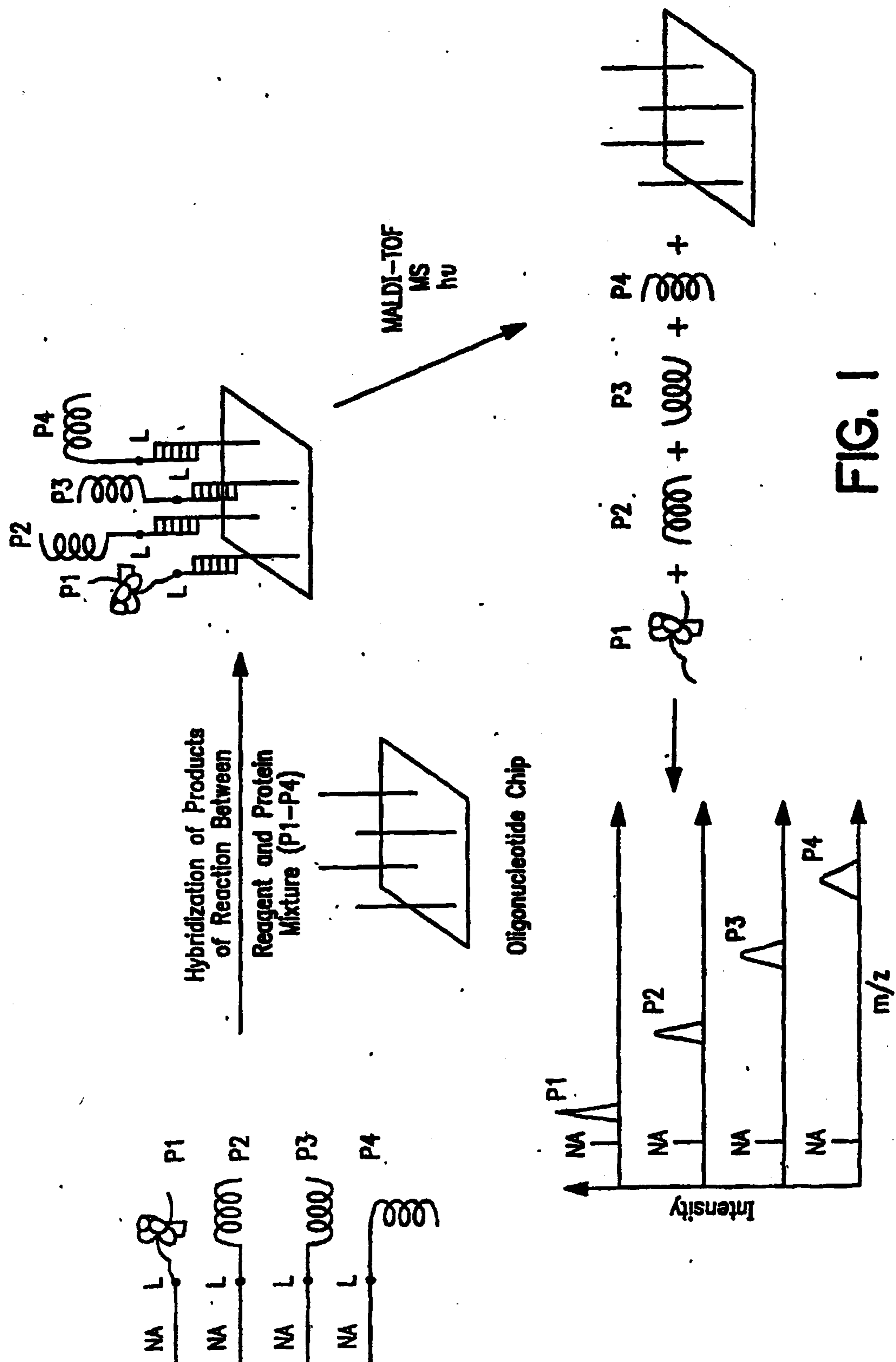


FIG. 1

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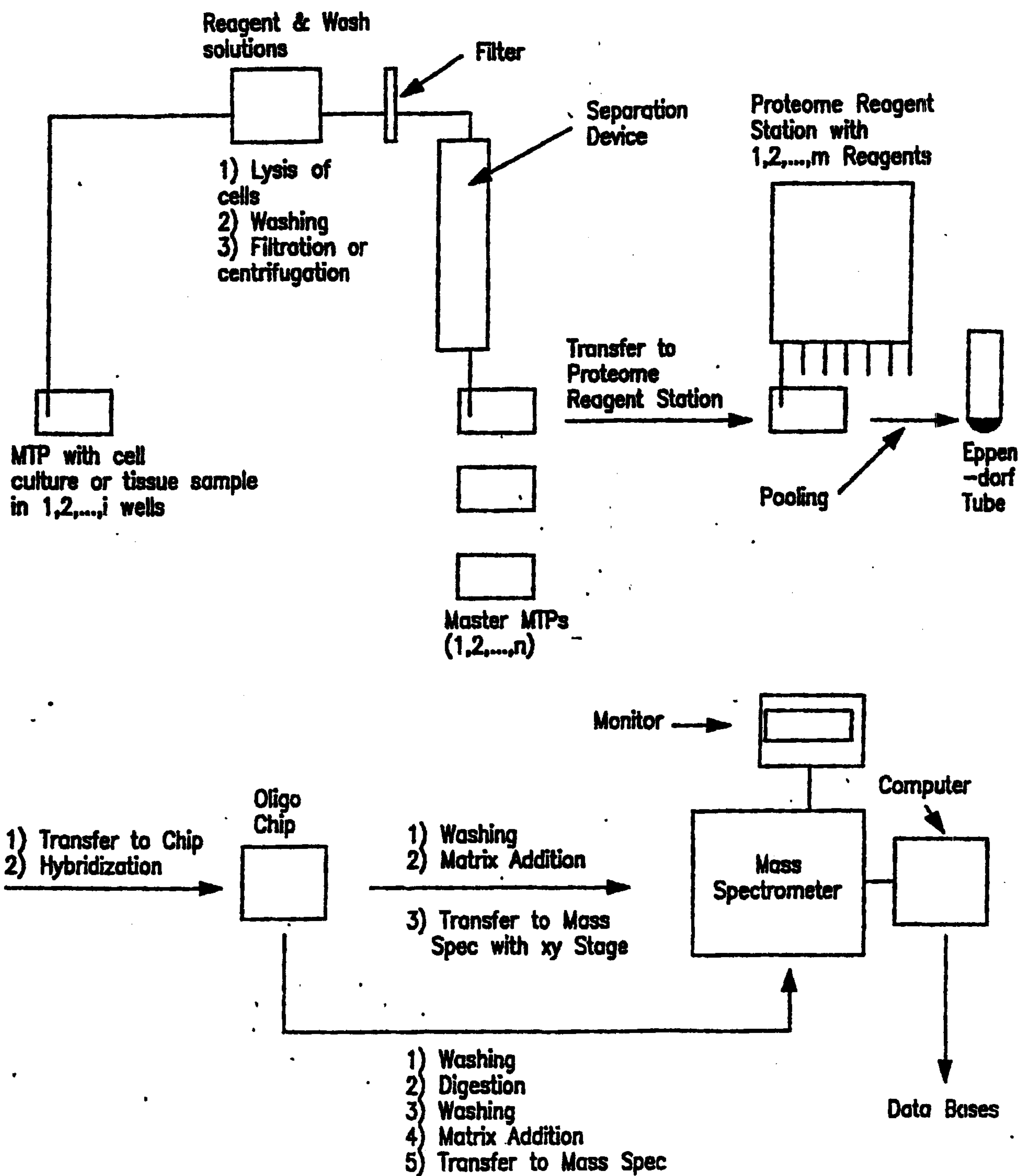


FIG. 2

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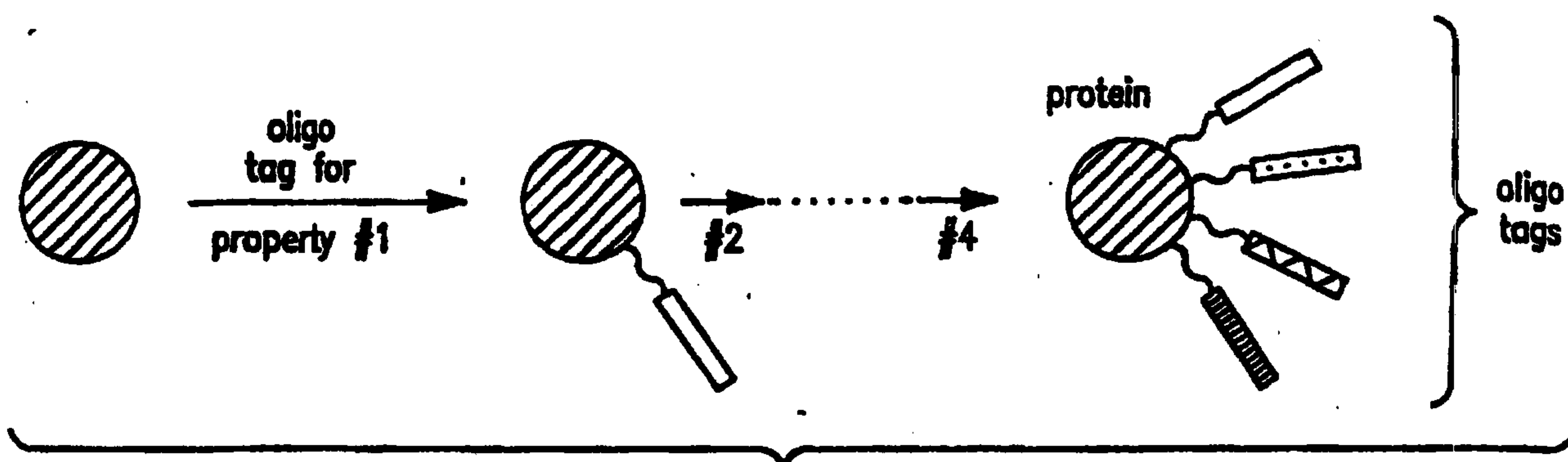


FIG. 3

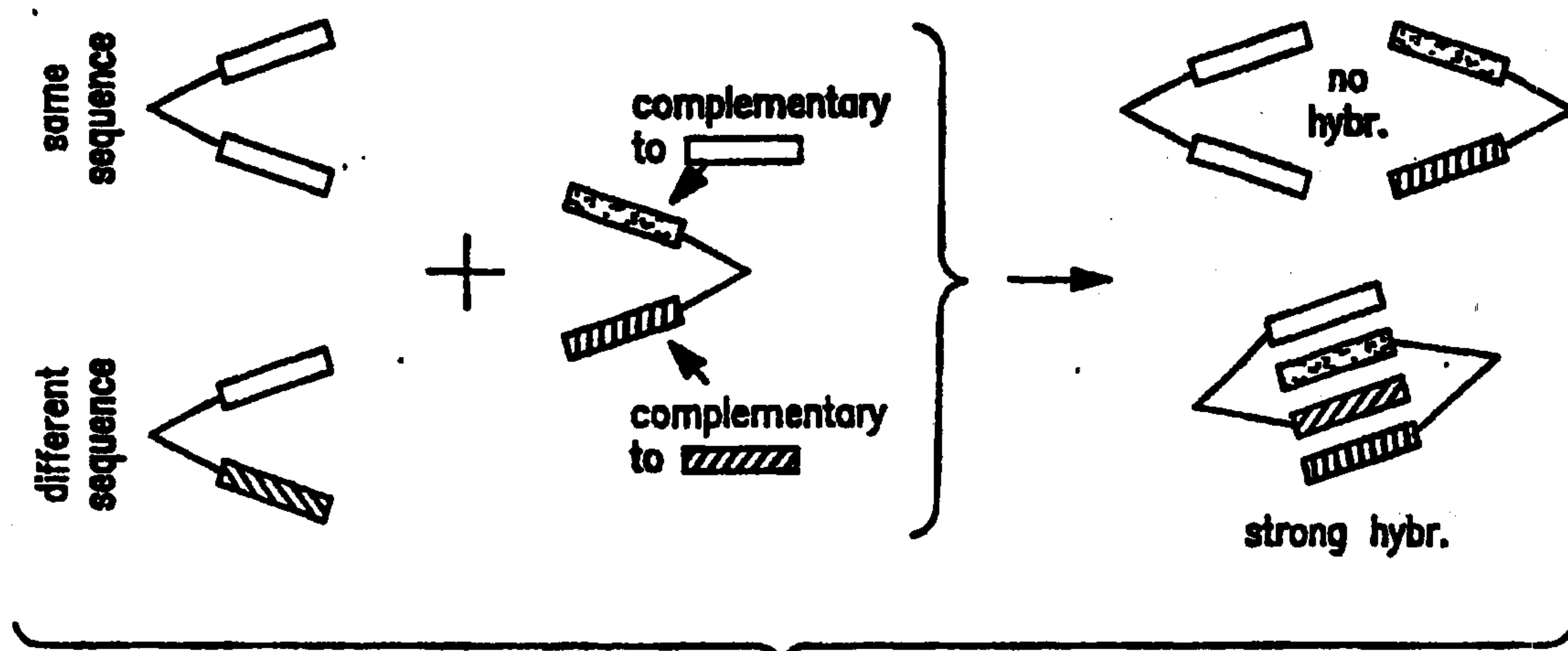


FIG. 4

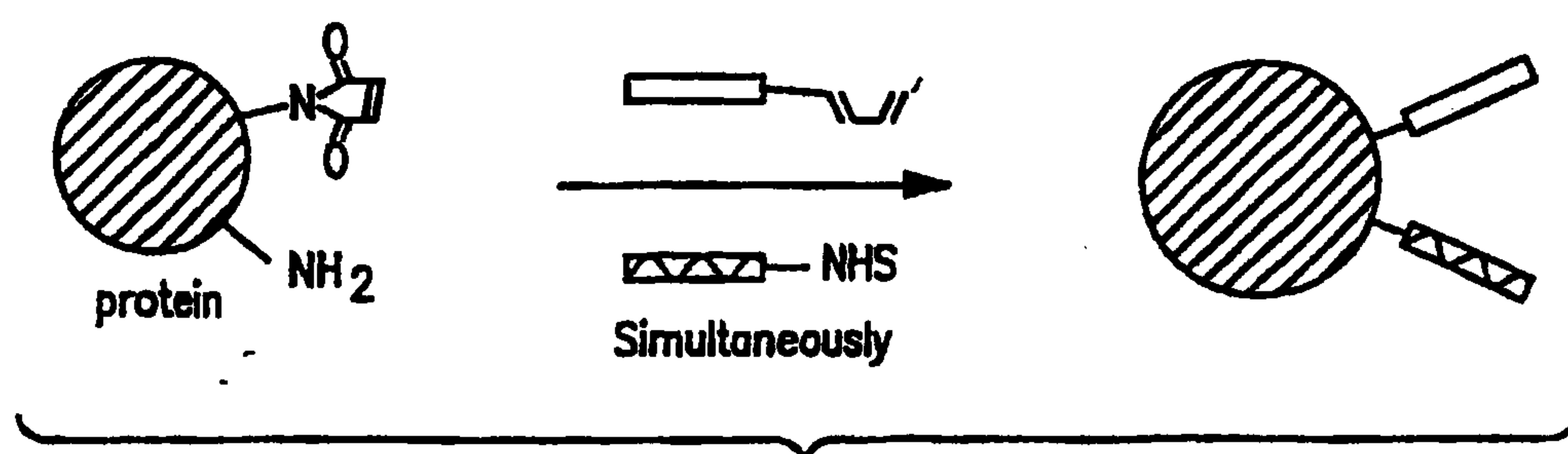


FIG. 5

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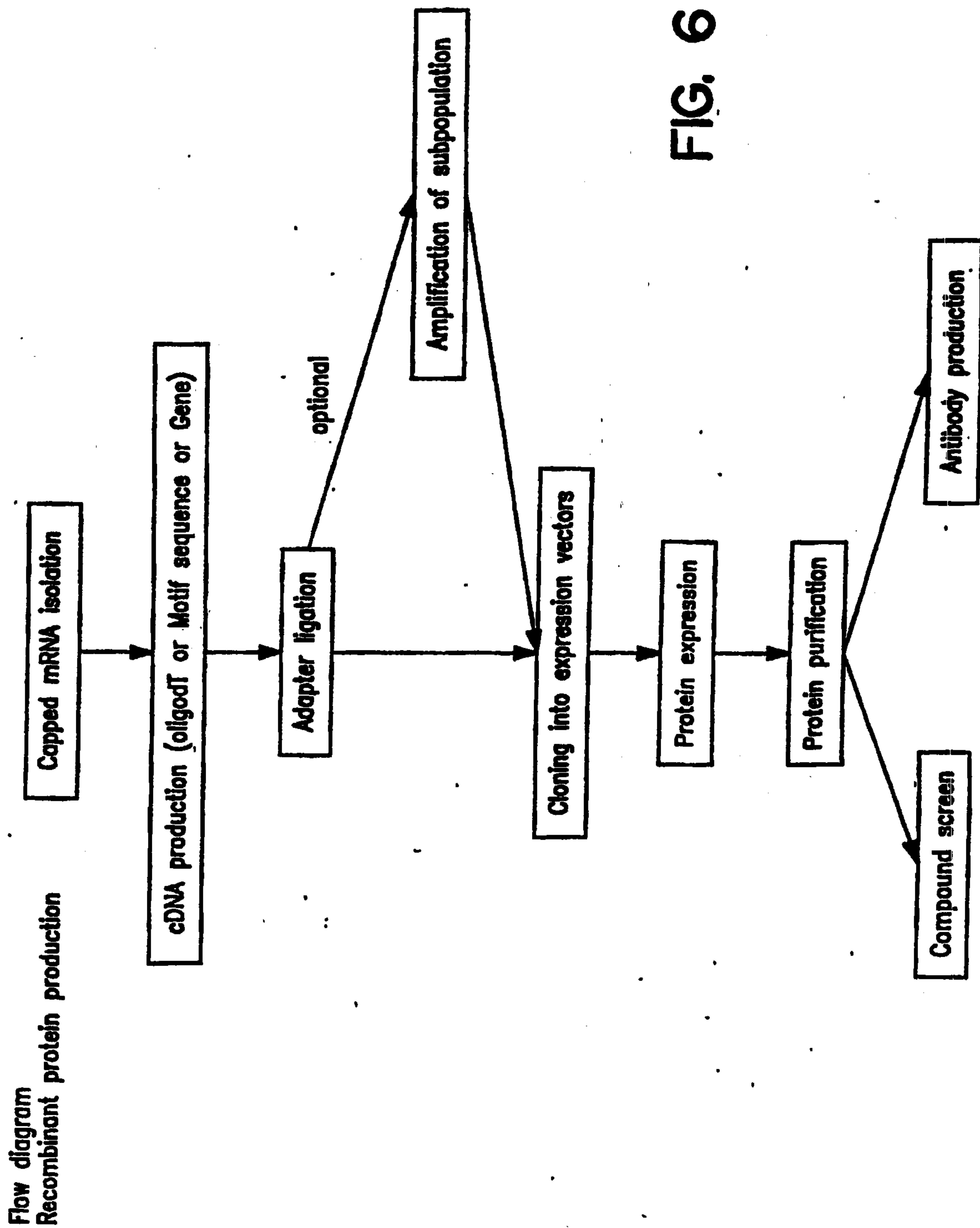


FIG. 6

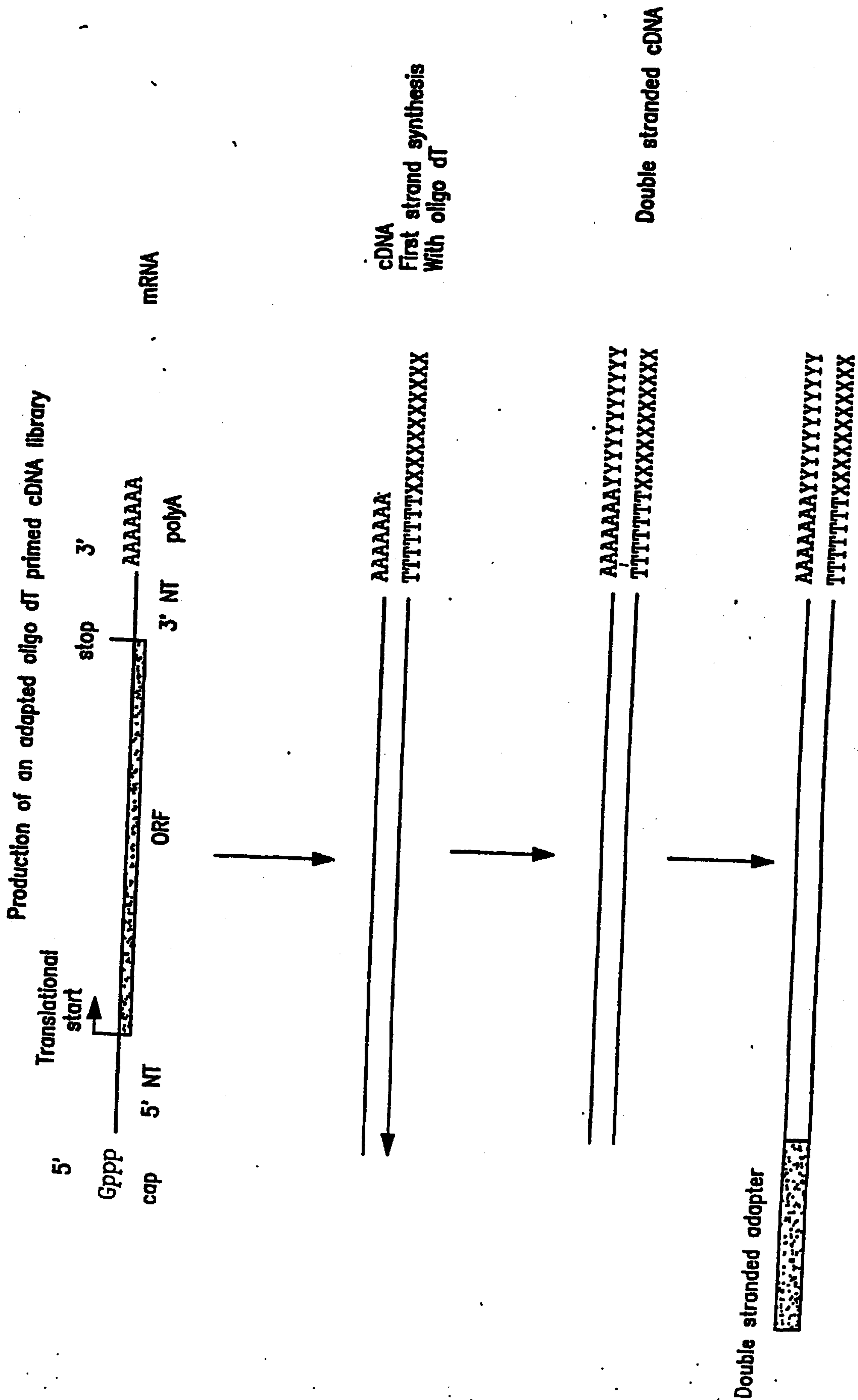
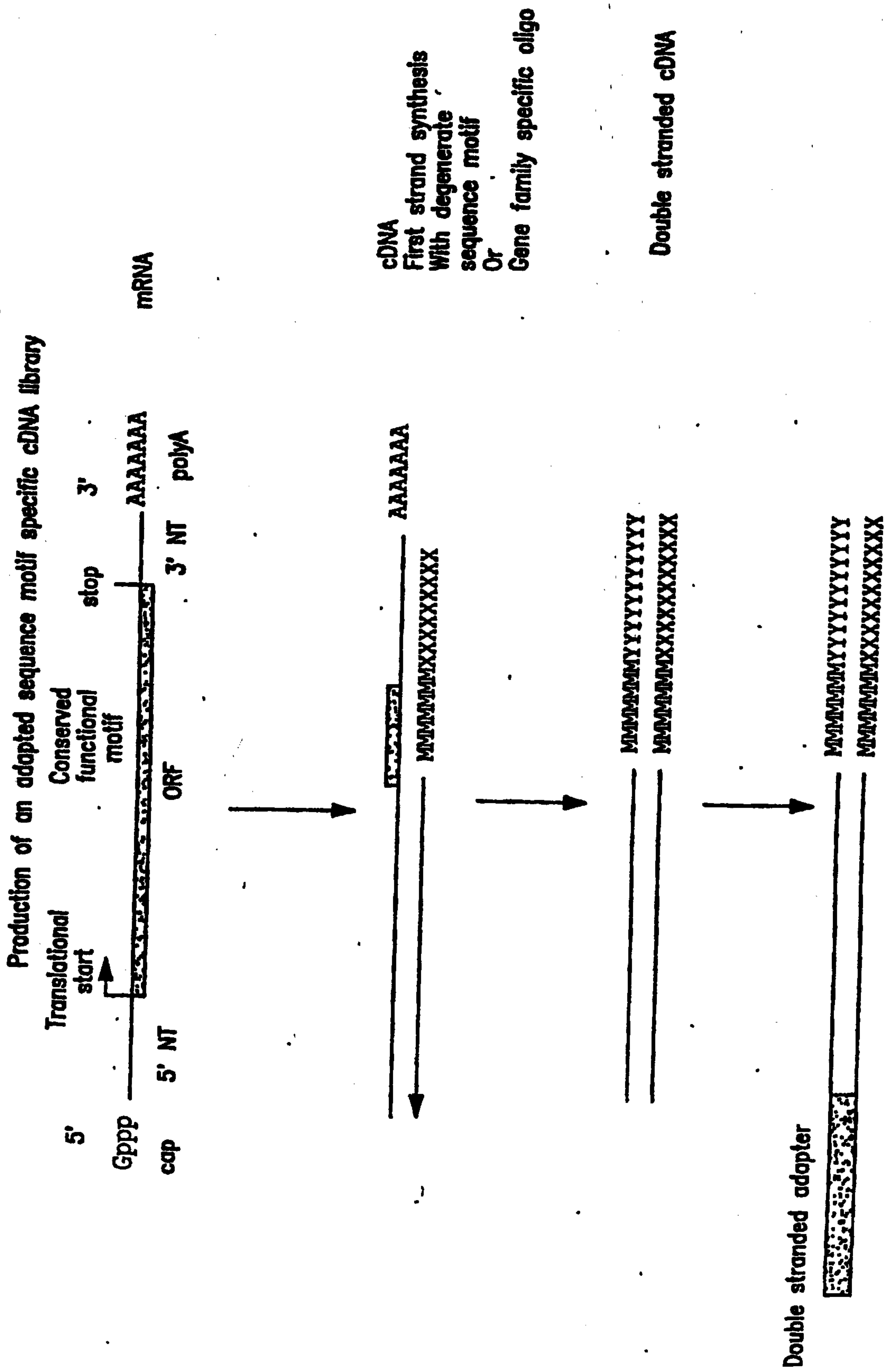


FIG. 7



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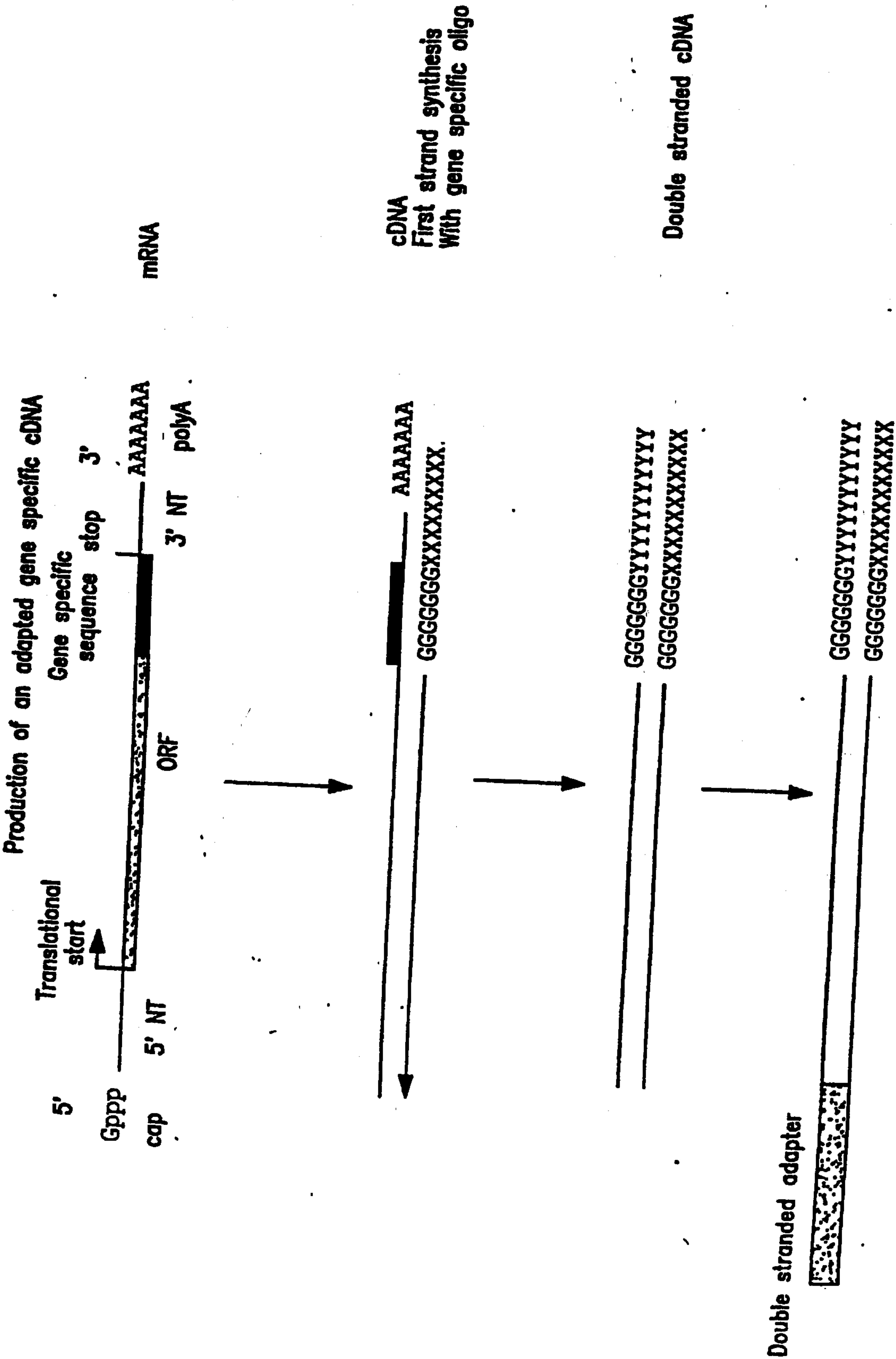


FIG. 9

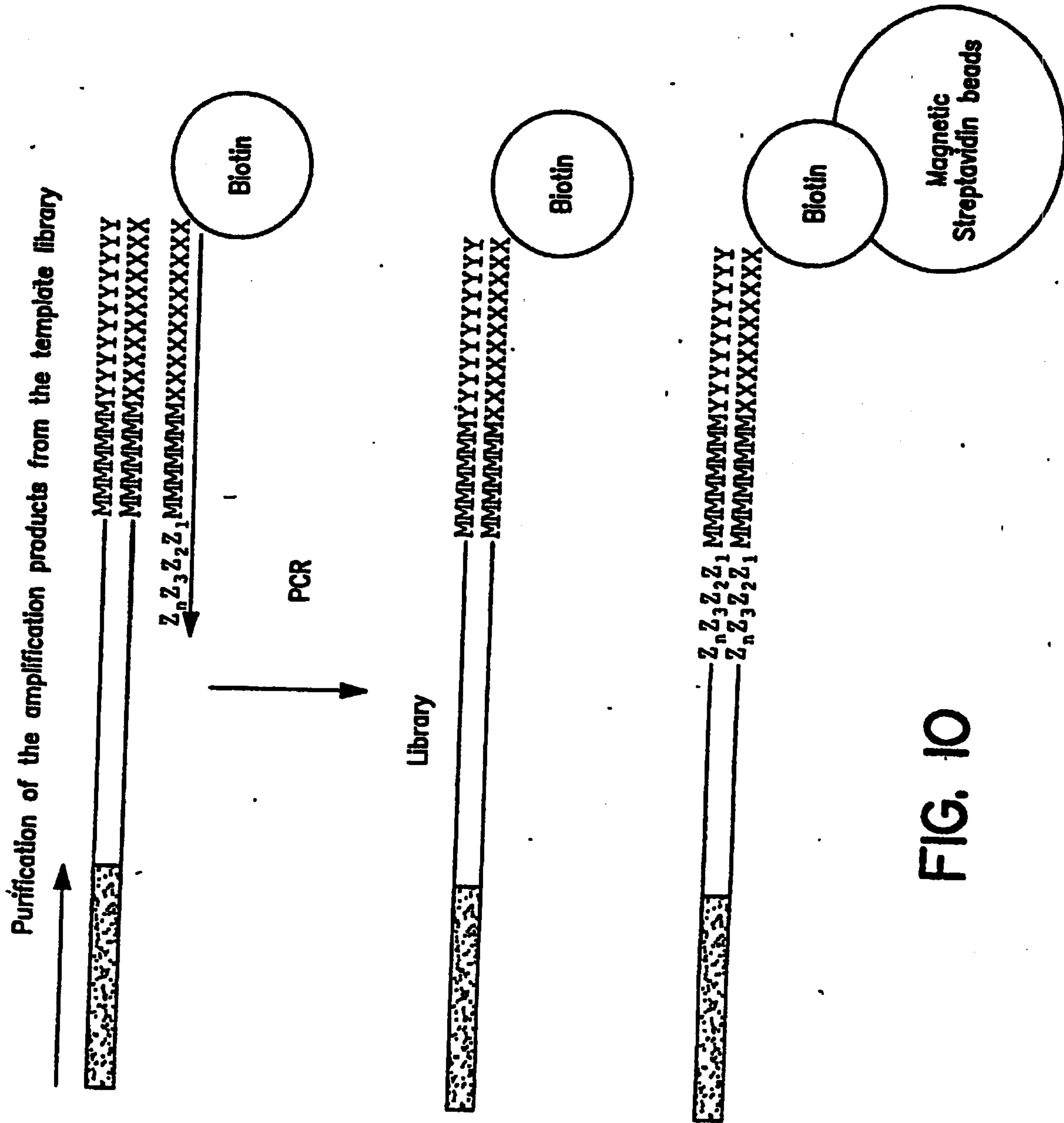
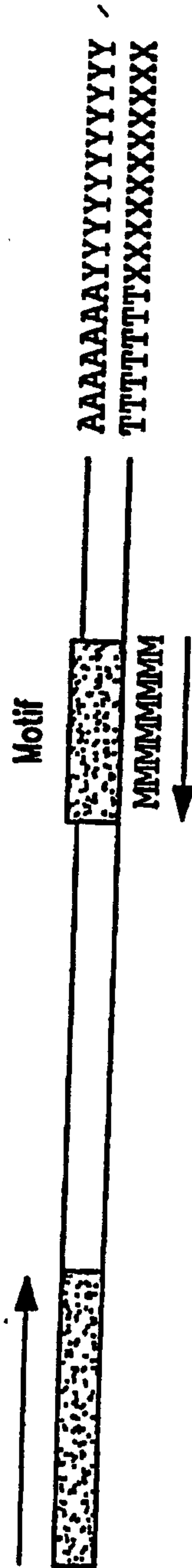


FIG. 10

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Adapted oligo dT primed cDNA library as universal template for the  
amplification of gene subpopulations



PCR amplification



Adapter

motif  
Amplification of all genes with the same functional motif

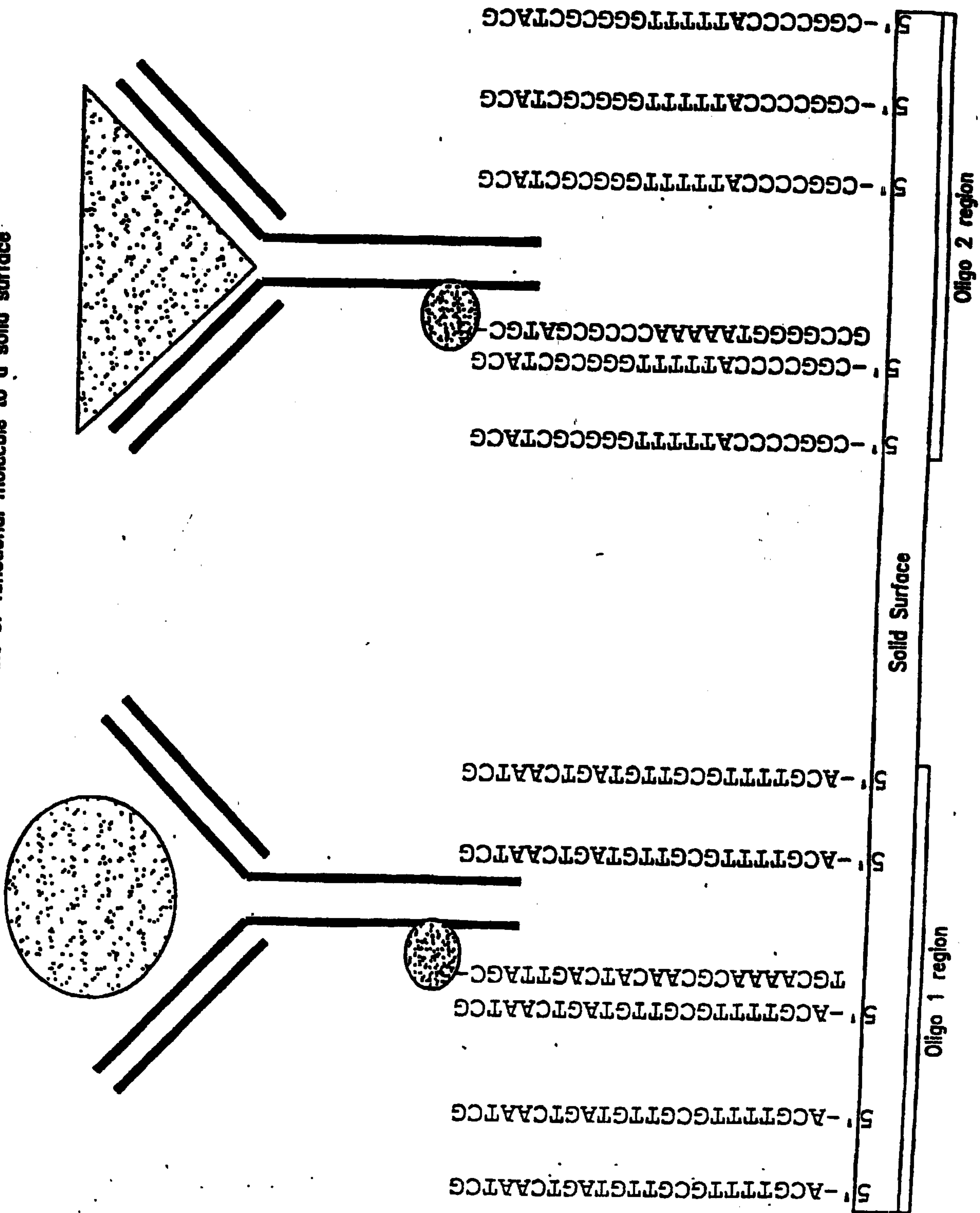
FIG. 11



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FIG. 13

Attachment of the bi-functional molecule to a solid surface



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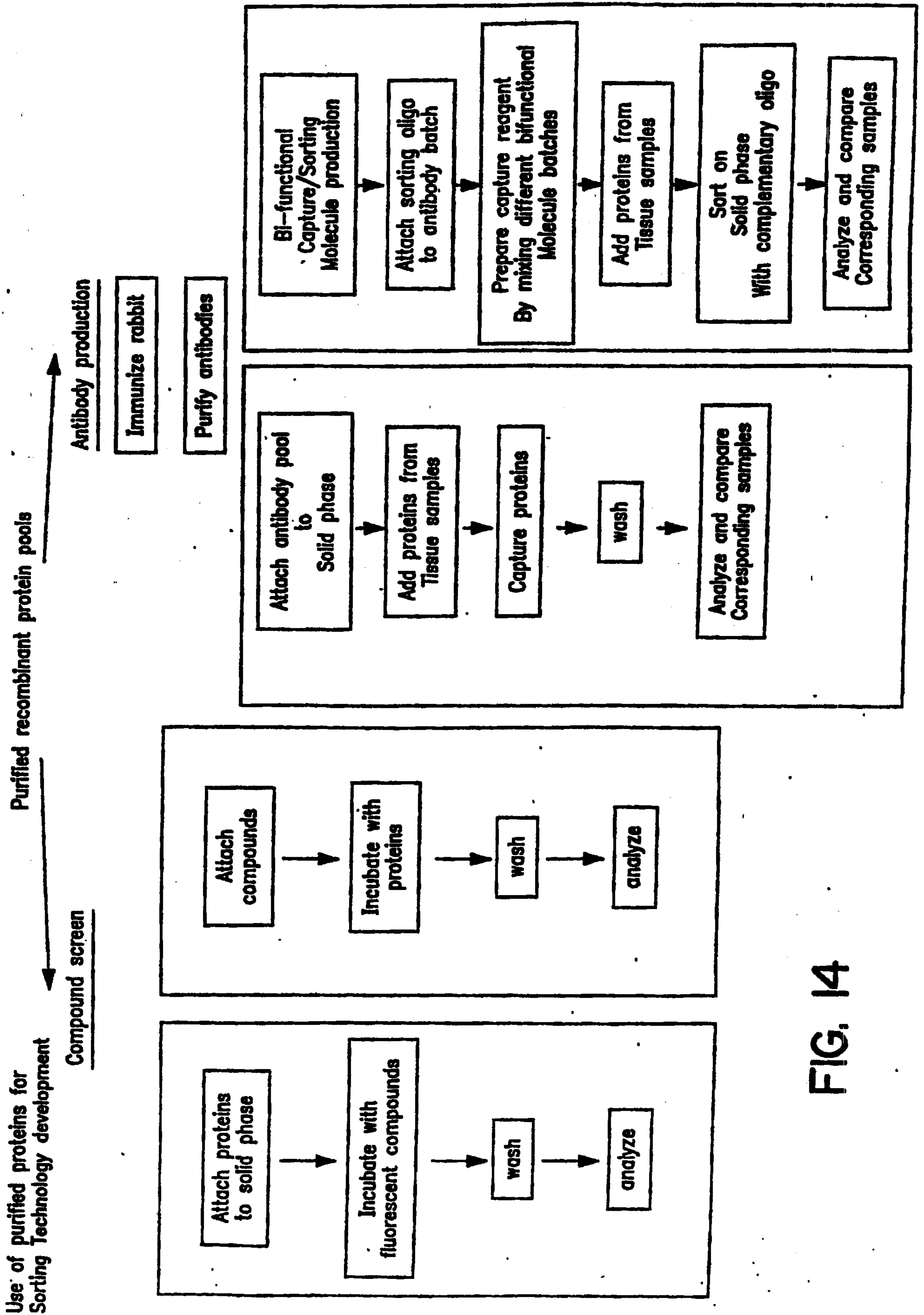


FIG. 14

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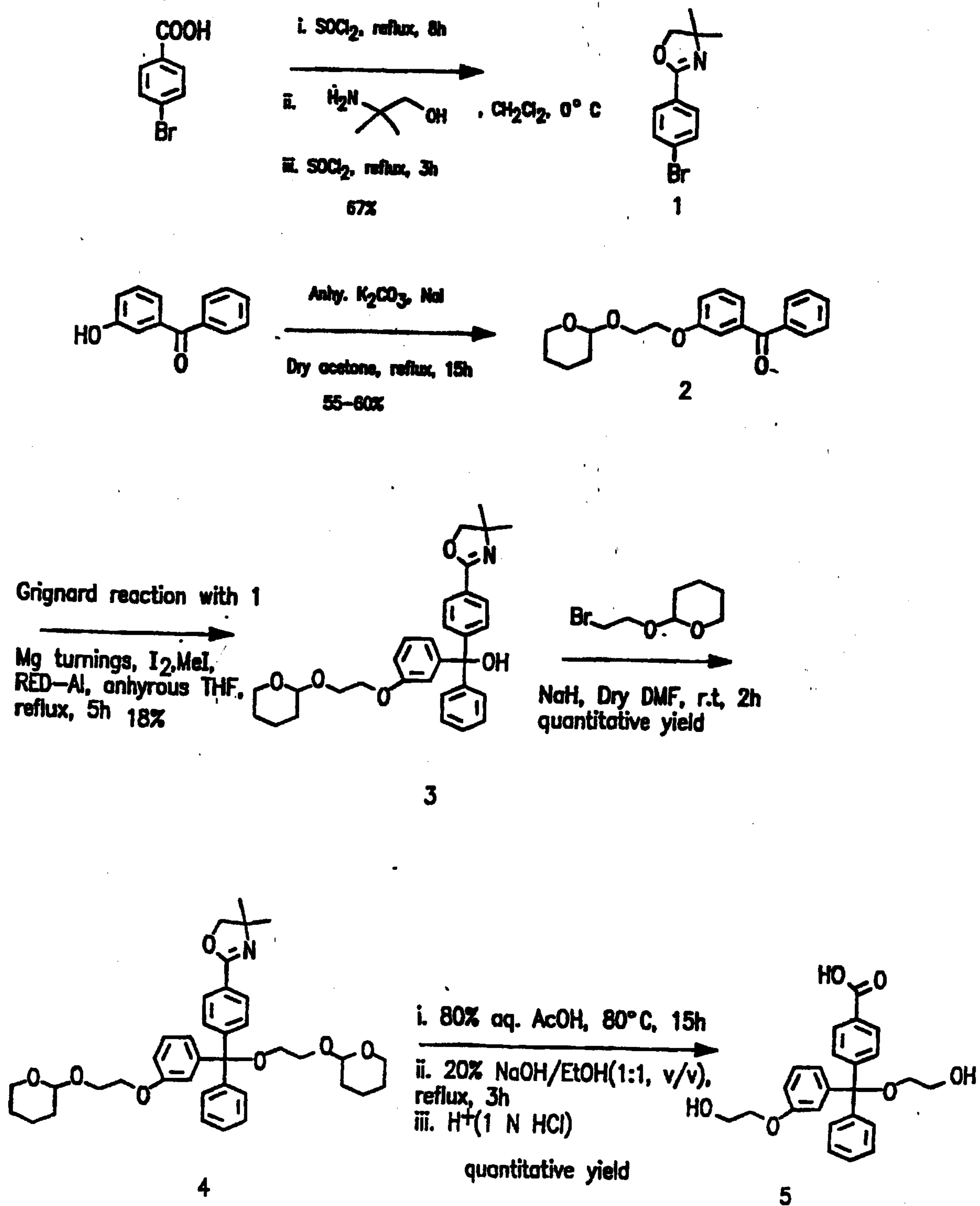


FIG. 15a

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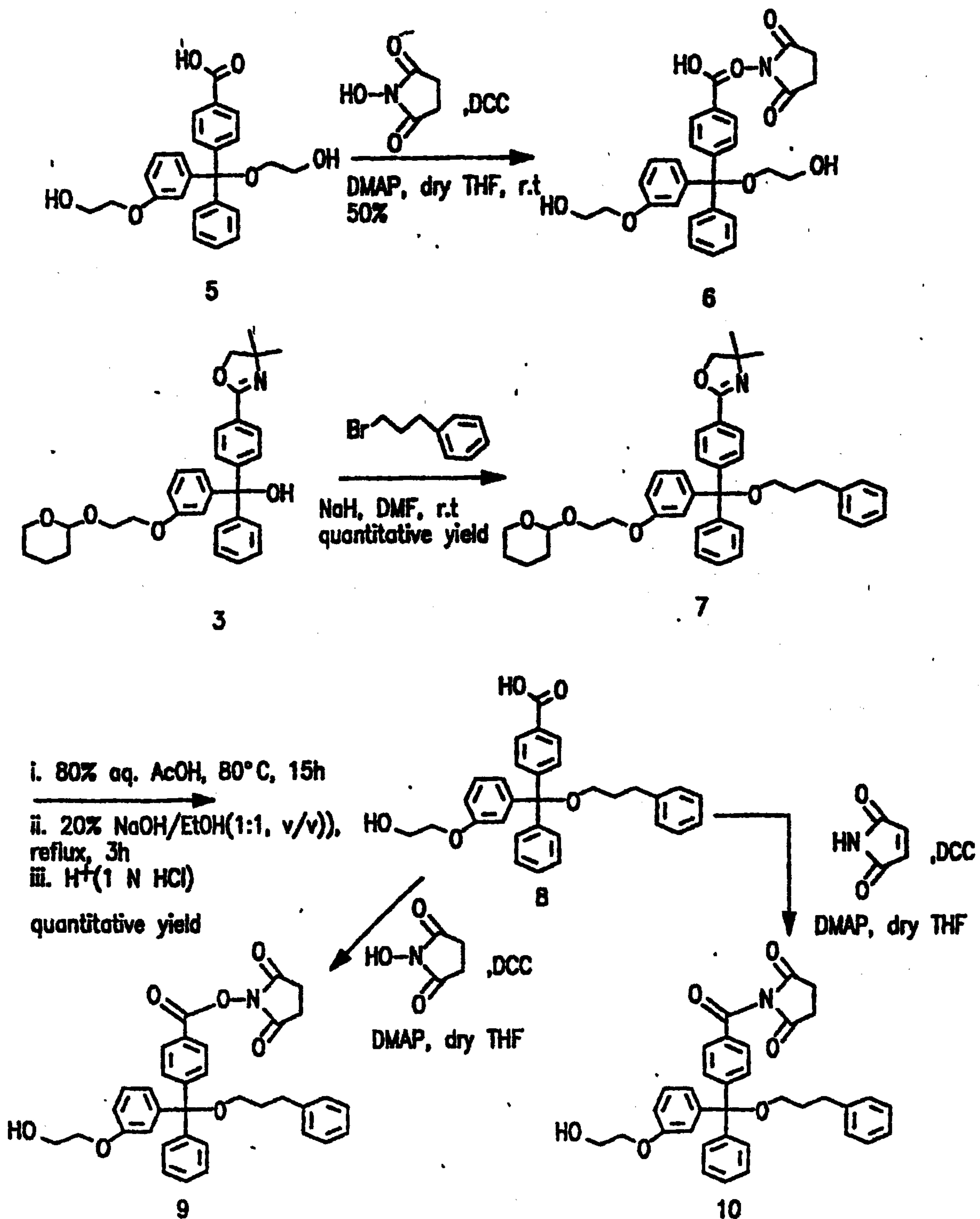


FIG. 15b

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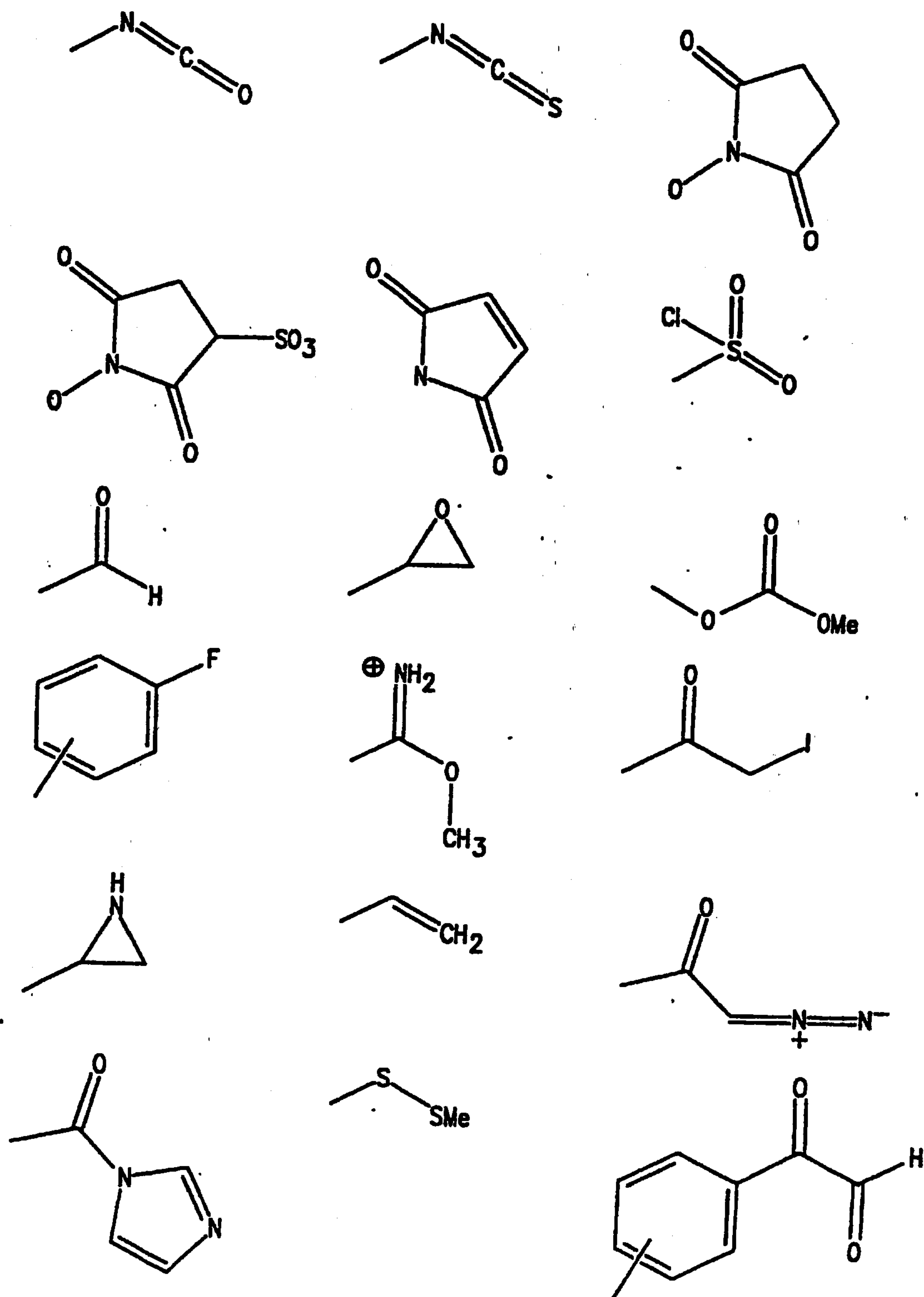


FIG. 16a

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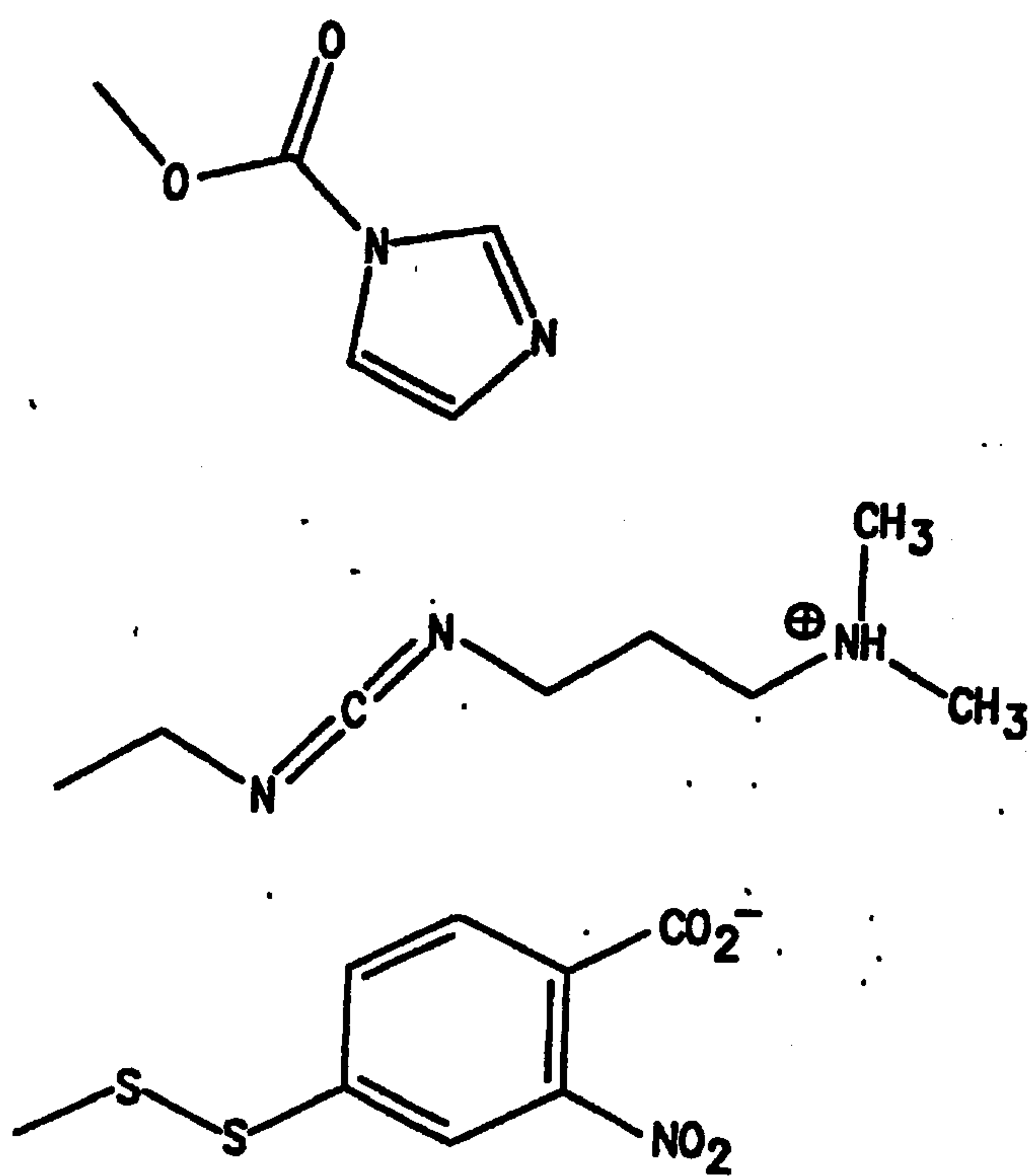
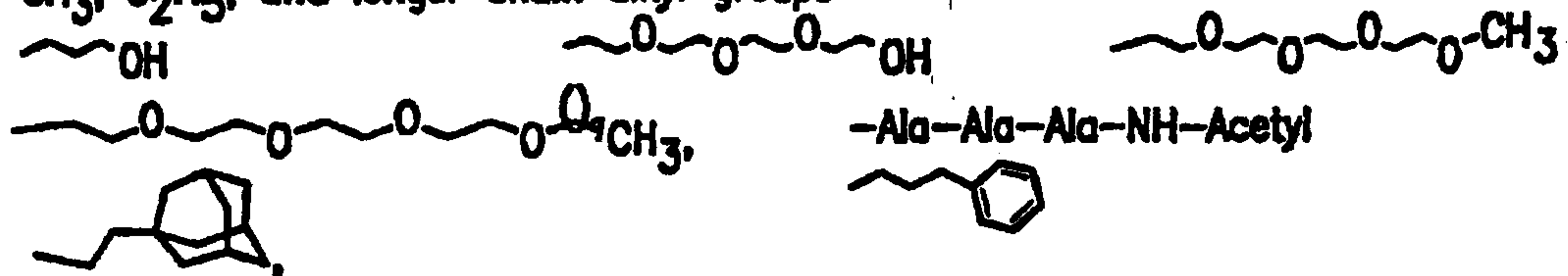


FIG. 16b

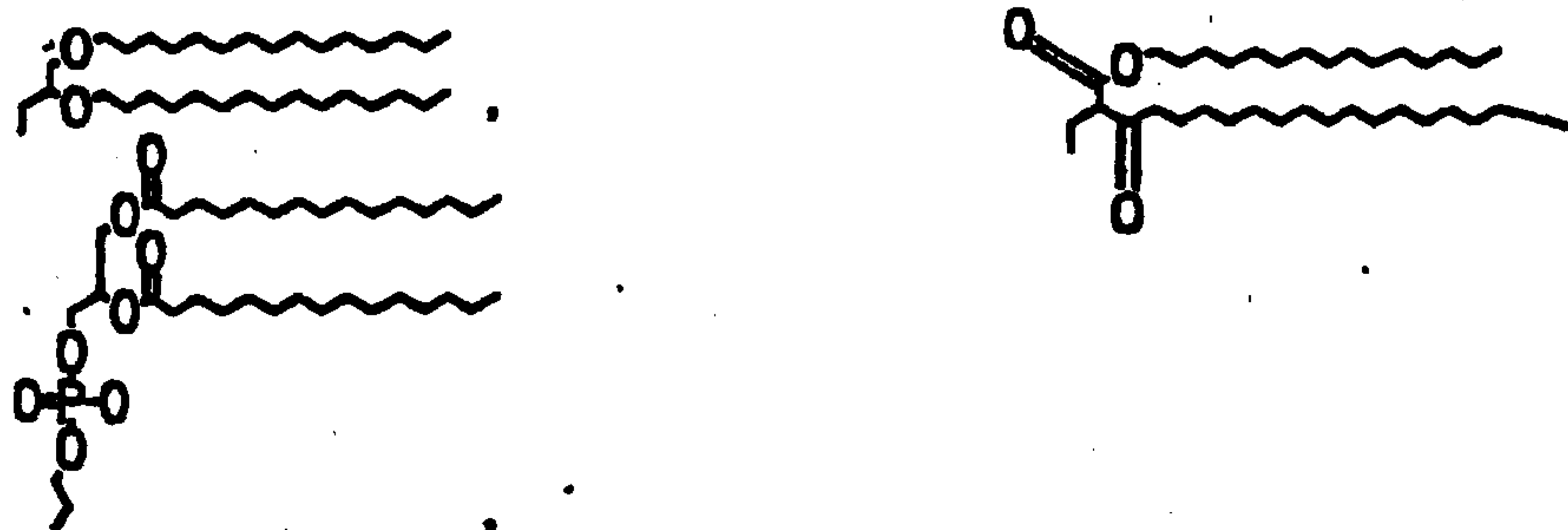
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## SMALL MOLECULES

### CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, and longer chain alkyl groups



## NATURAL PRODUCTS



**Cholesterol, Steroids, alkaloids, flavonoids, prostaglandin, peptides, EGF, rapamycin**

## PROTEIN AGONISTS AND ANTAGONISTS

**1,1,1-Trifluoro-6Z,9Z,12Z,15Z-heneicosatetraen-2-one,**

**trans-4-[3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol,**

**Arachidonyl-2'-chloroethylamide/  
(all Z)-N-(2-cycloethyl)-5,8,11,14-eicosatetraenamide,**

**Arachidonylcyclopropylamide/(all Z)-N-(cyclopropyl)-5,8,11,14-eicosatetraenamide,**

**N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.**

**1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide,**

**(all Z)-N-(4-Hydroxyphenyl)-5,8,11,14-eicosatetraenamide,**

**6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl) methanone.**

**Arachidonylethanolamide/(all Z)-N-(2-Hydroxyethyl)-5,8,11,14-eicosatetraenamide,**

FIG. 17a

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Arachidonylethanolamide/(all Z)-N-(2-Hydroxyethyl)-5,8,11,14-eicosatetraenamide,

N-(2-Hydroxyethyl)-[5,6,8,9,11,12,14,15-H]-5Z,8Z,11Z,14Z-eicosatetraenamide,

2-AG/(5Z,8Z,11Z,14Z)-5,8,11,14-Eicosatetraenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester,

(-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol,

Docosatetraenylethanolamide/N-(2-Hydroxyethyl)-7Z,10Z,13Z,16Z-docosatetraenamide,

(6aR)-trans-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol,

[6aR-(6aα,9α,10αβ)]-3-(1,1-Dimethylheptyl)-6a,7,8,9,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-[7,8-H]-9-methanol,

(2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone,

(6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran,

Methyl arachidonyl fluorophosphonate/(5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenyl-methyl ester phosphonofluoridic acid,

[R-(all-Z)]-N-(2-Hydroxy-1-methylethyl)-5,8,11,14-eicosatetraenamide, 2-[(5Z,8Z,11Z,14Z)-Eicosatetraenyloxy]-1,3-propanediol,

N-(bis-3-chloro-4-hydroxybenzyl)-5Z,8Z,11Z,14Z-eicosatetraenamide,

(9Z)-N-(2-Hydroxyethyl)-9-octadecenamide,

N-(2-Hydroxyethyl)hexadecanamide,

(5Z,8Z,11Z,14Z)-N-(4-Hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide,

(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone

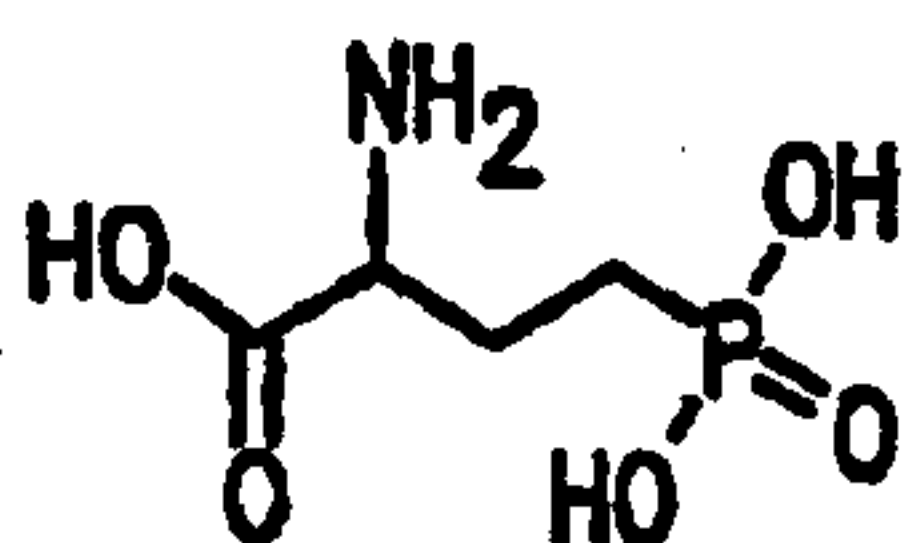


FIG. 17b

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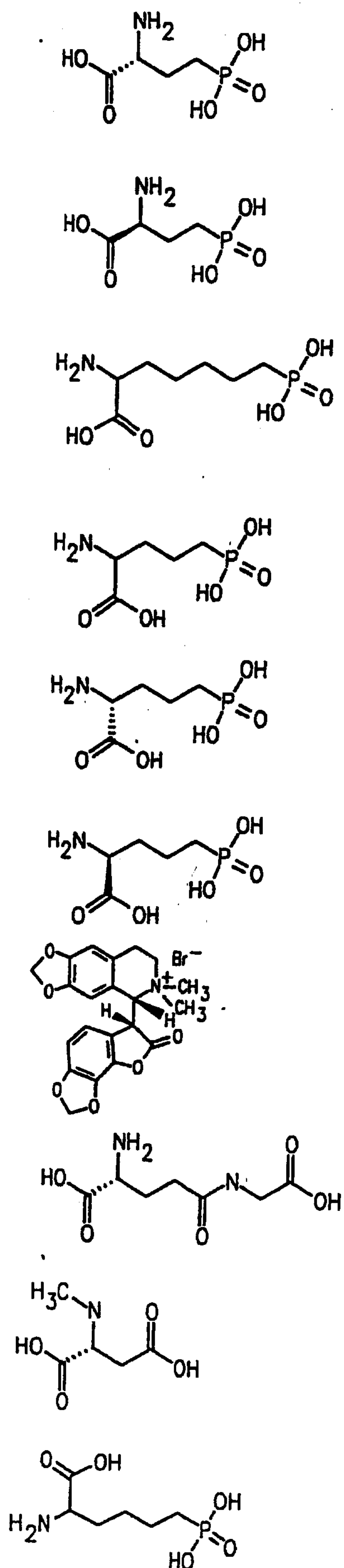


**FIG. 17c**

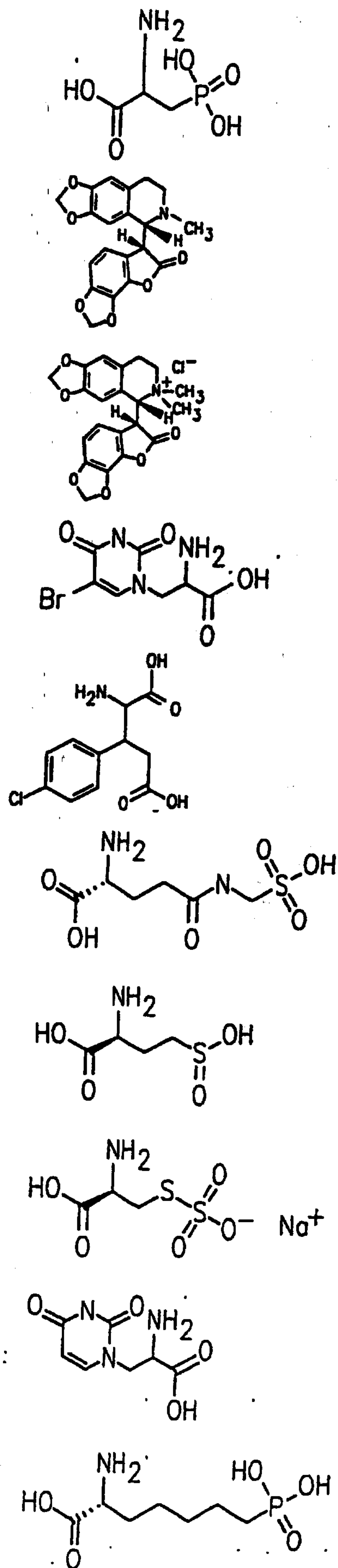


**FIG. 17d**

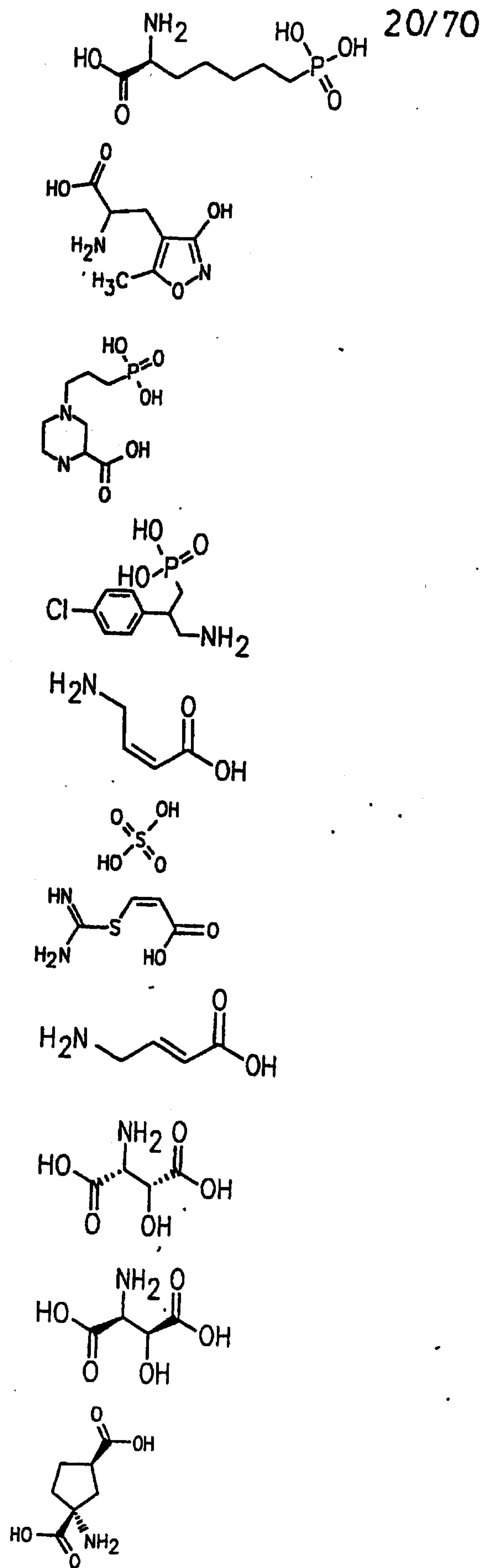


FIG. 17e

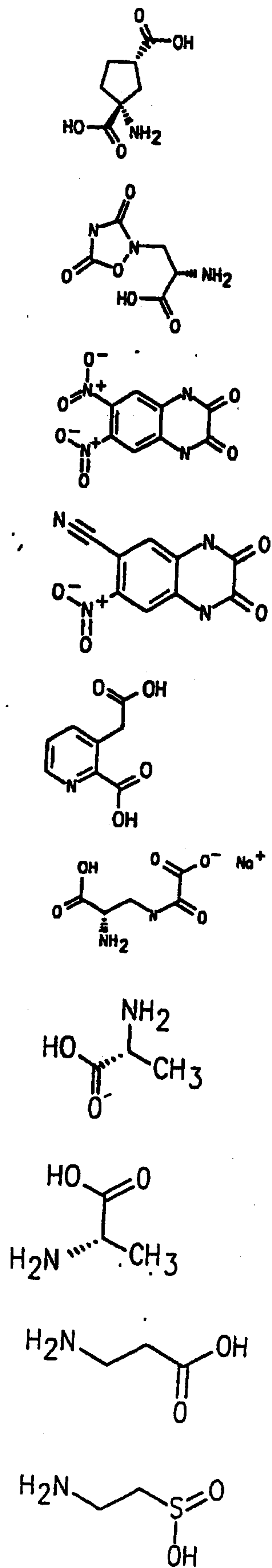


FIG. 17f

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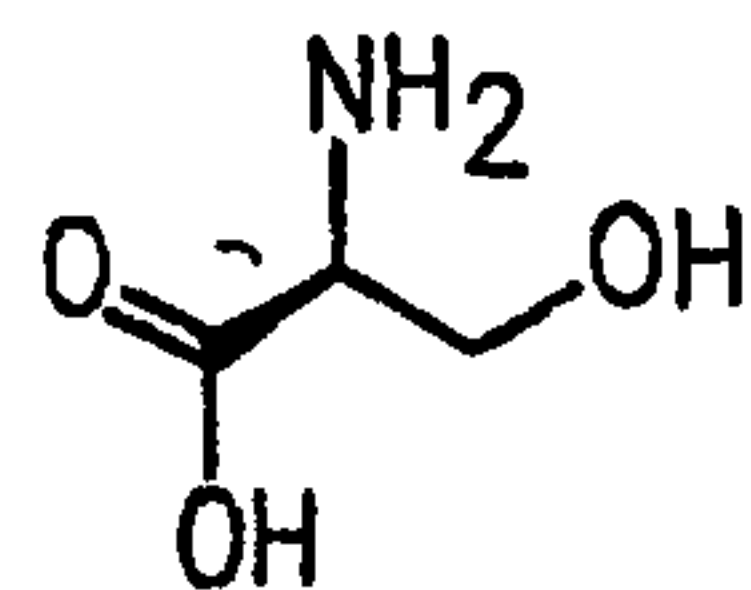
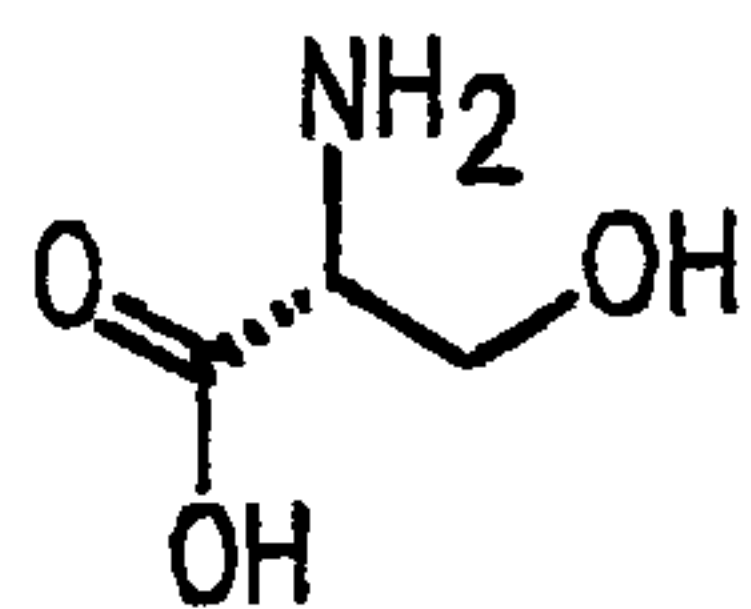
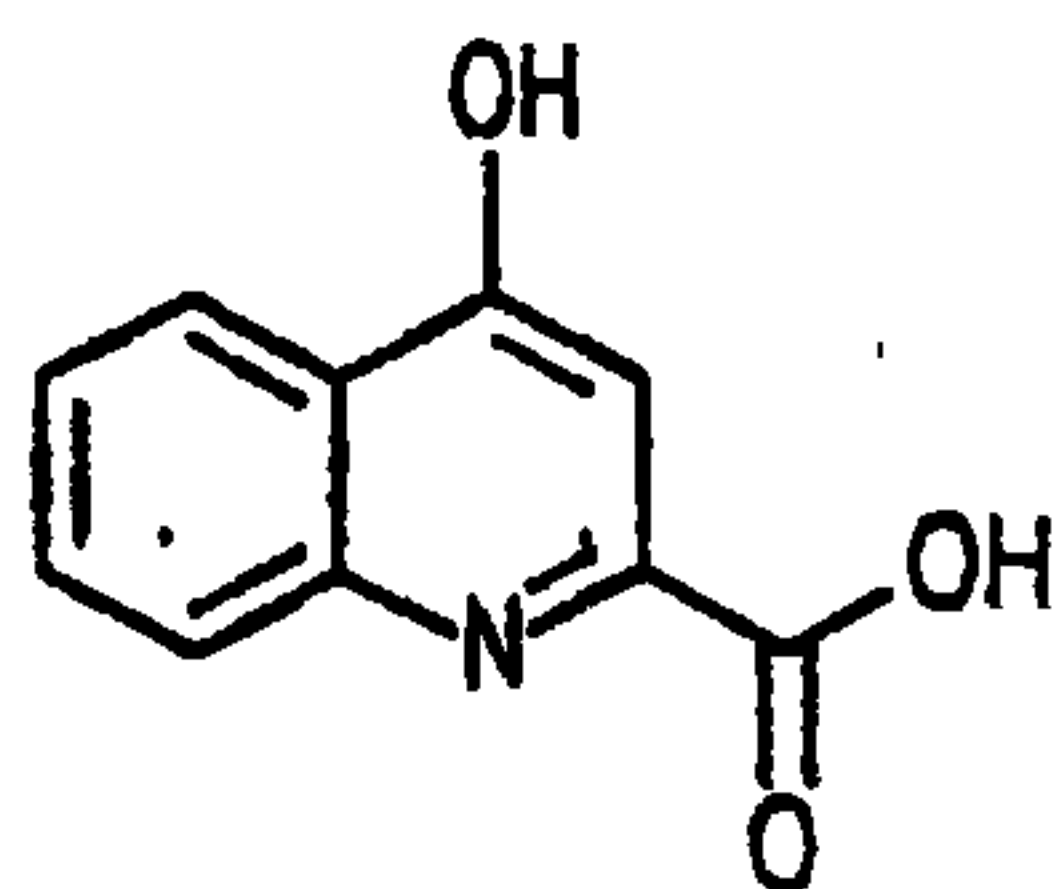
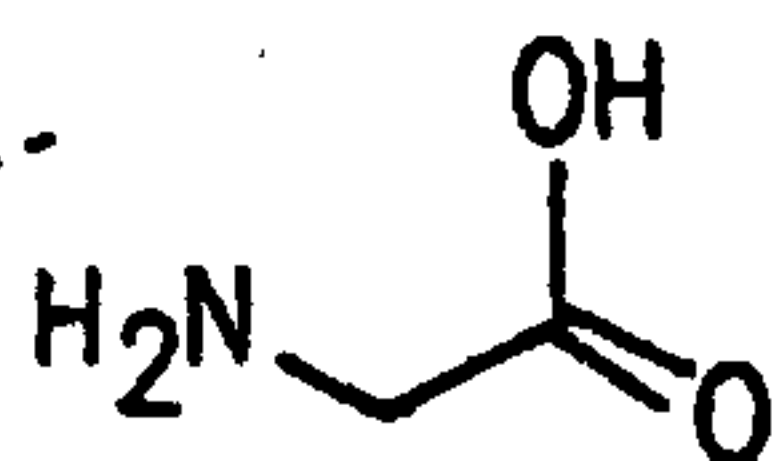
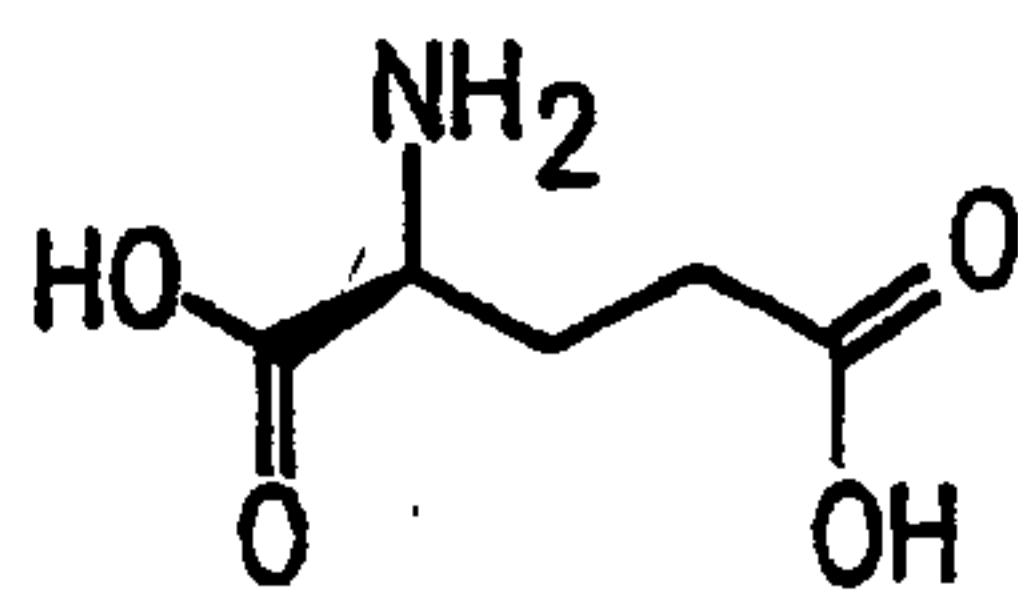
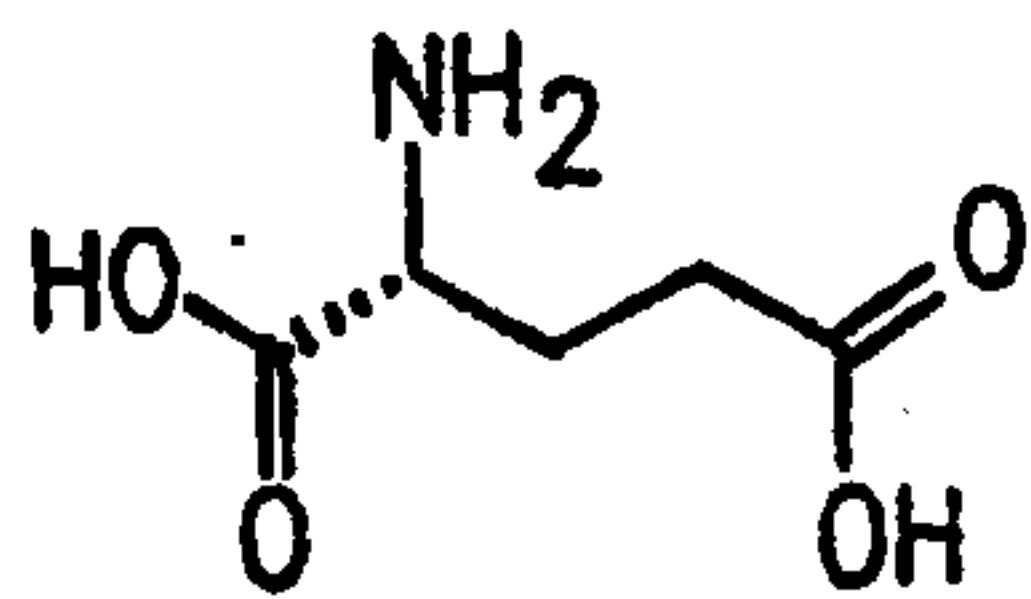
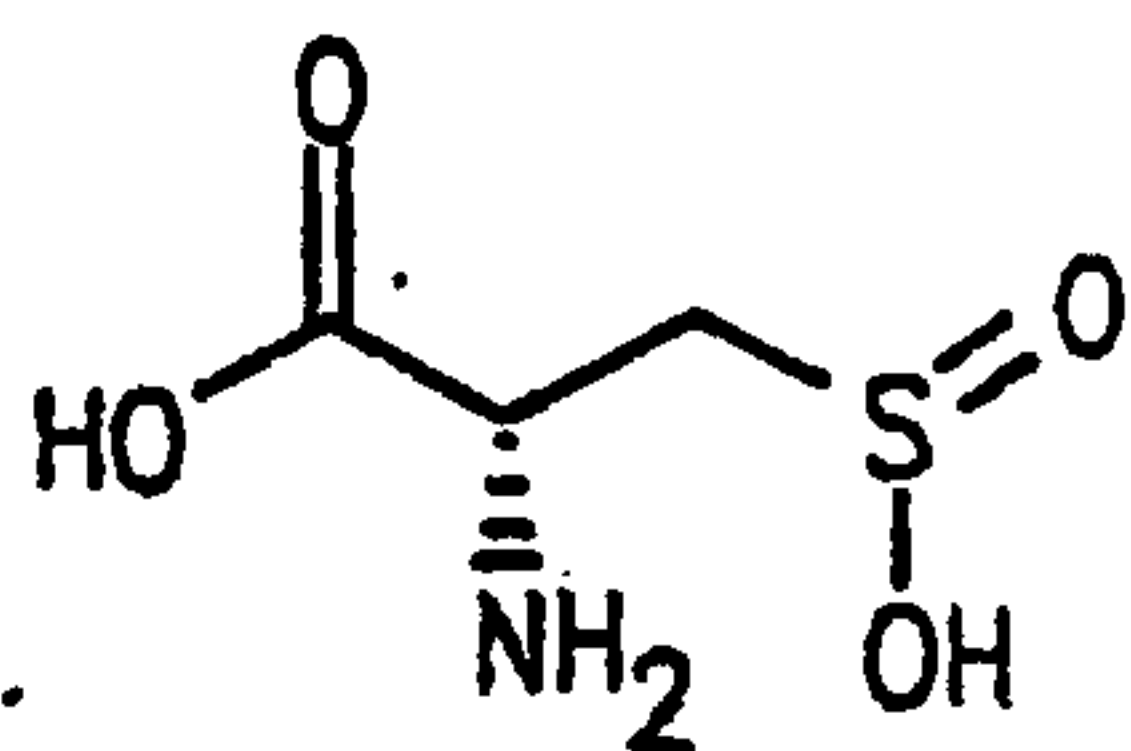
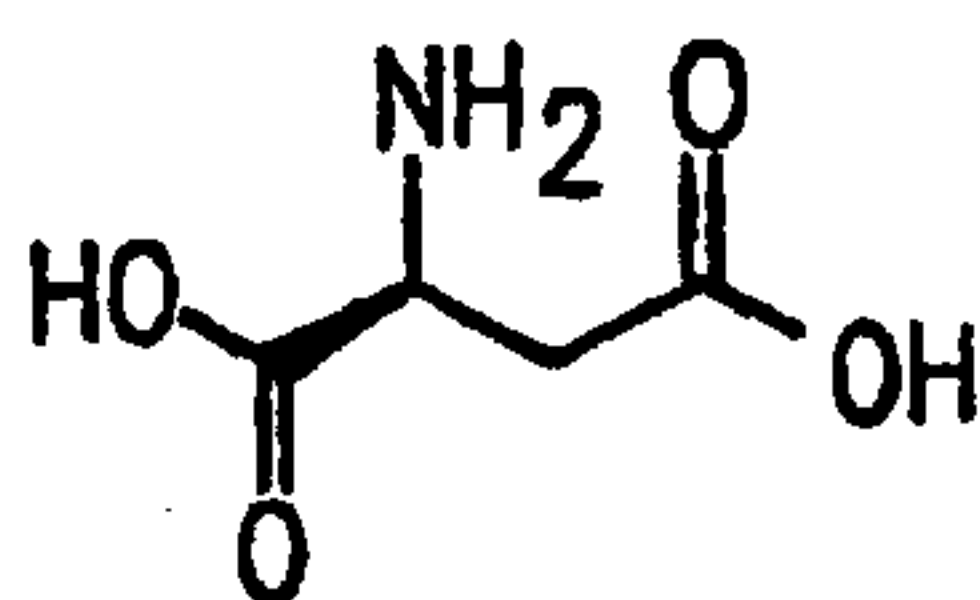
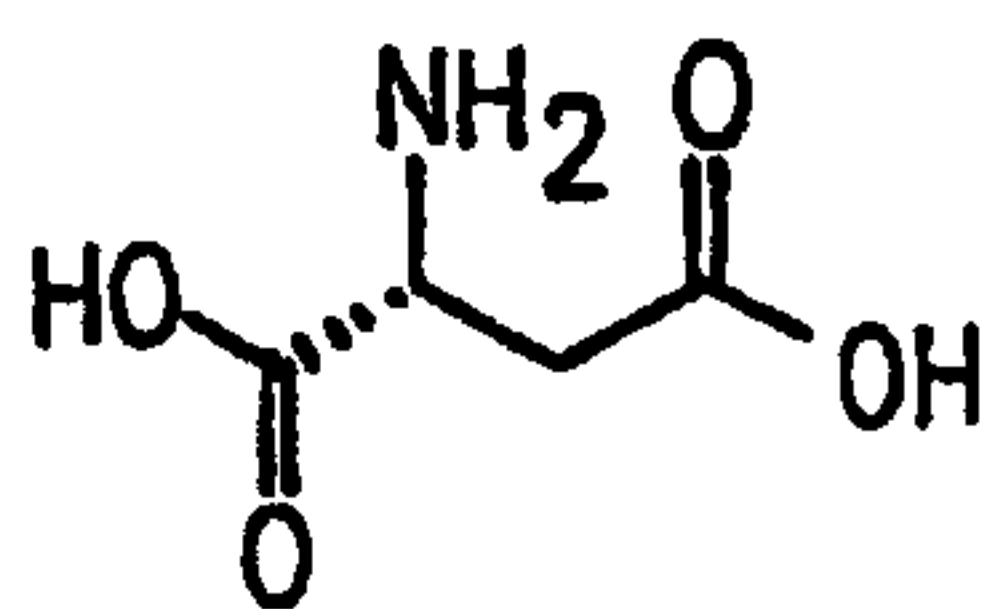
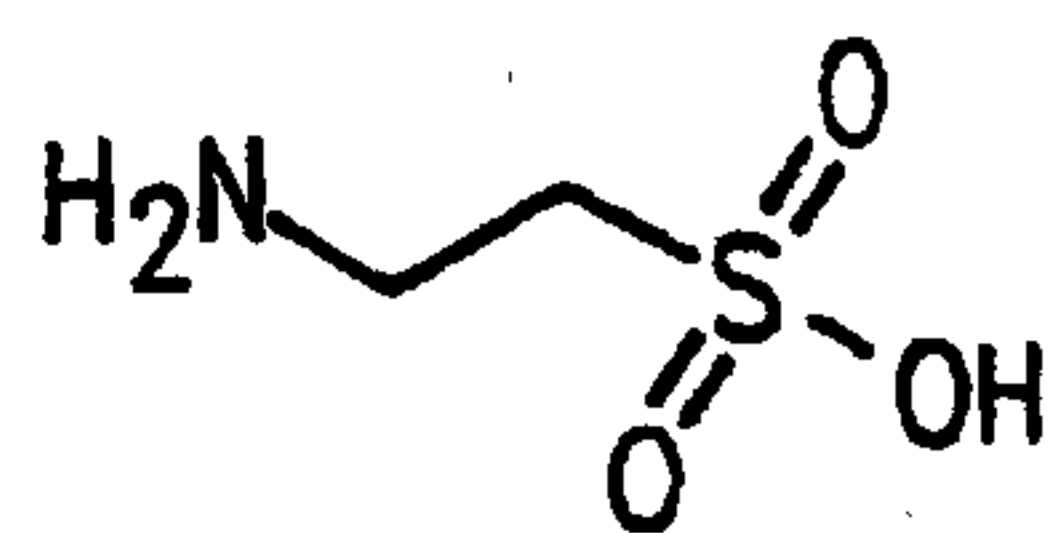


FIG. 17g

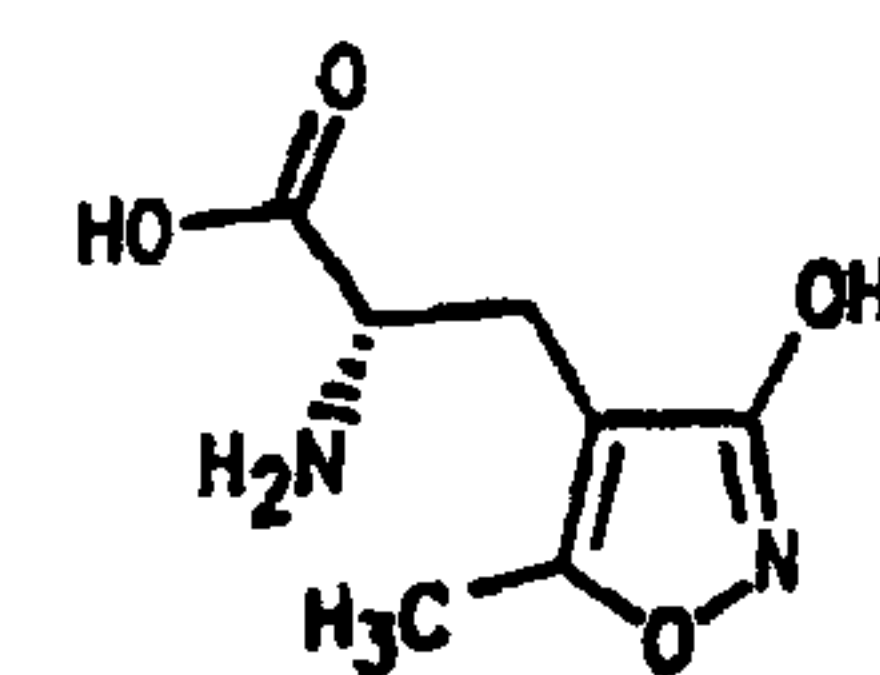
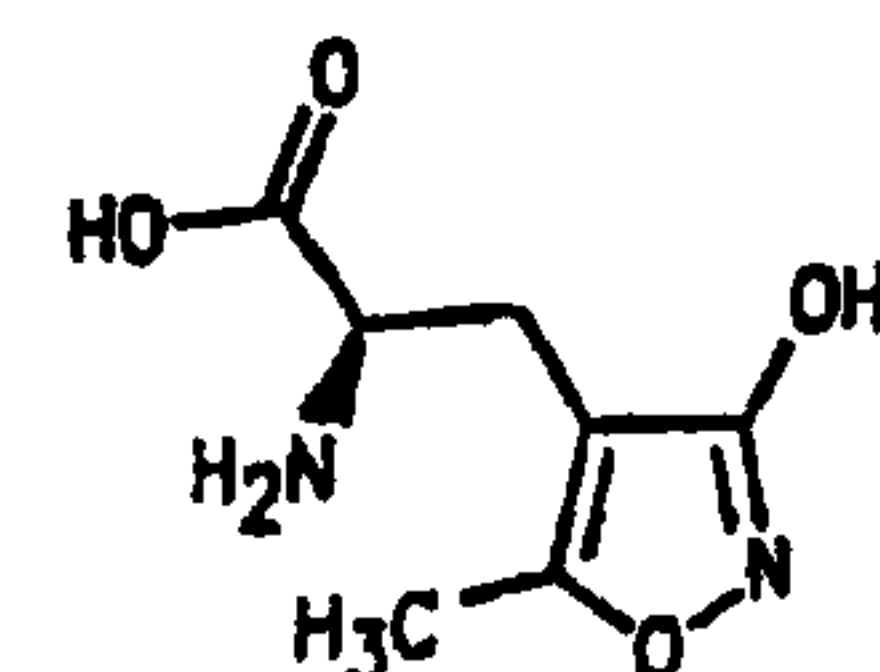
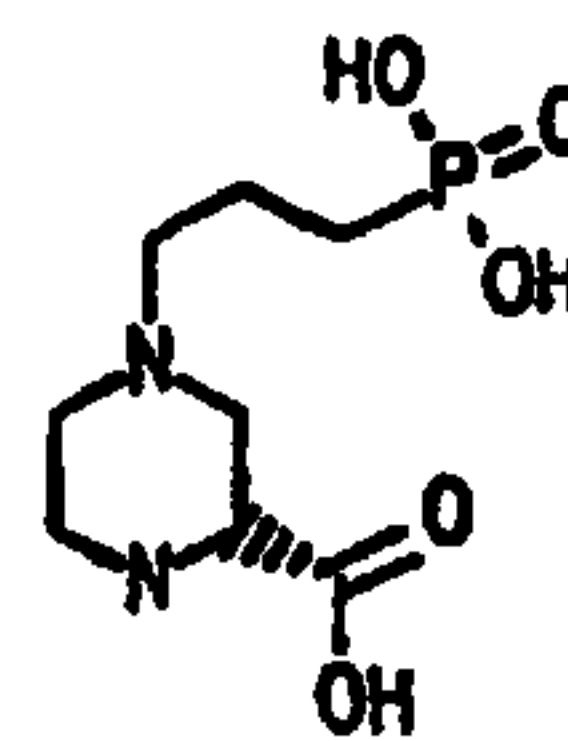
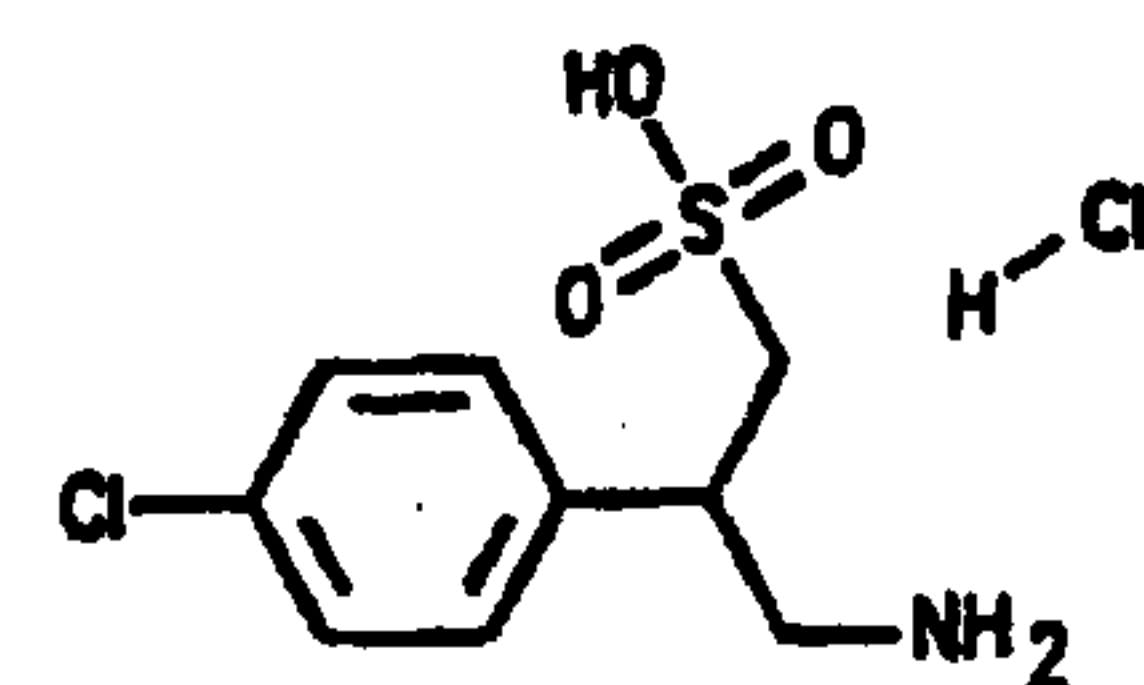
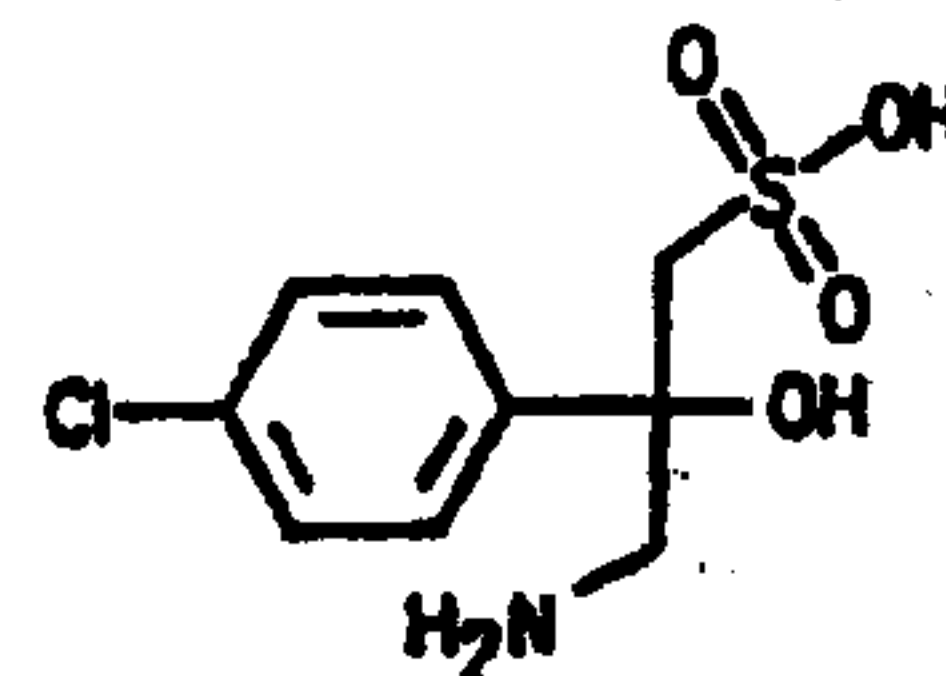
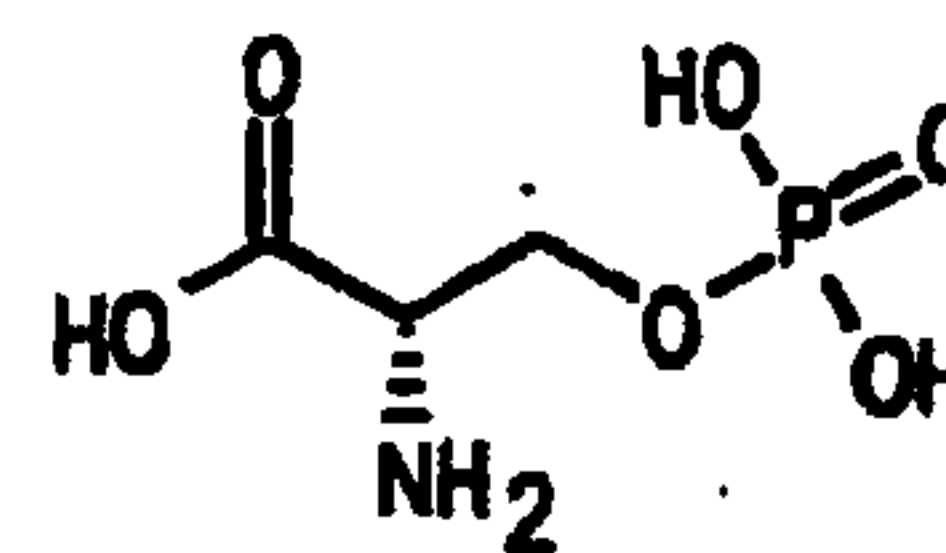
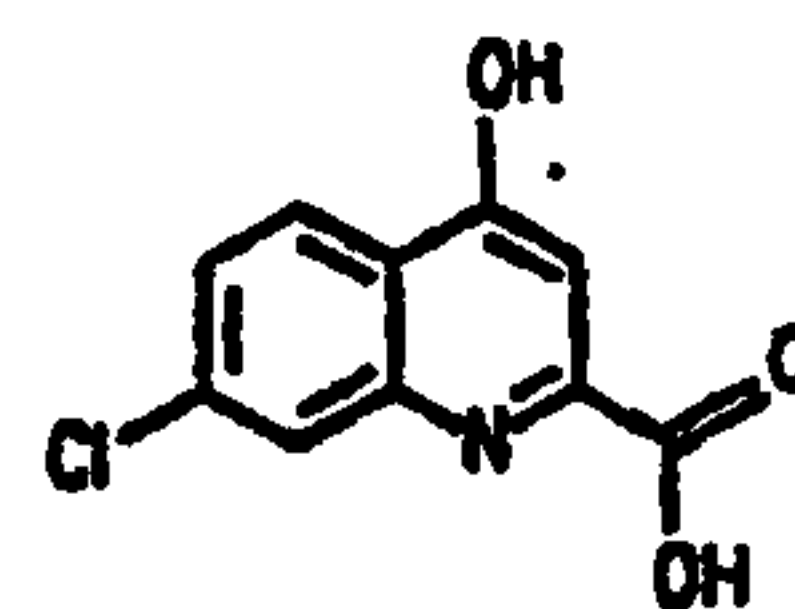
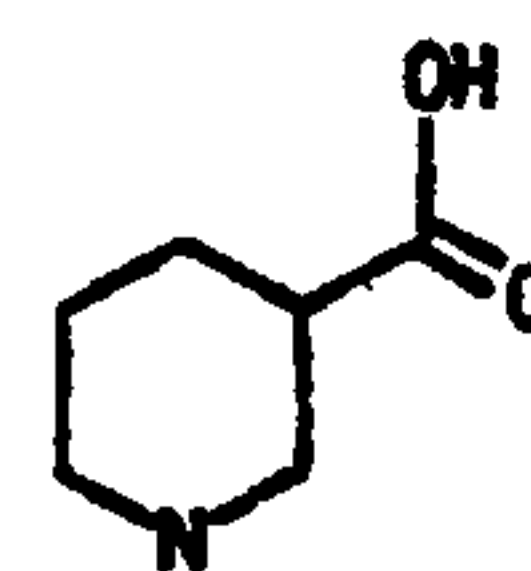
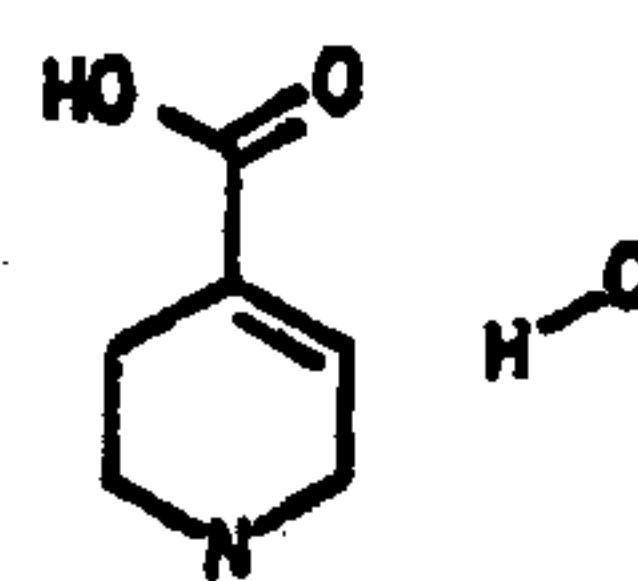
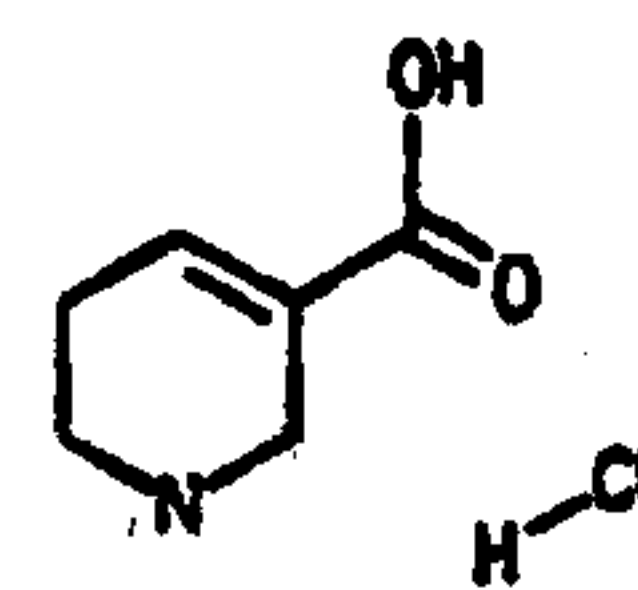


FIG. 17h

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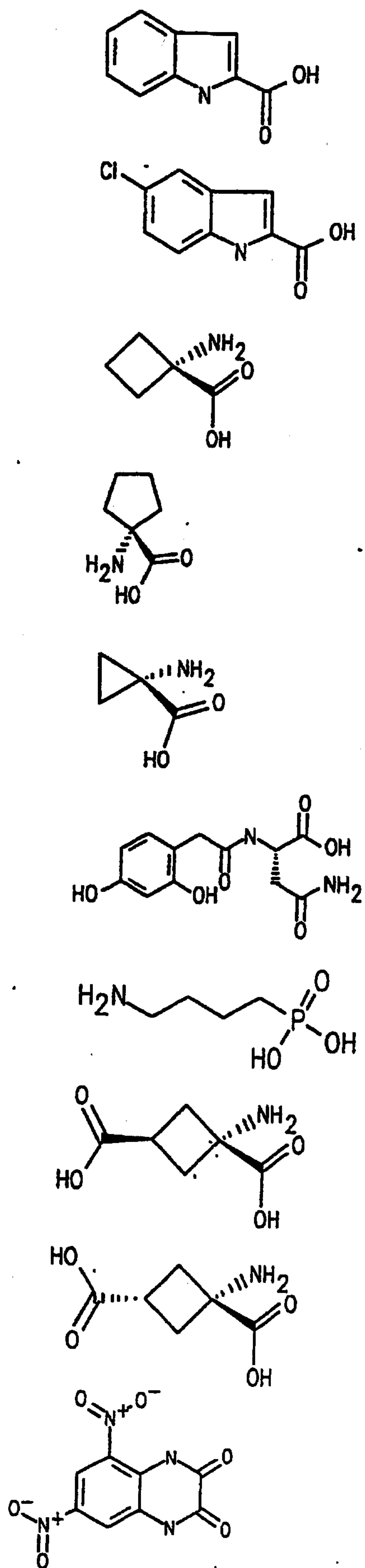


FIG. 17i

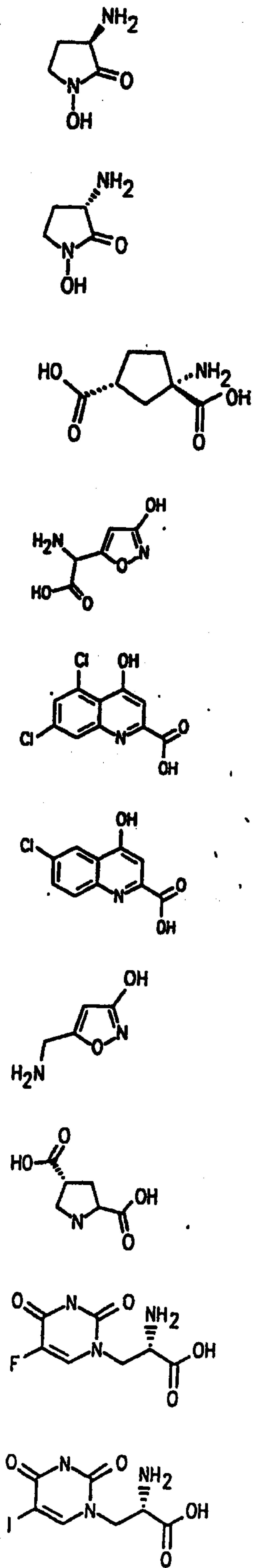


FIG. 17j

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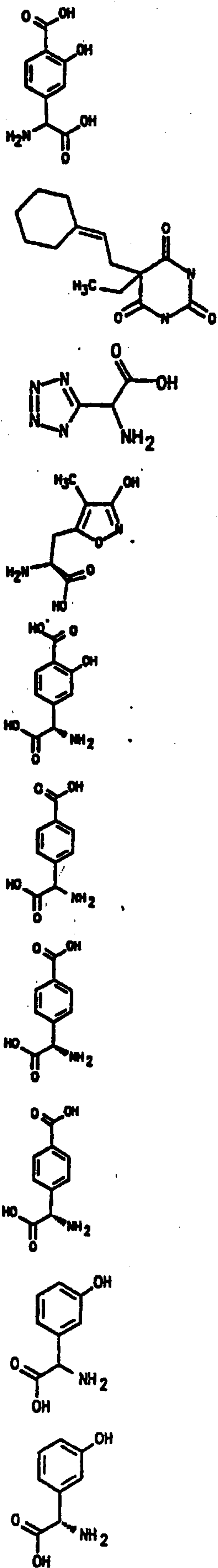


FIG. 17k

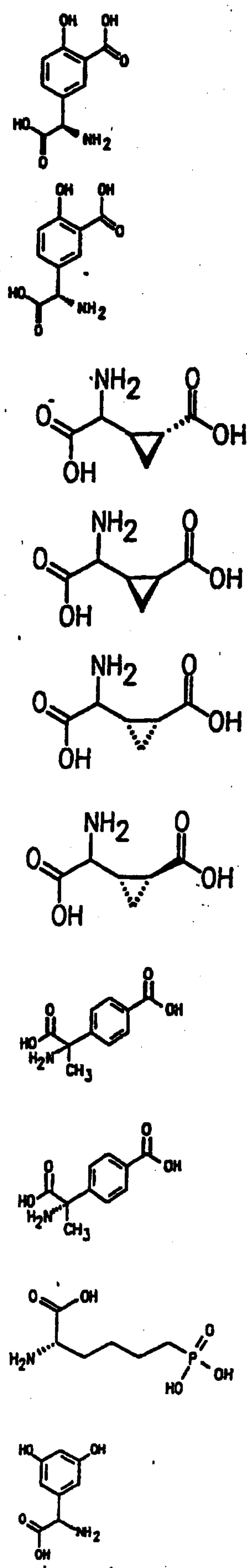


FIG. 17l

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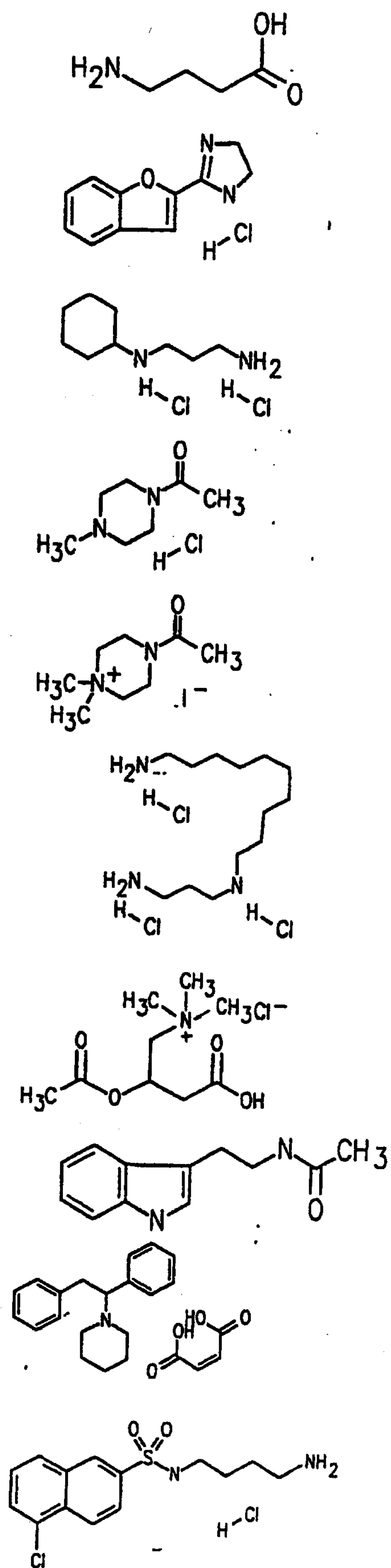


FIG. 17m

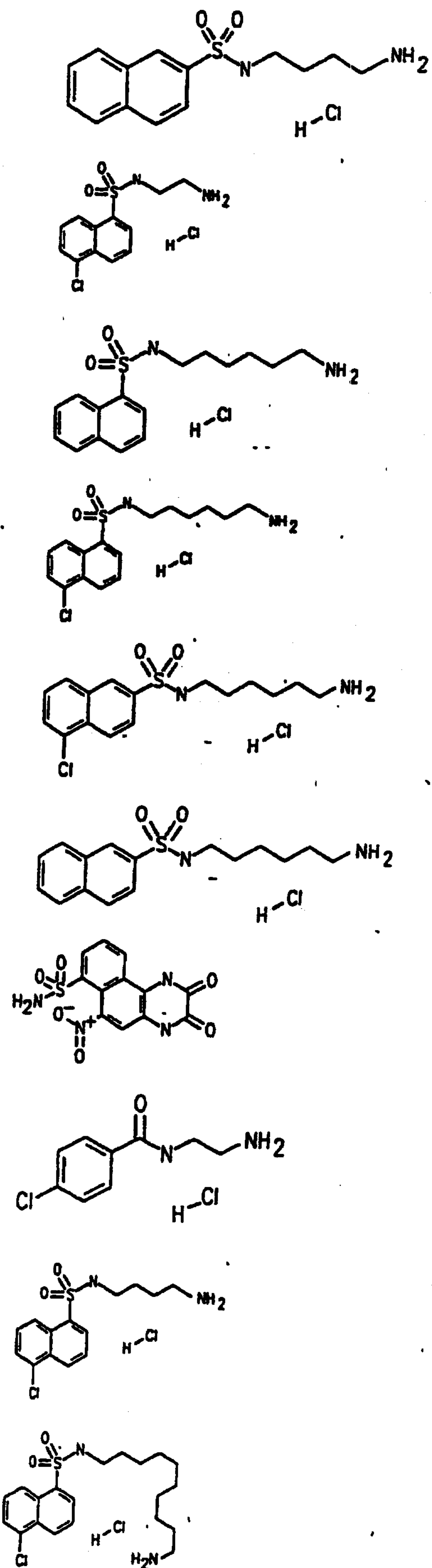


FIG. 17n

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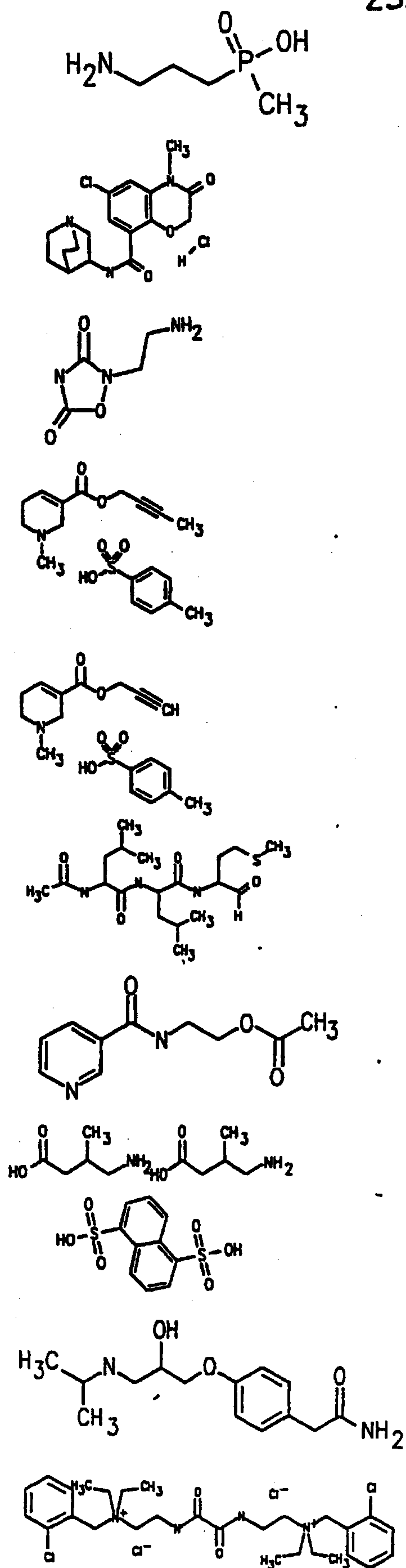


FIG. 17o

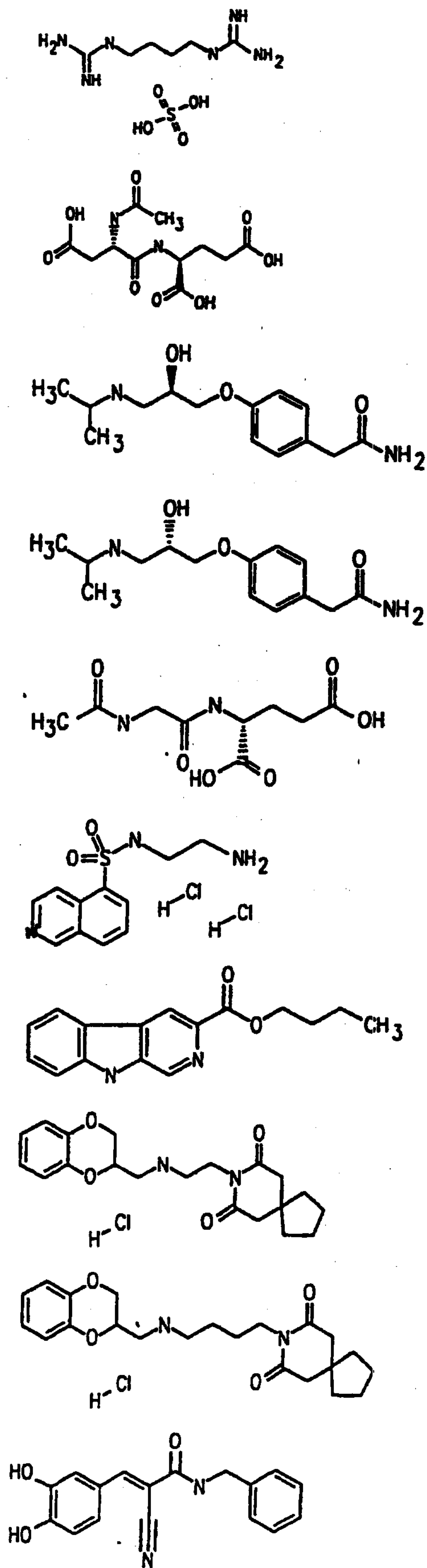


FIG. 17p

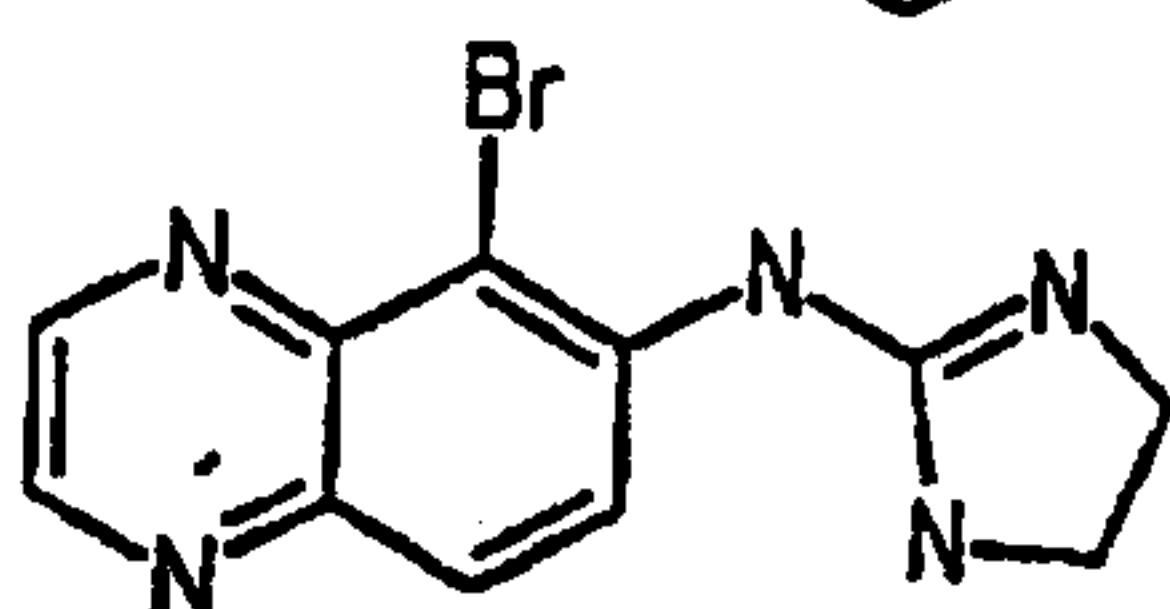
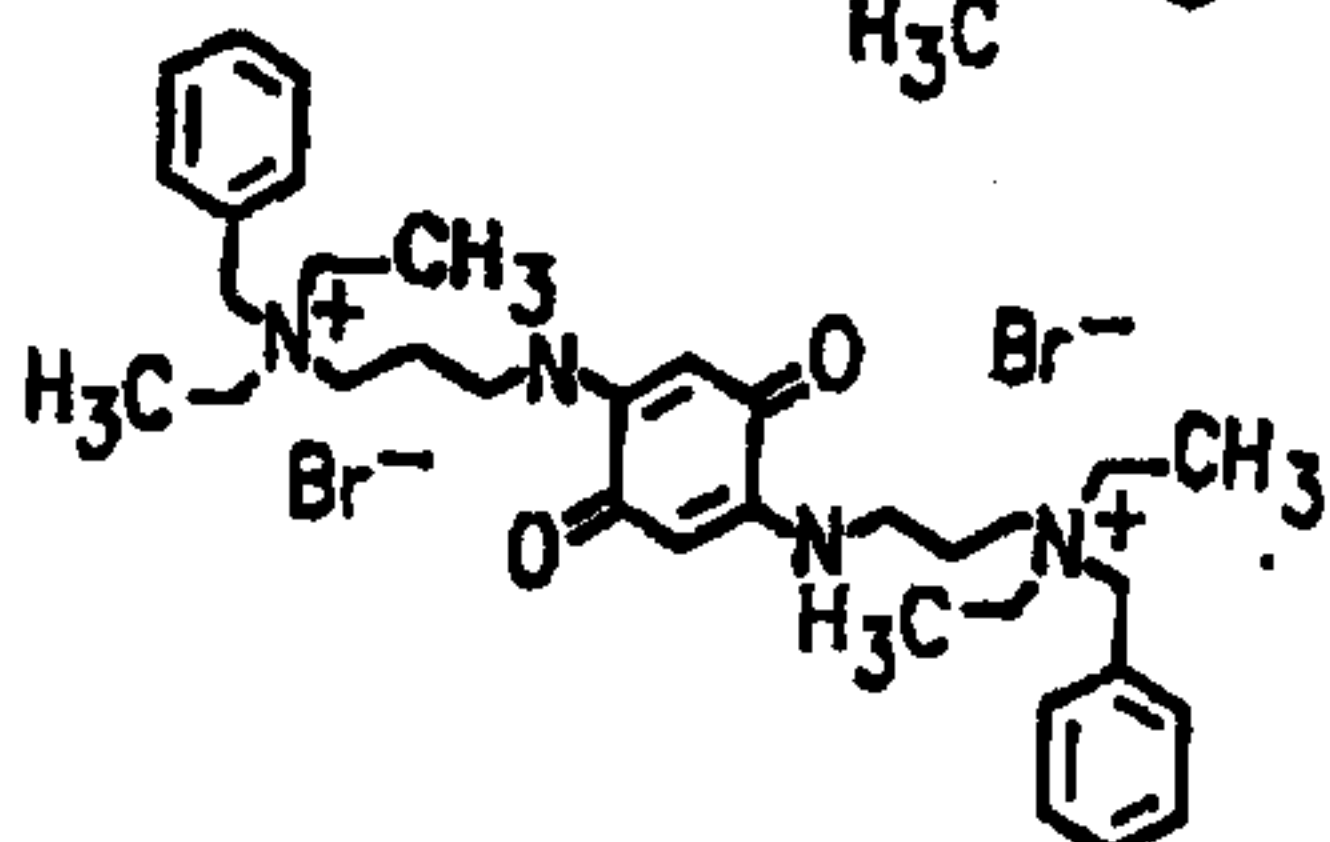
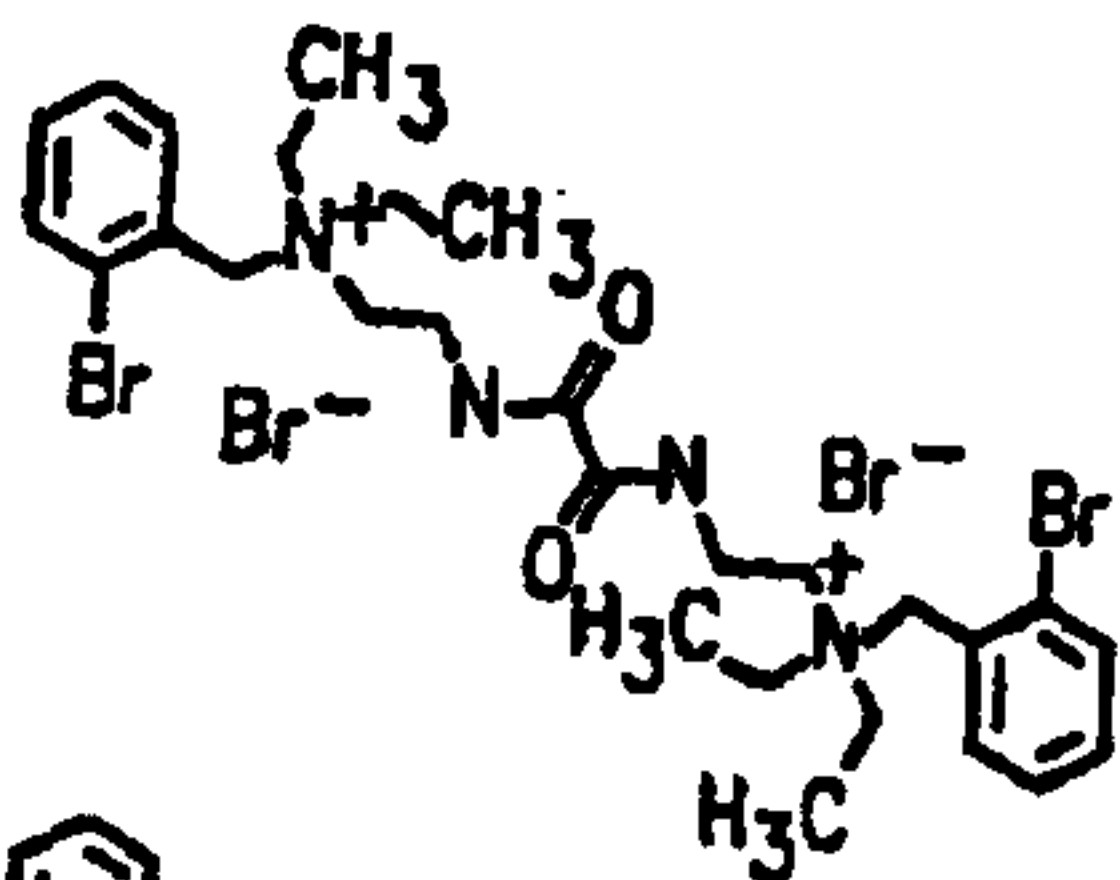
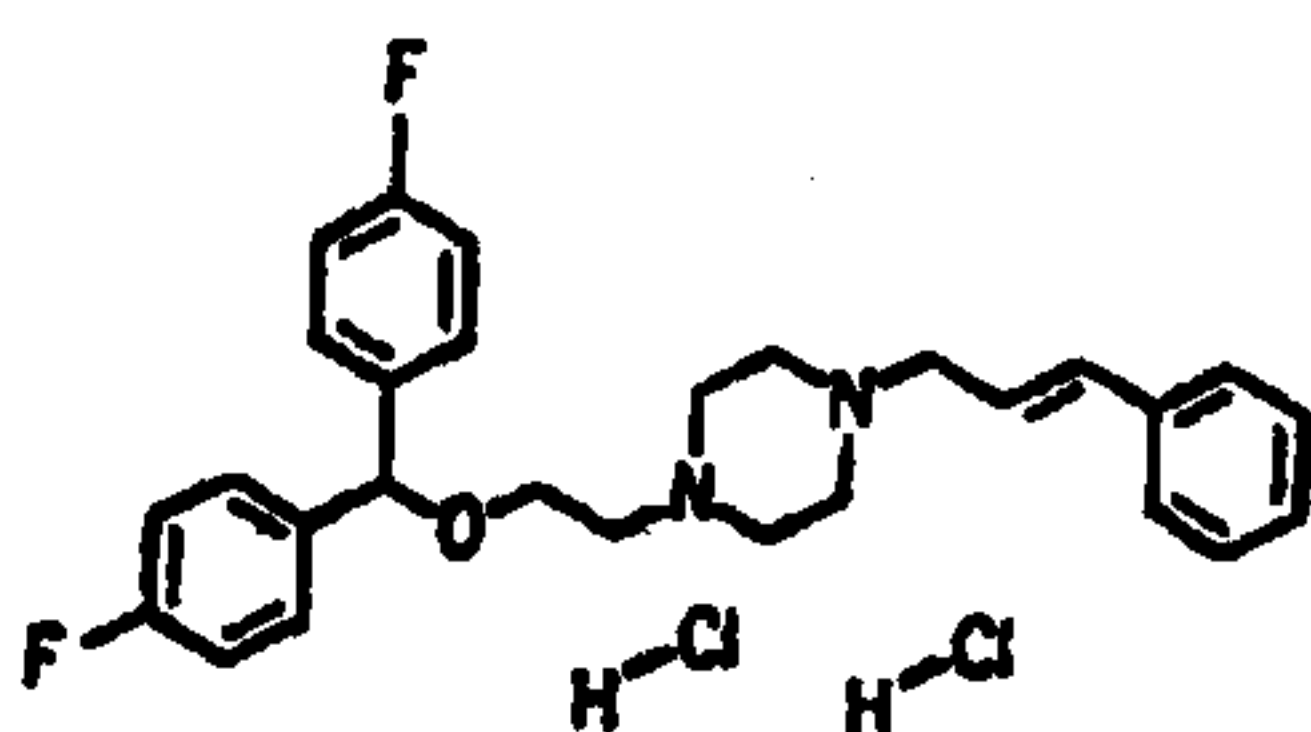
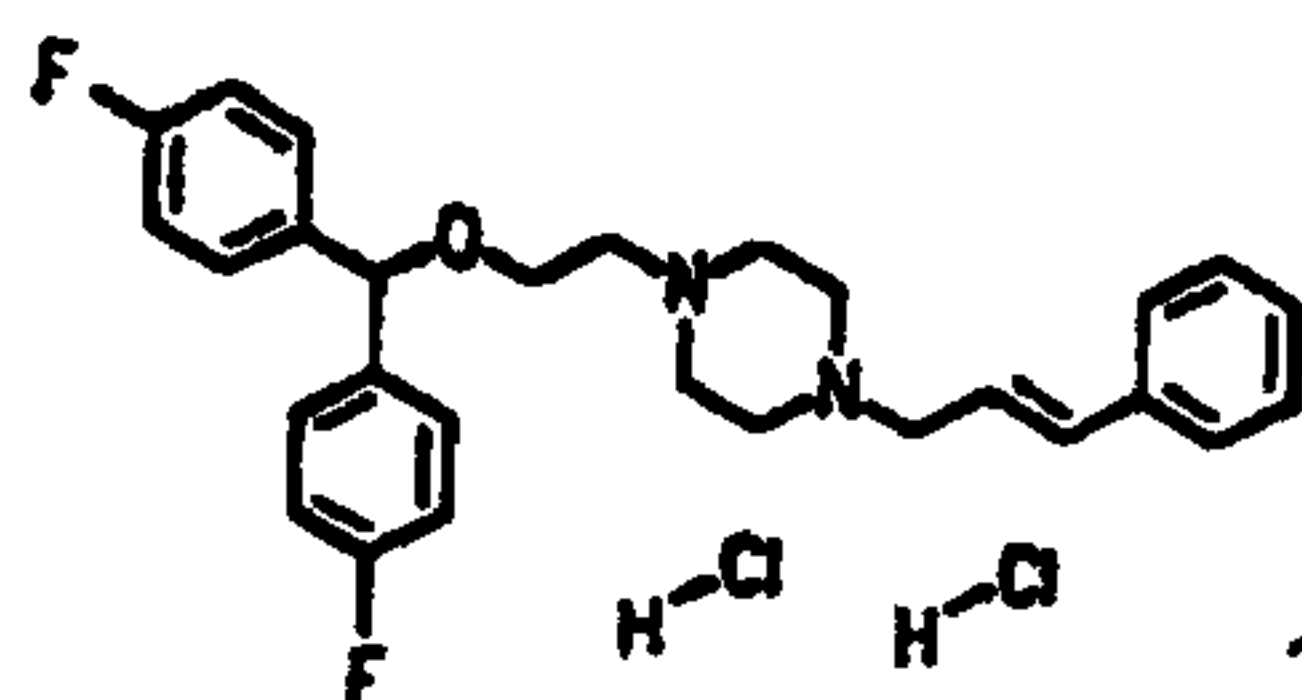
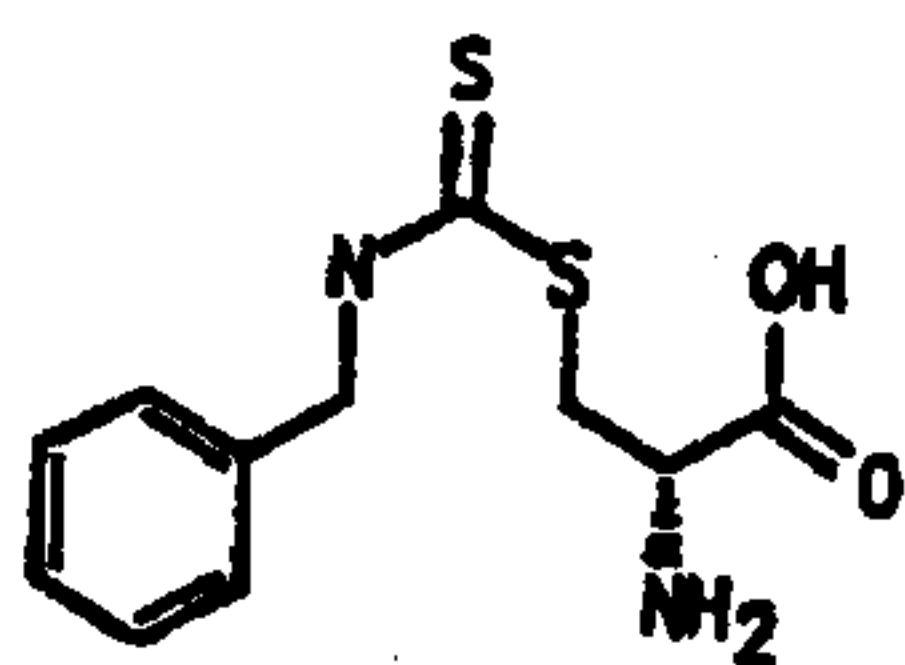
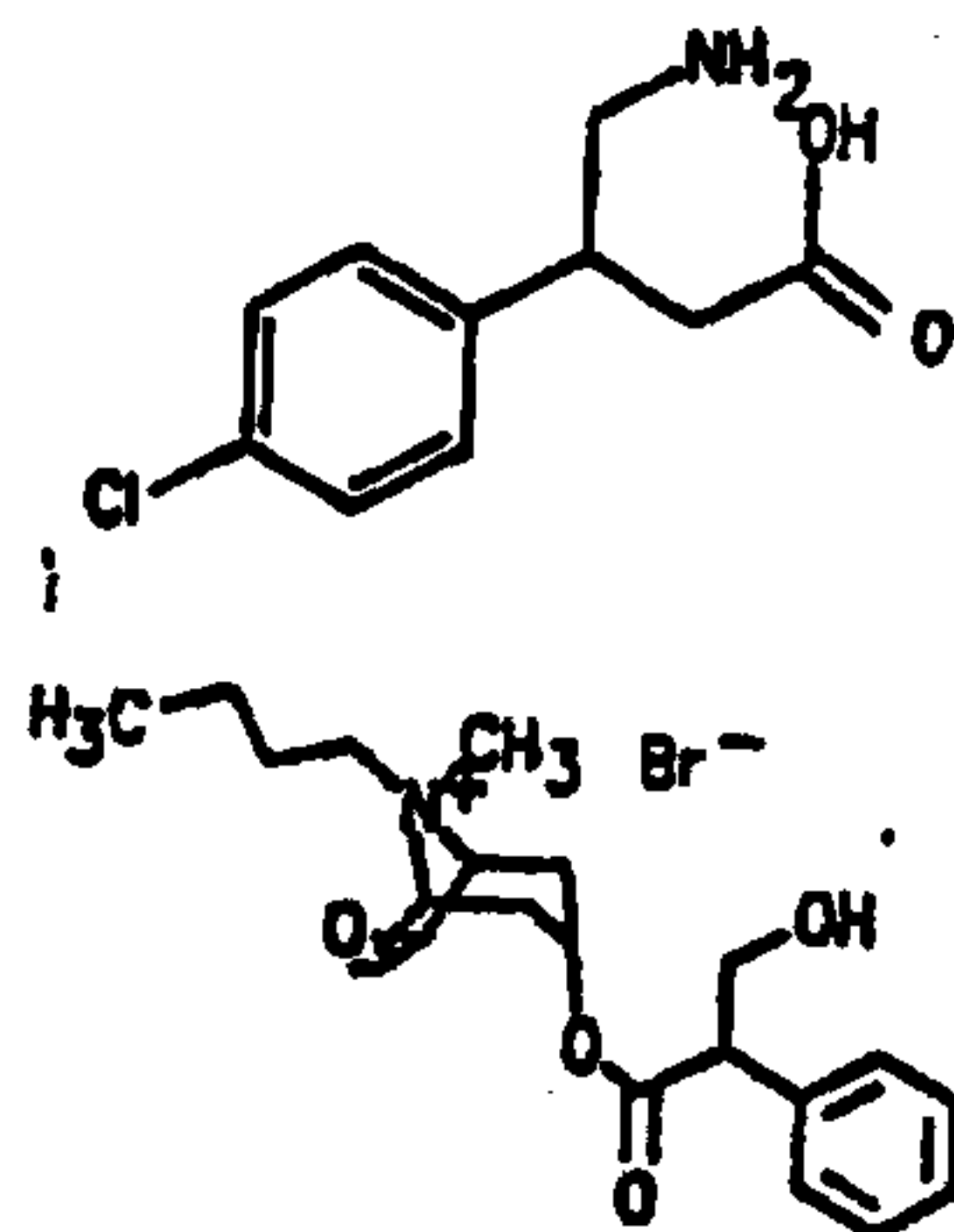
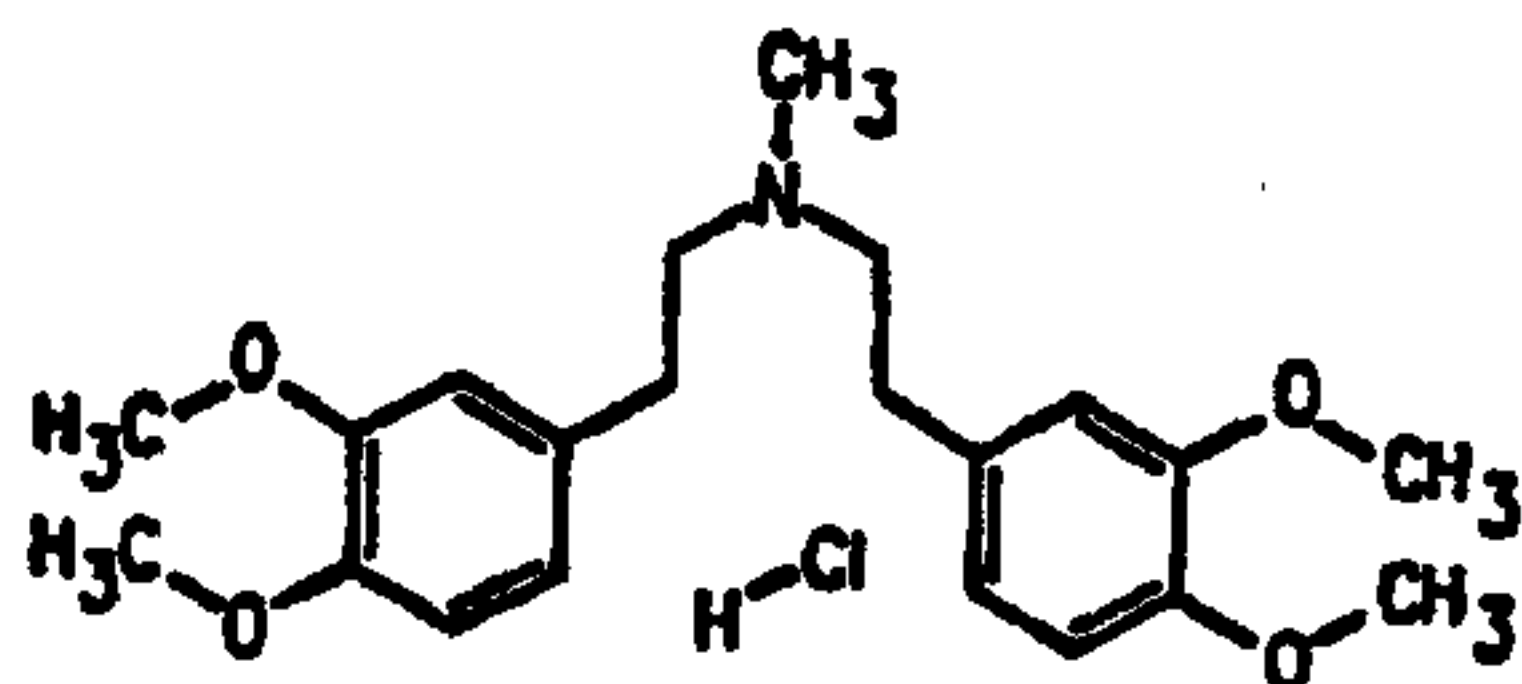
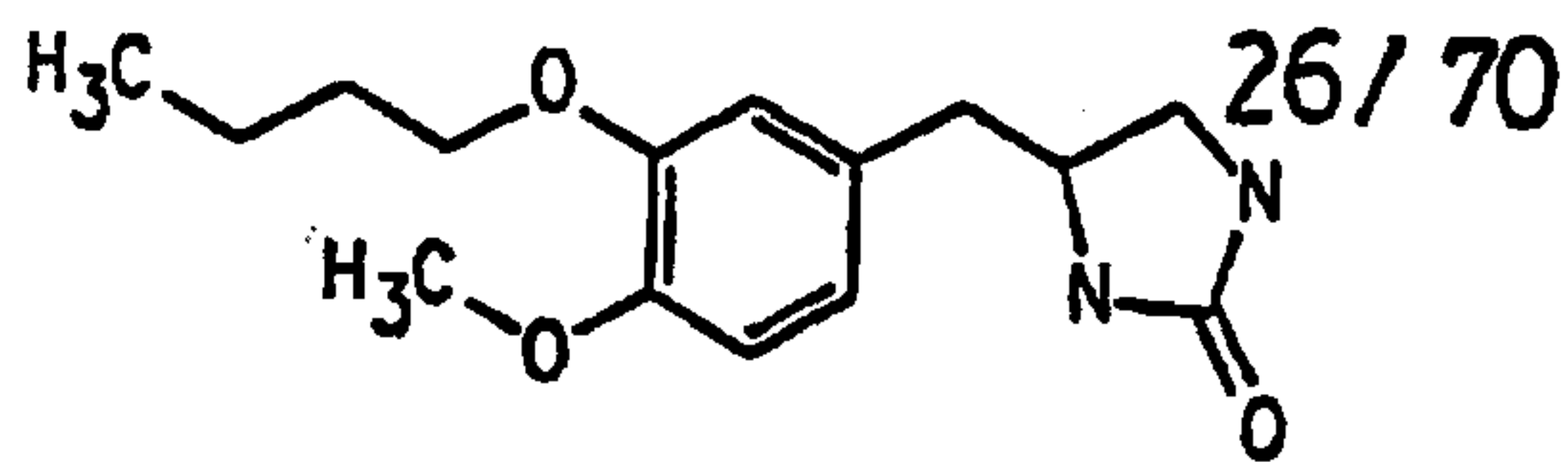


**FIG. 17q**

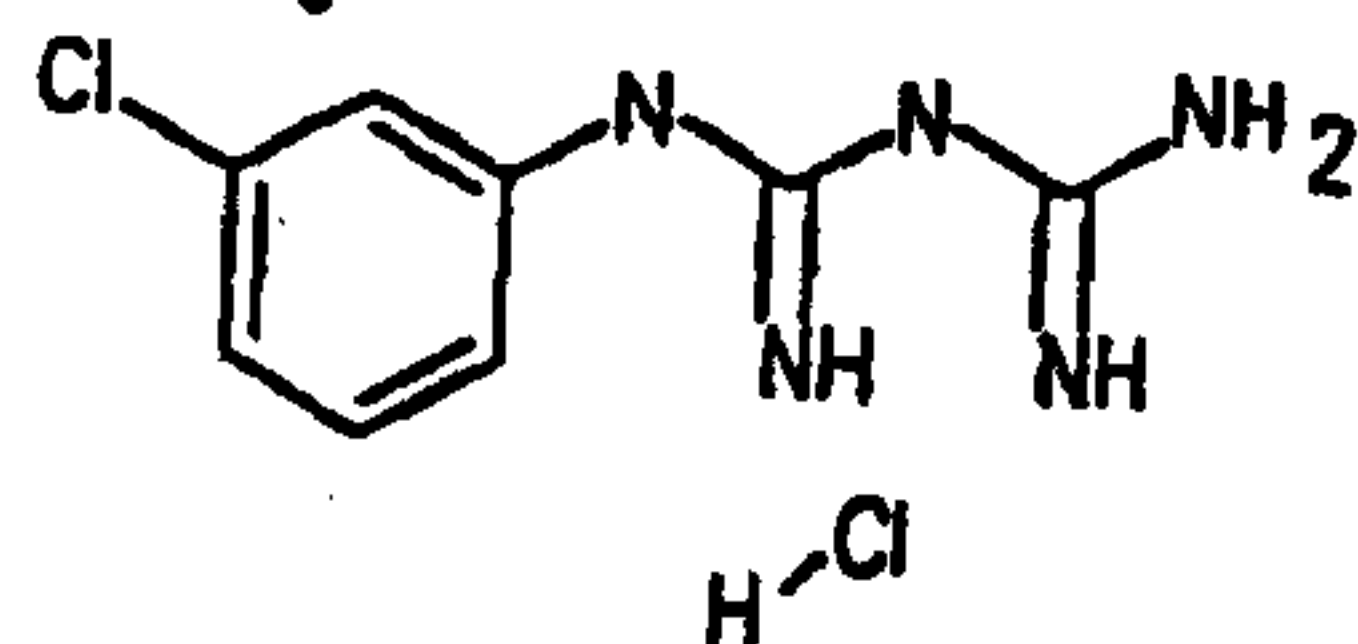
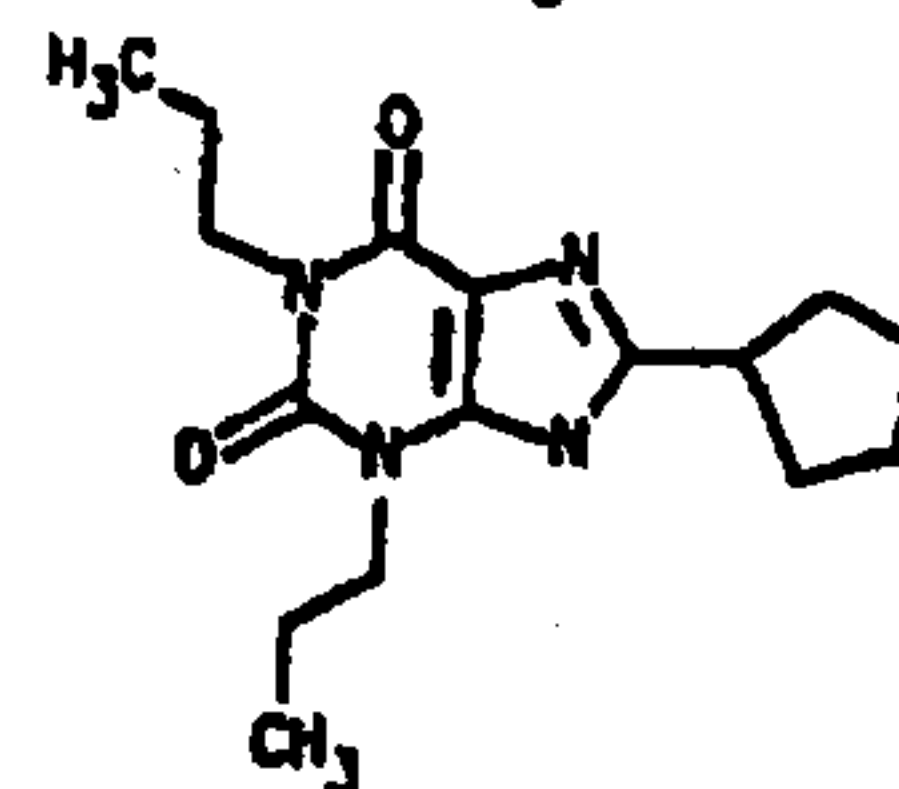
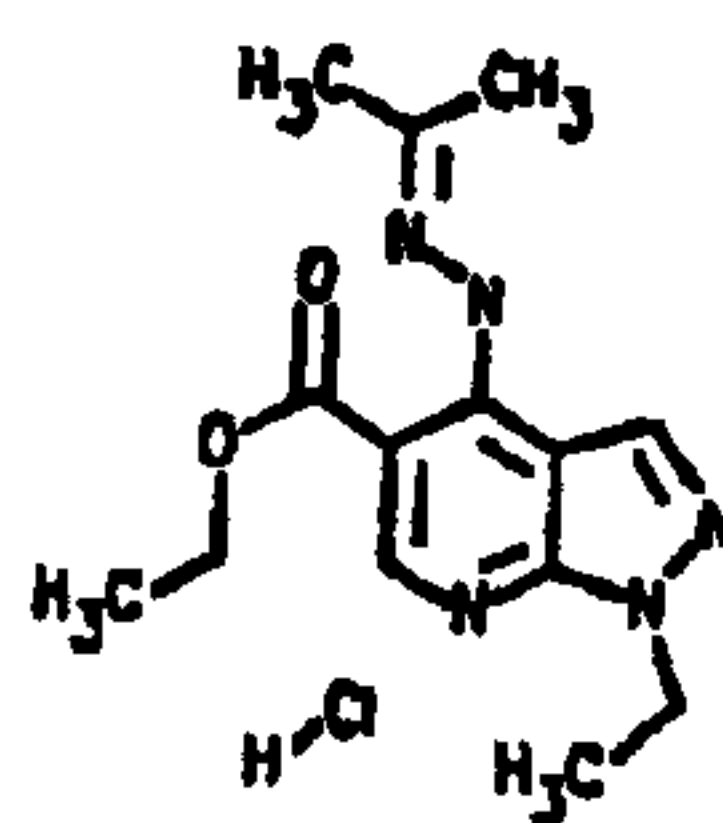
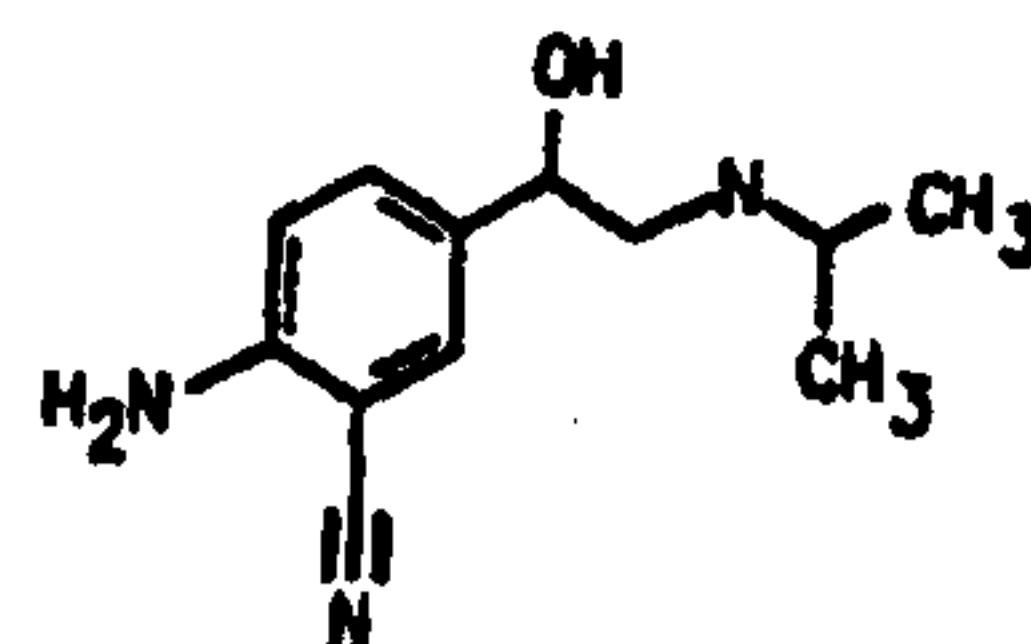
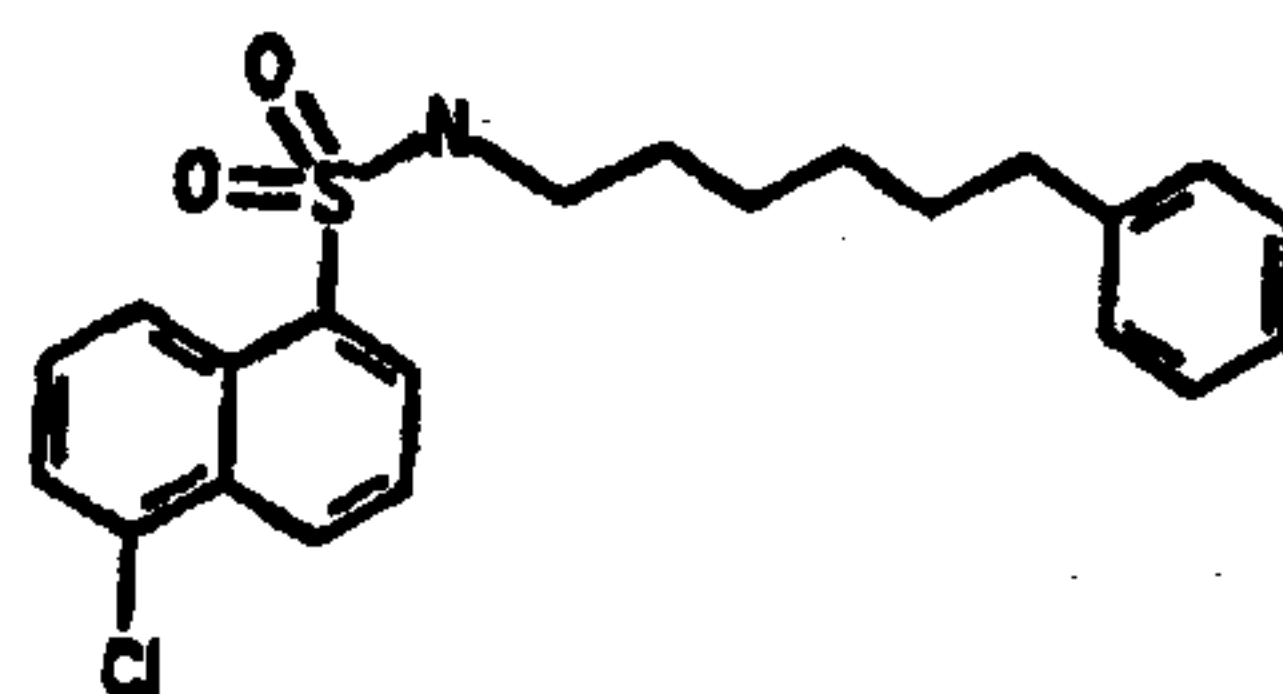
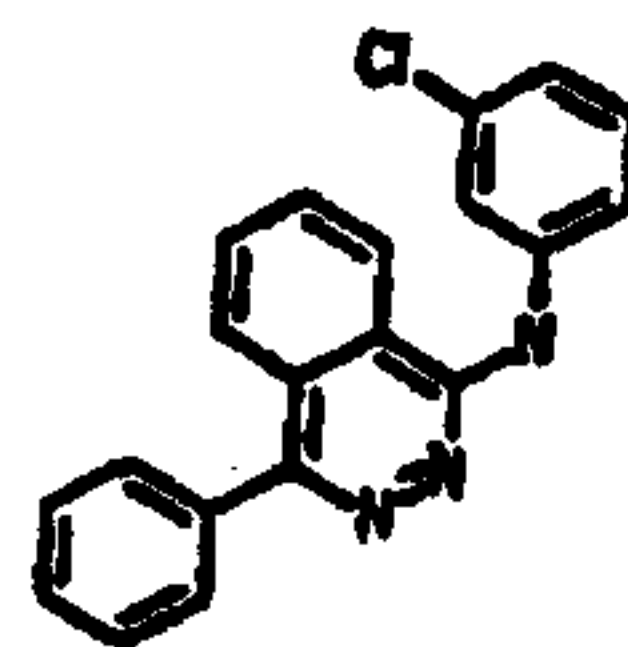
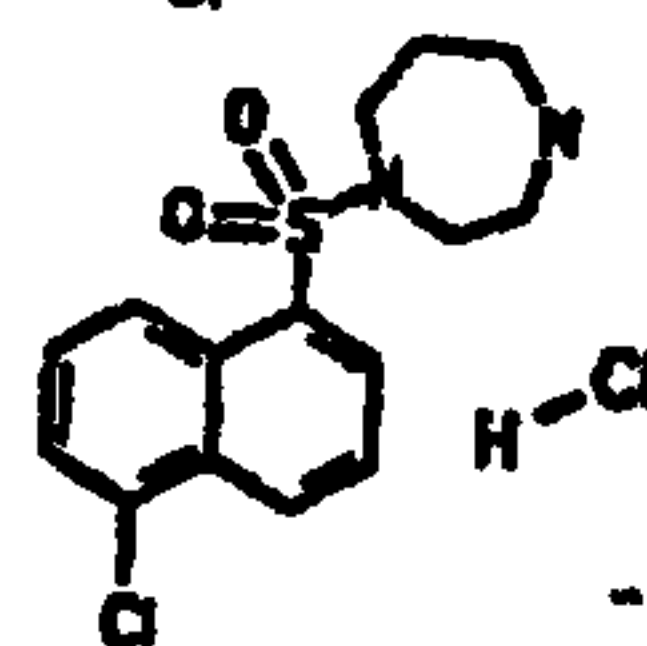
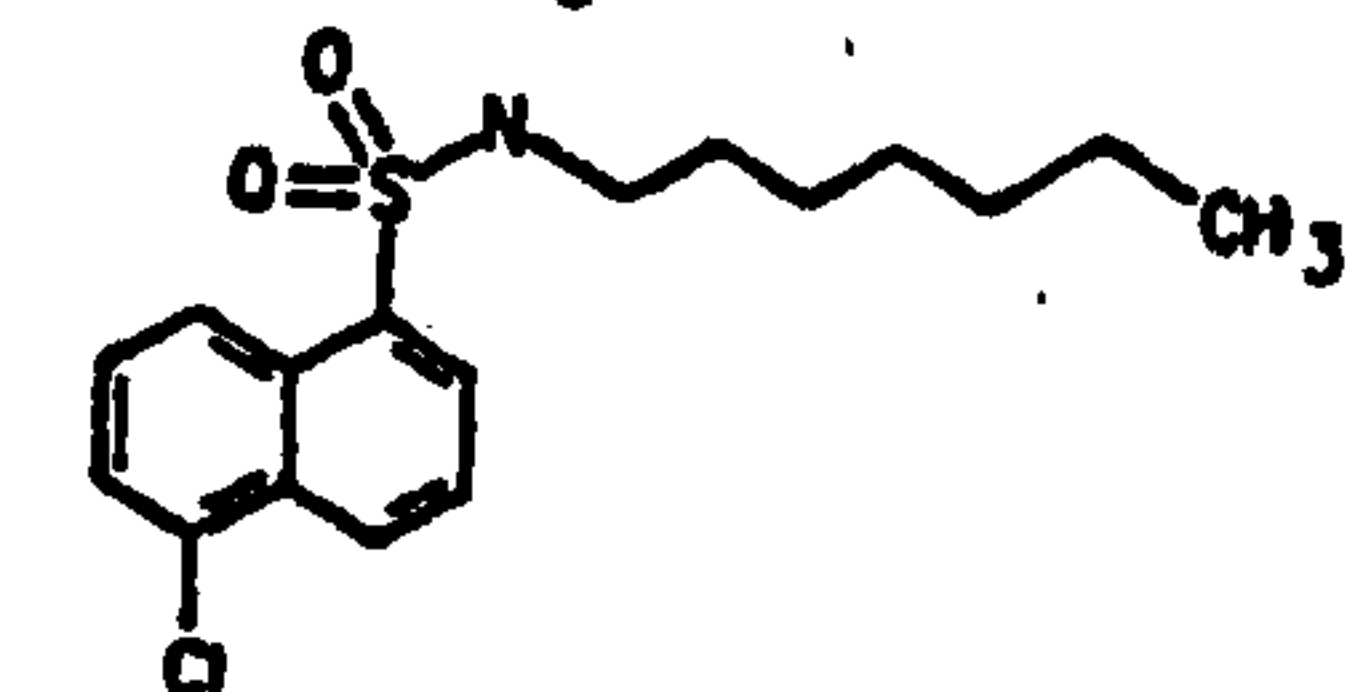
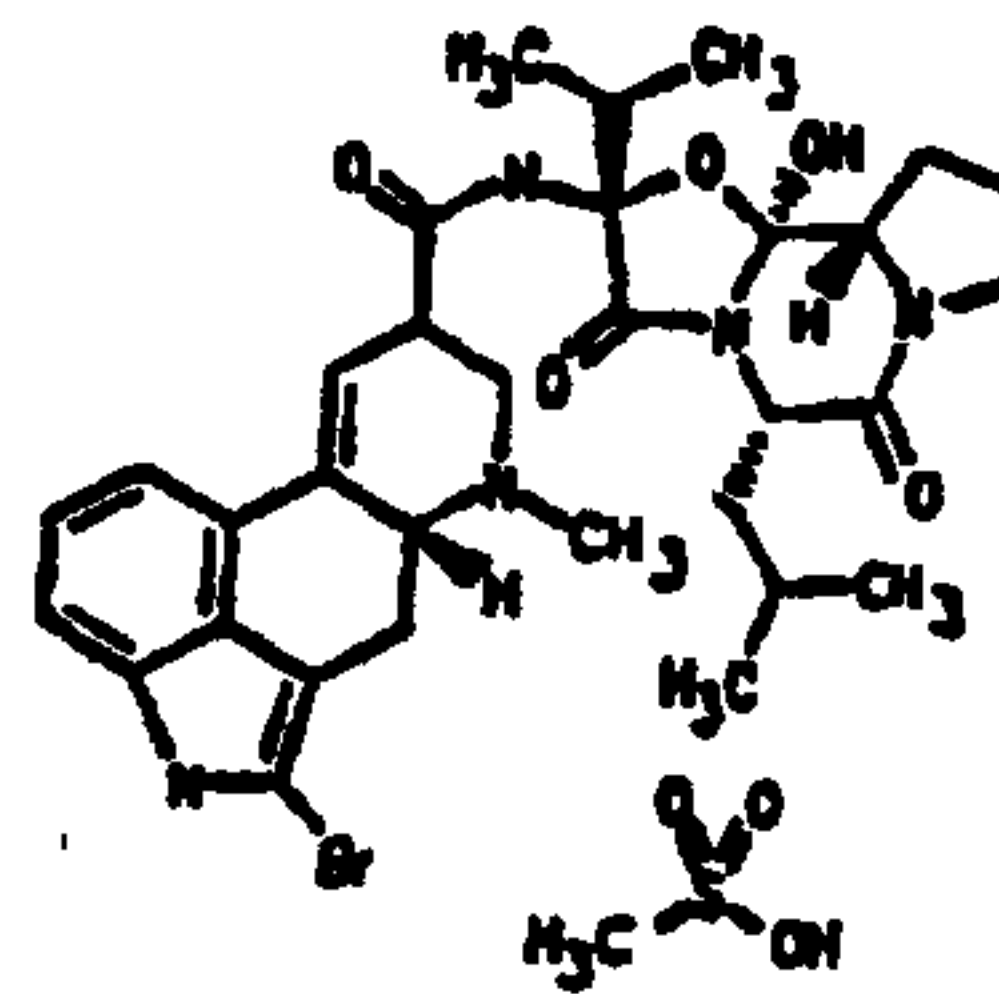
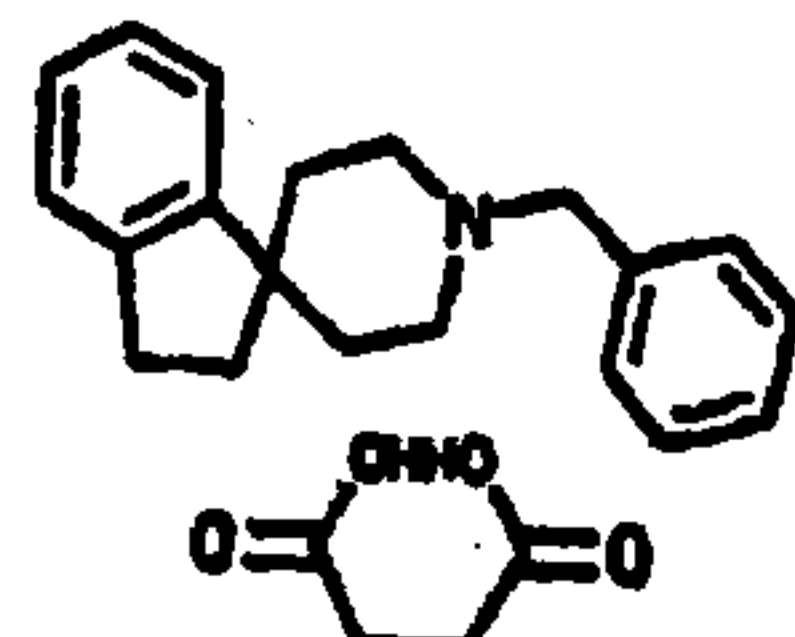


**FIG. 17r**

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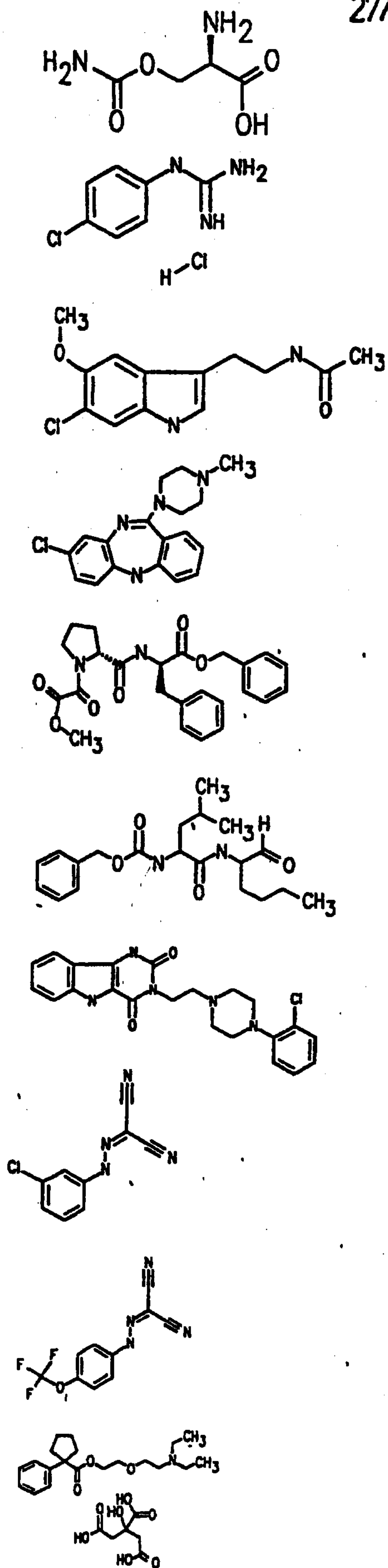


FIG. 17s

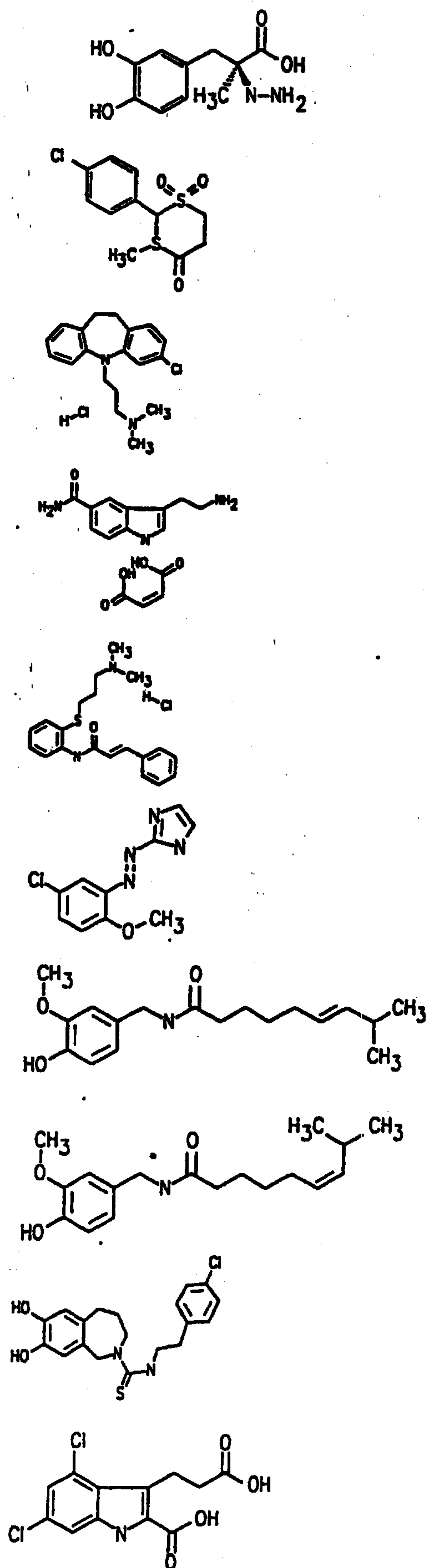


FIG. 17t

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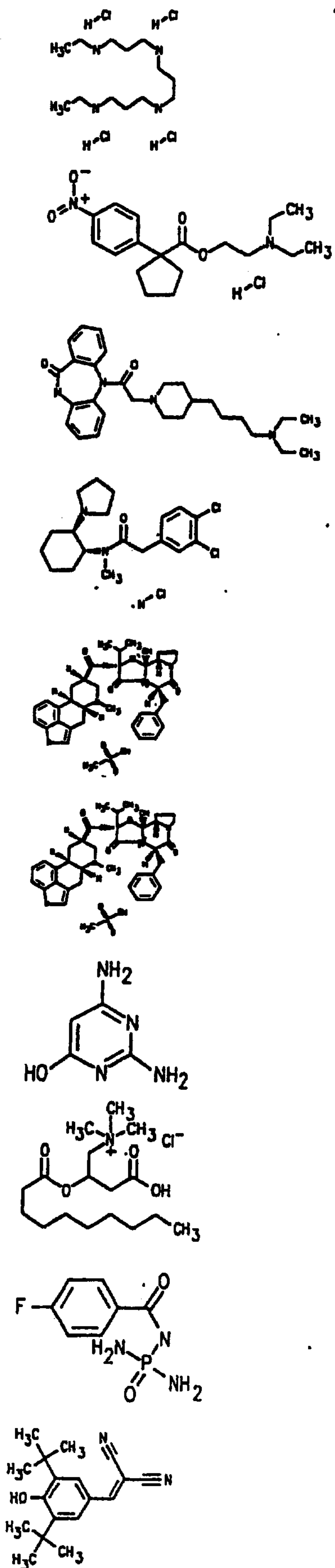


FIG. 17u

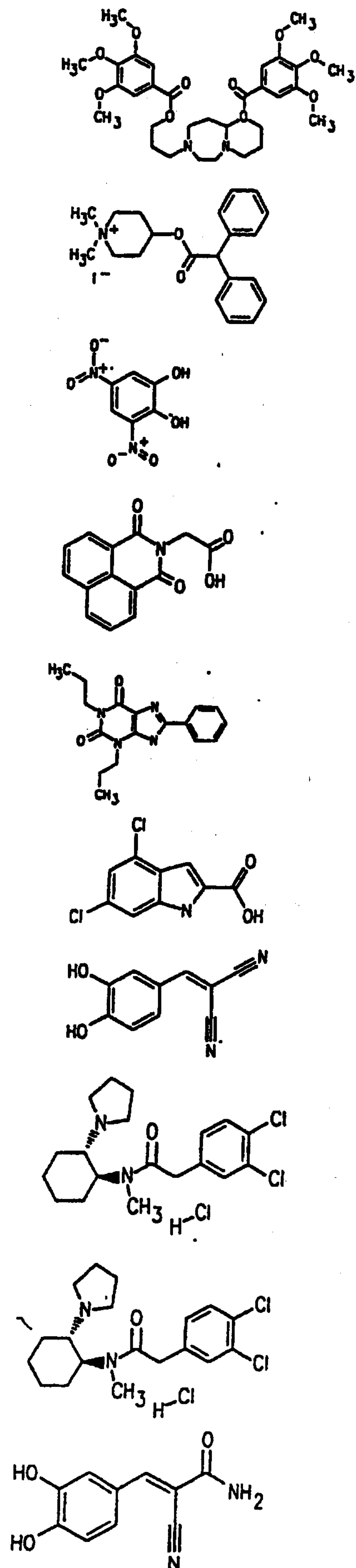


FIG. 17v

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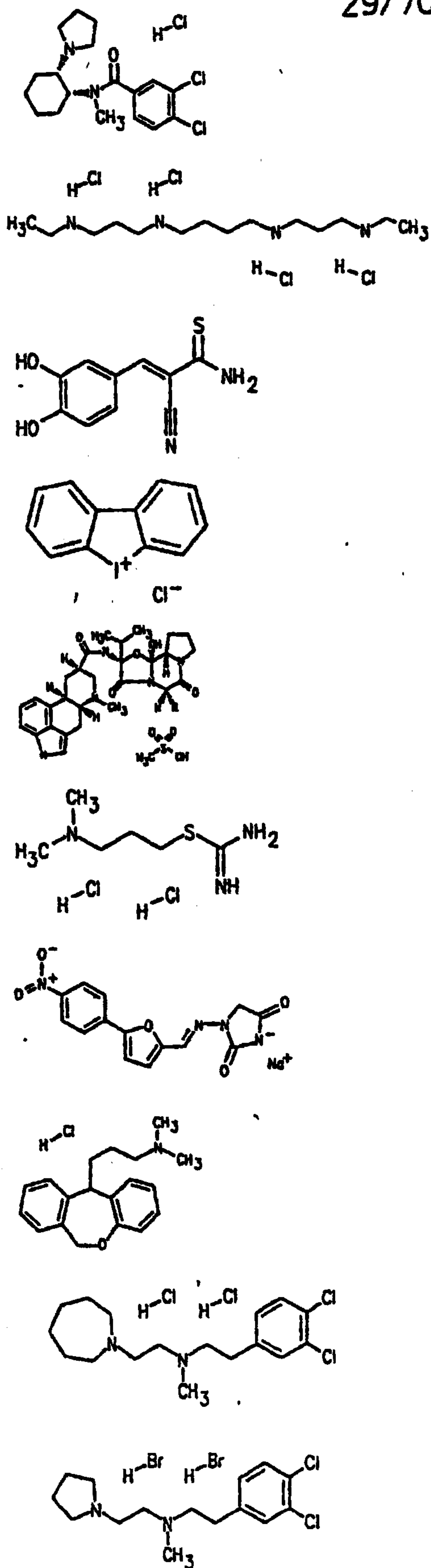


FIG. 17w

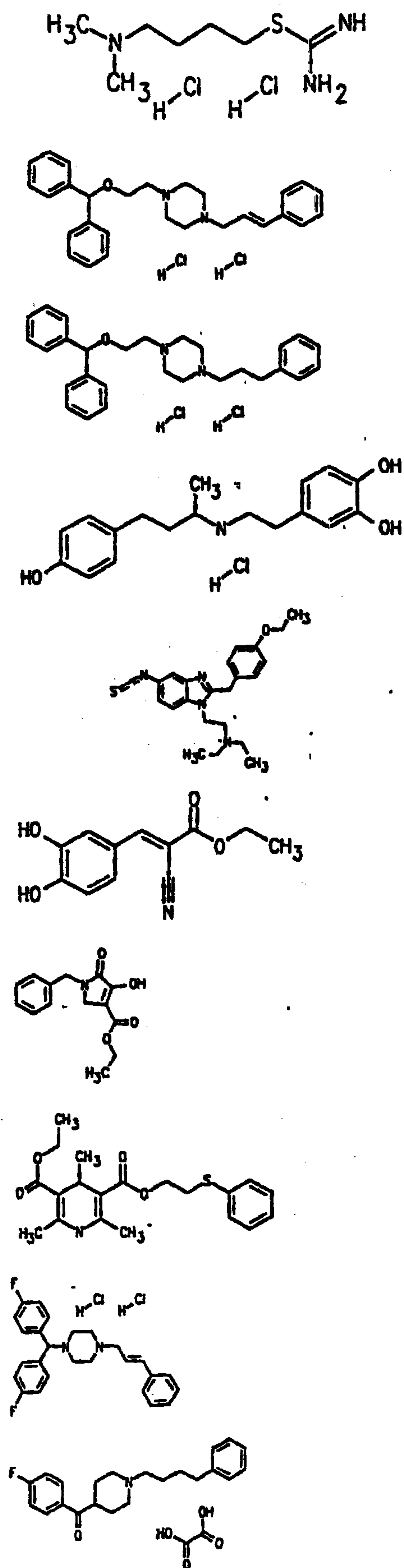


FIG. 17x

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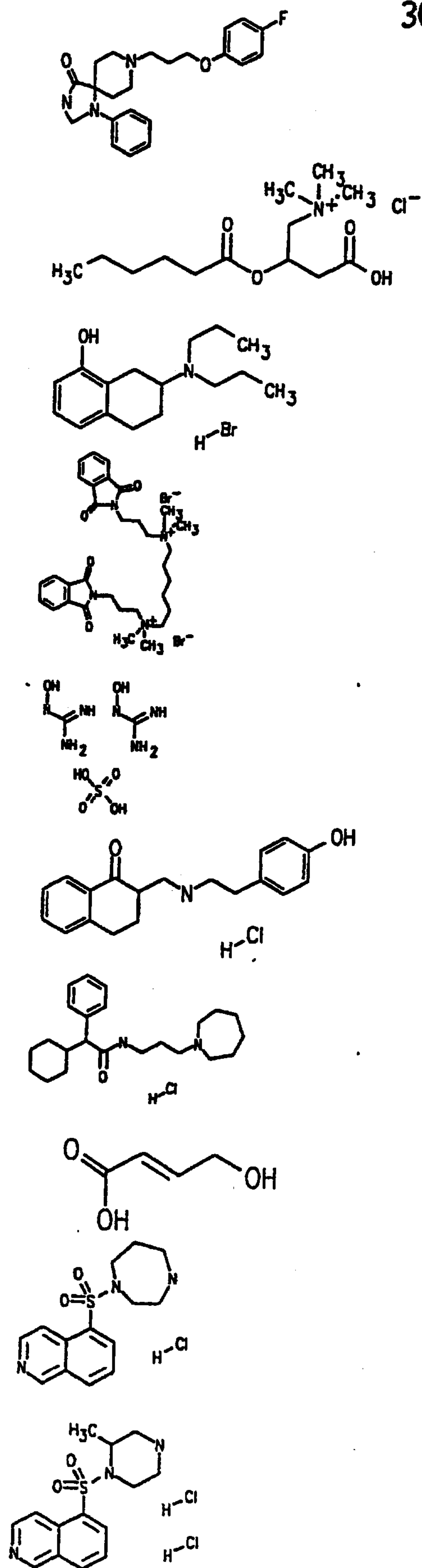


FIG. 17y

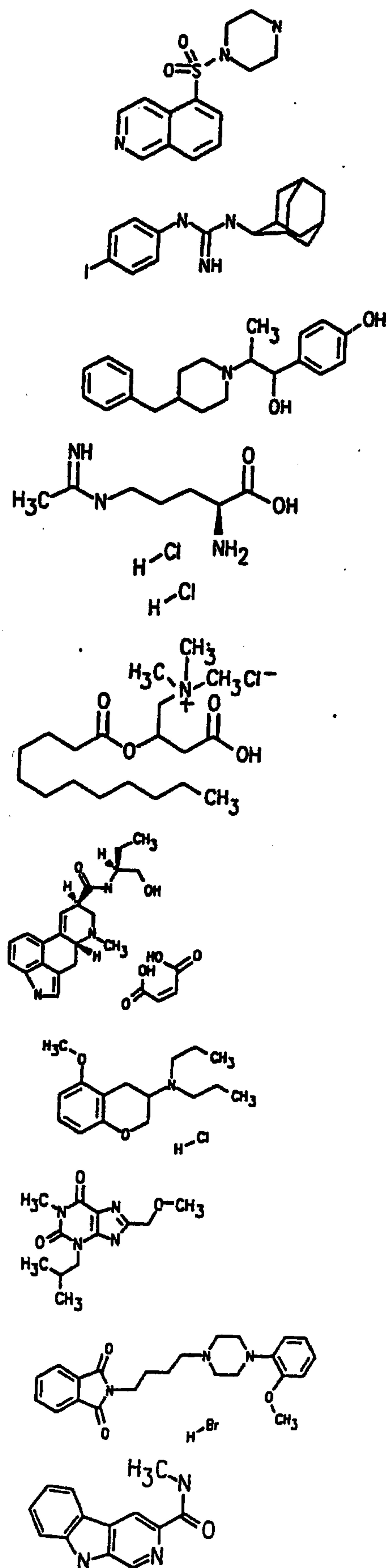


FIG. 17z

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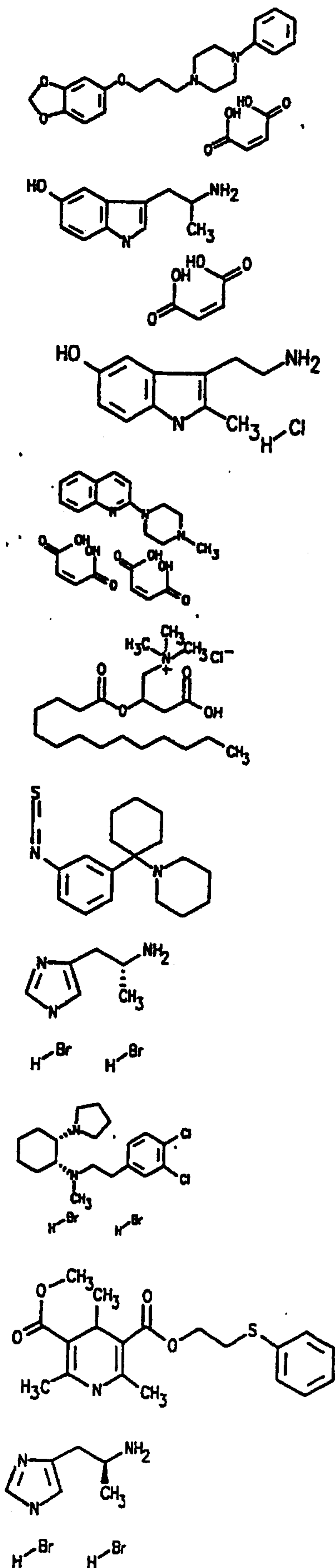


FIG. 17aa

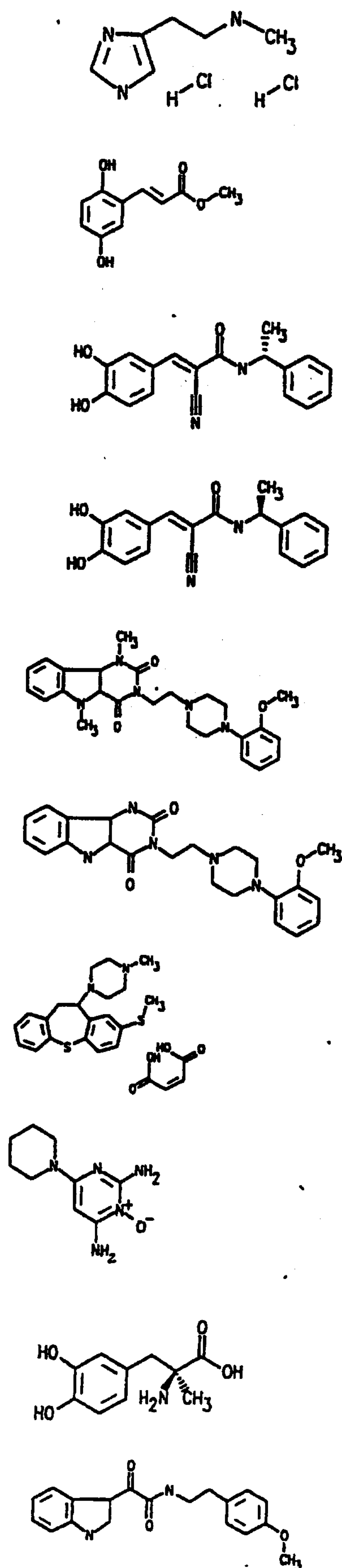


FIG. 17bb

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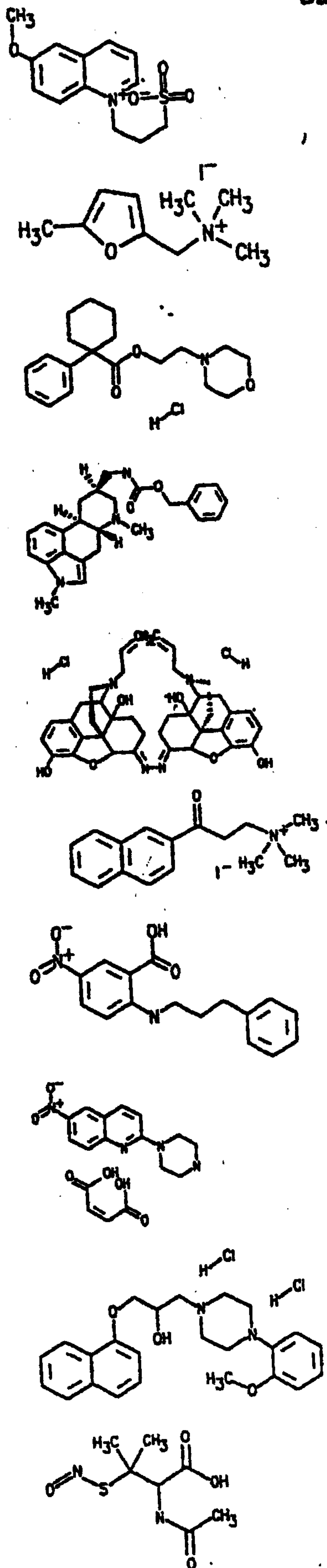


FIG. 17cc

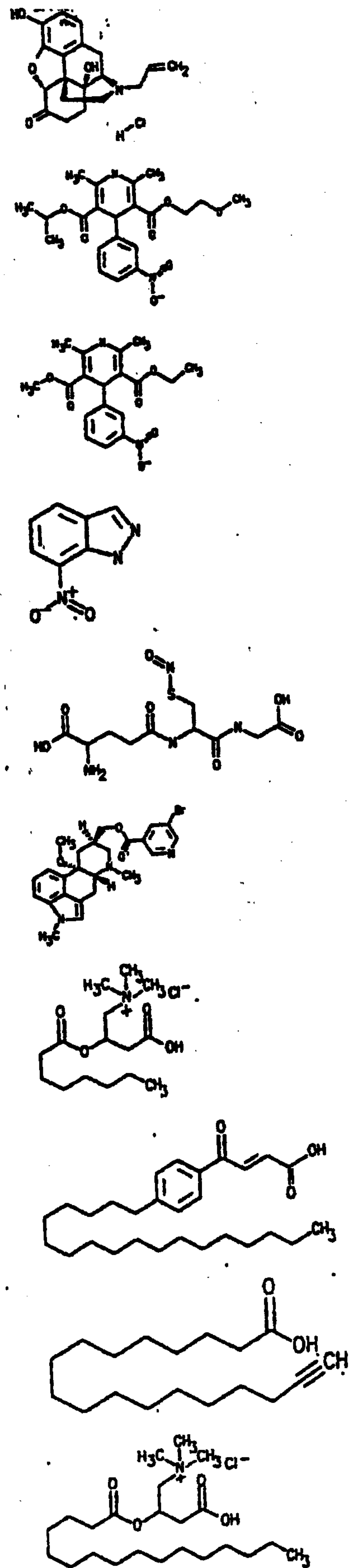


FIG. 17dd

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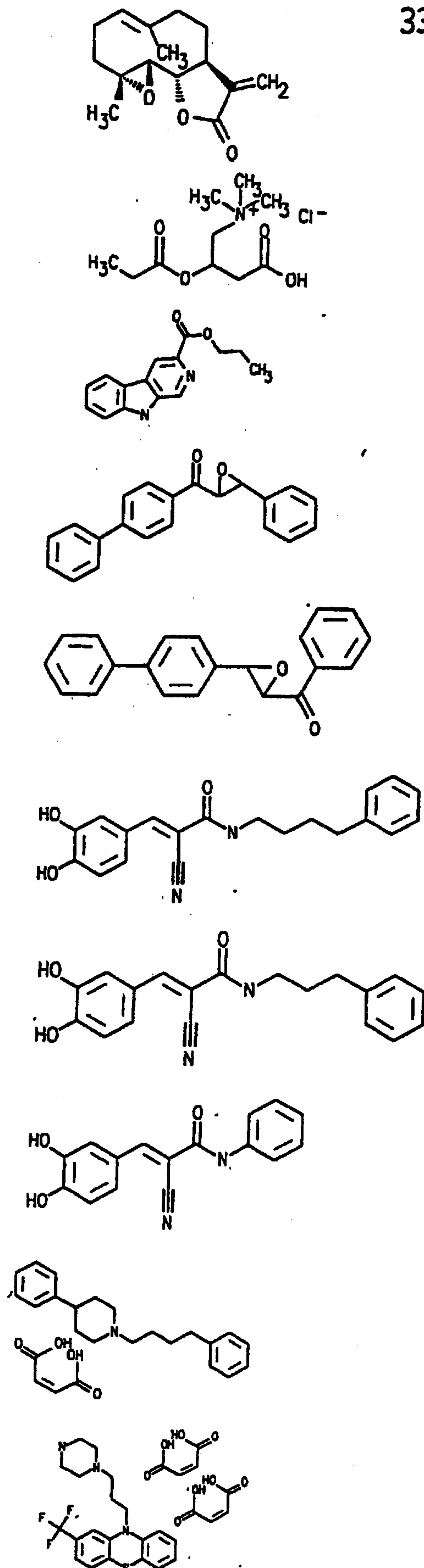


FIG. 17ee

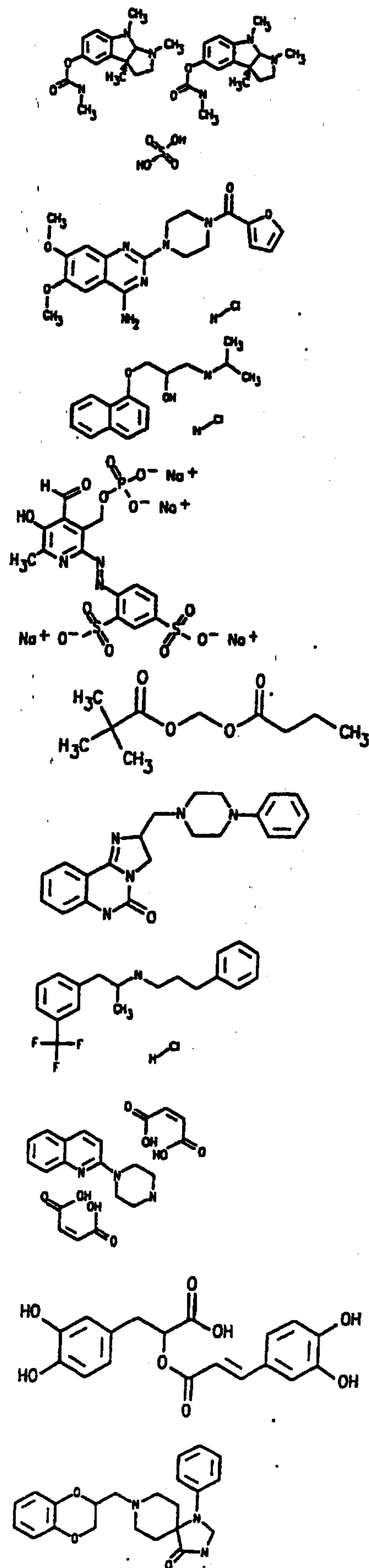


FIG. 17ff

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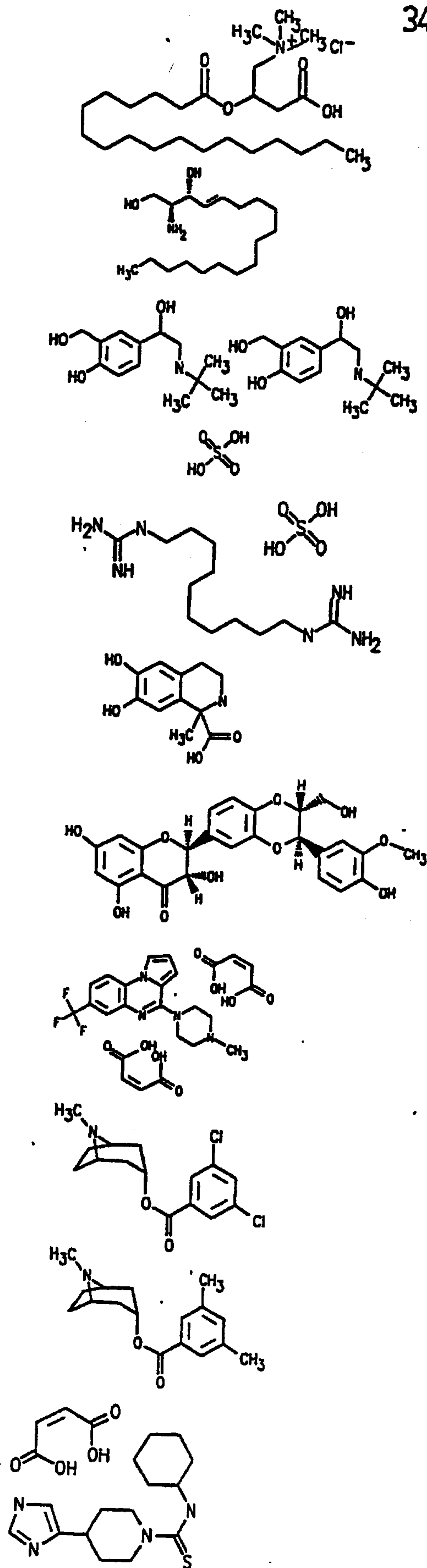


FIG. 17gg

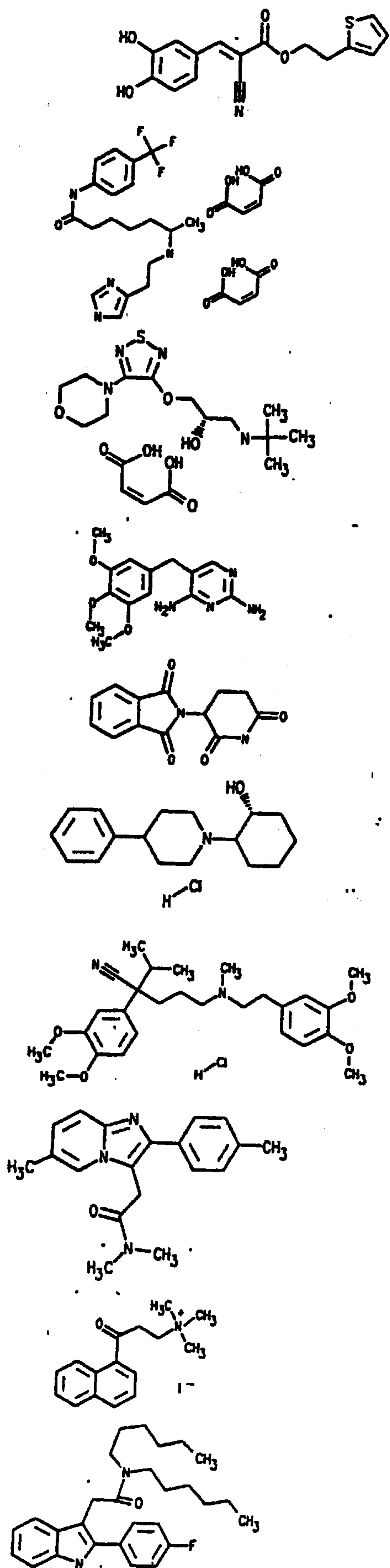


FIG. 17hh

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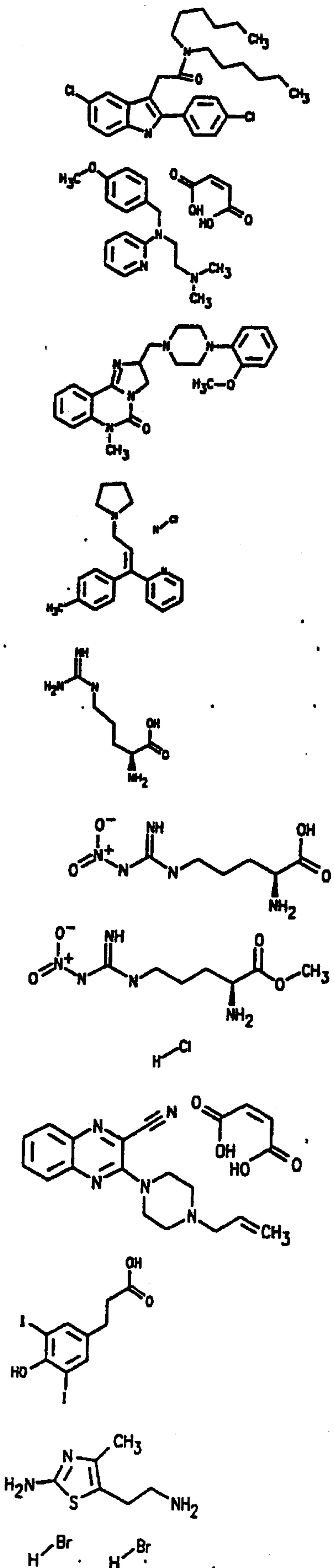


FIG. 17ii

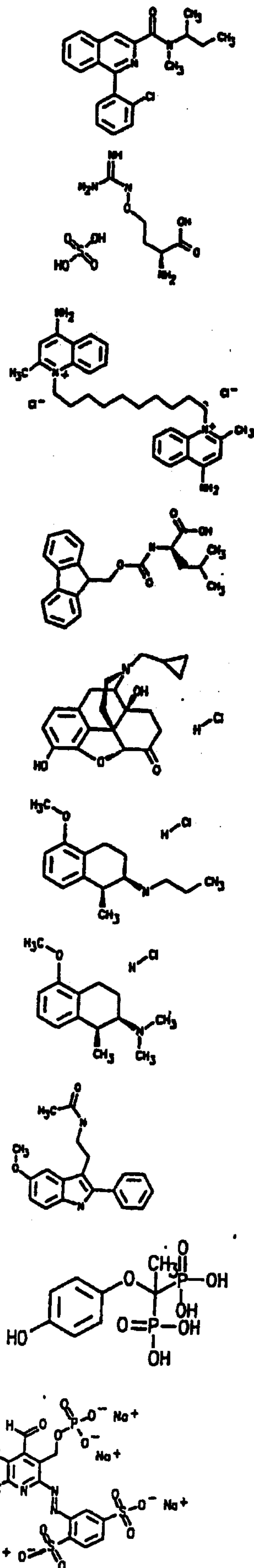


**FIG. 17ii**

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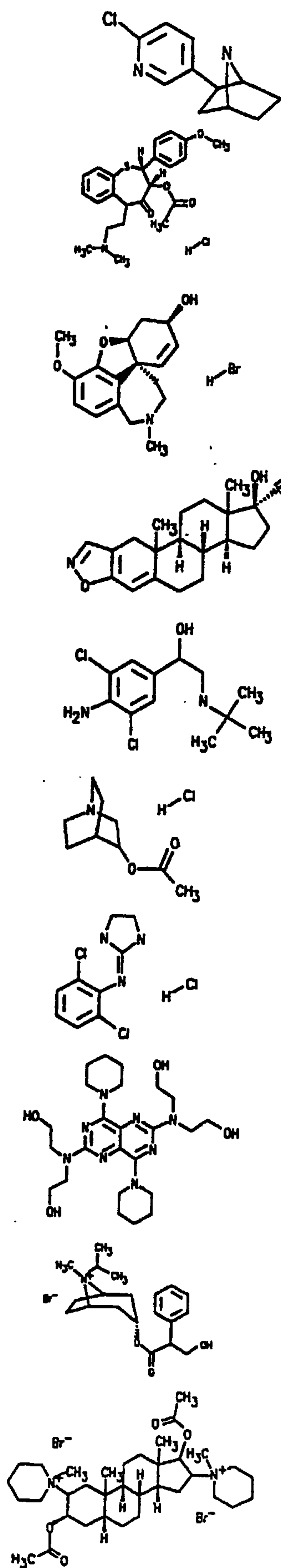


FIG. 17kk

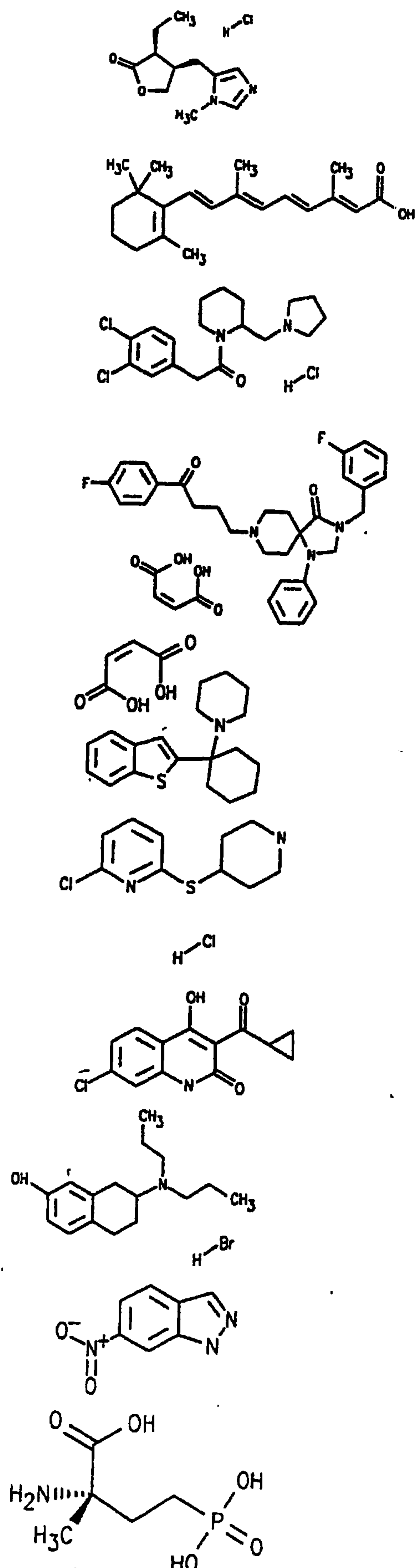


FIG. 17ll

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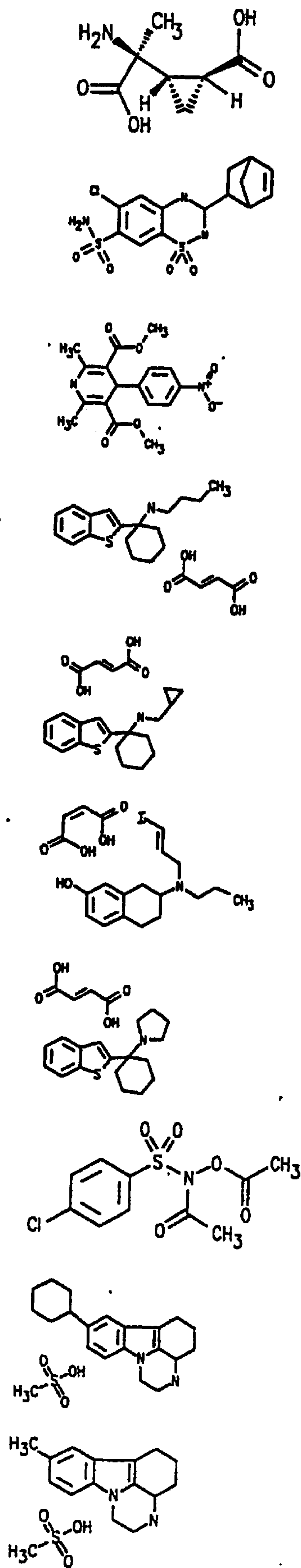


FIG. 17mm

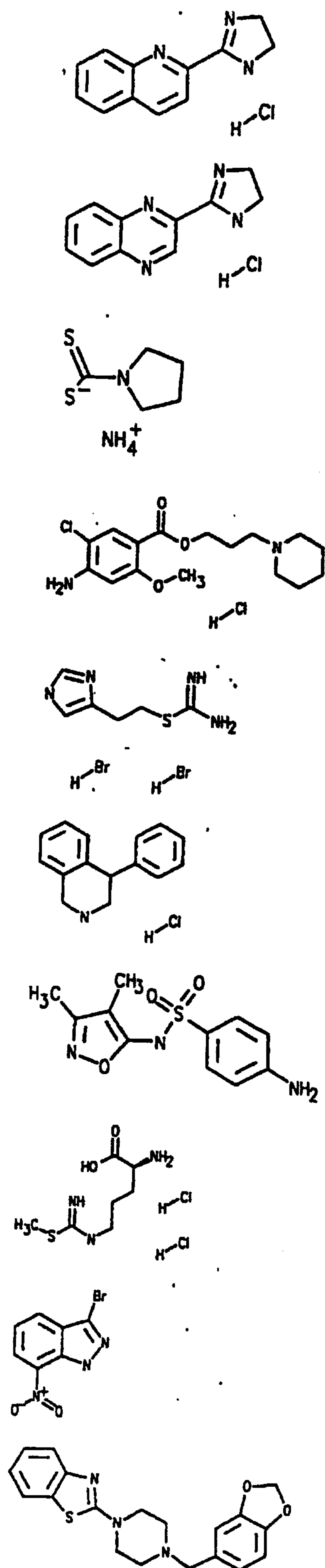


FIG. 17nn

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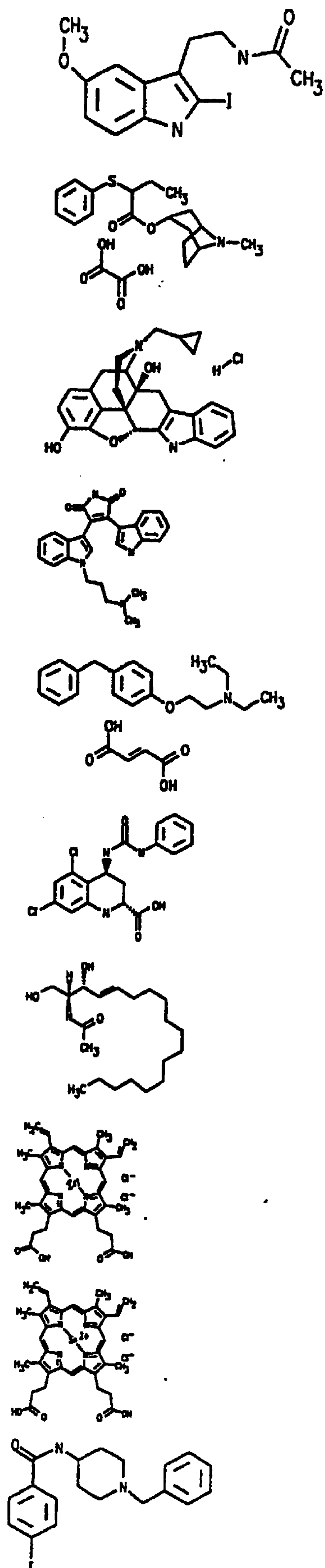


FIG. 1700

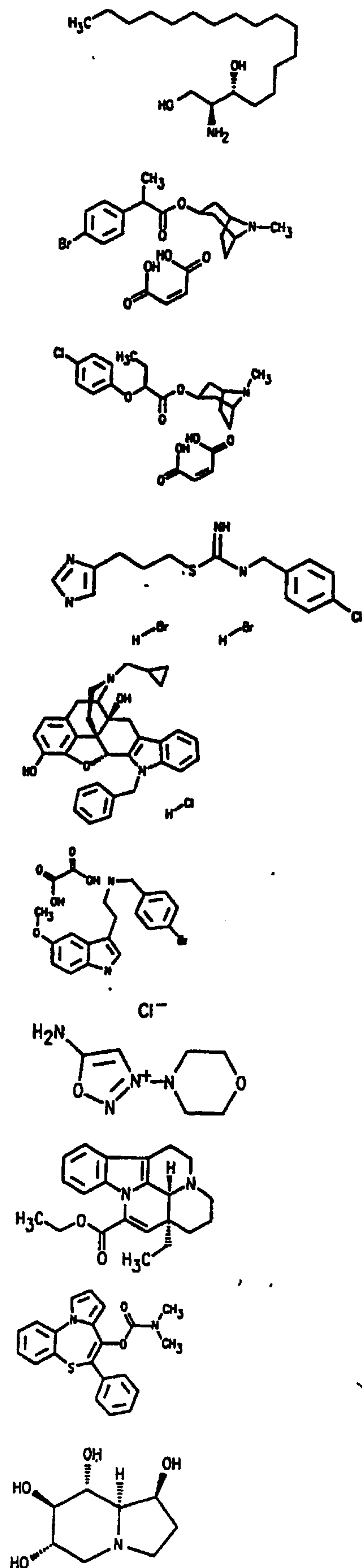


FIG. 17pp

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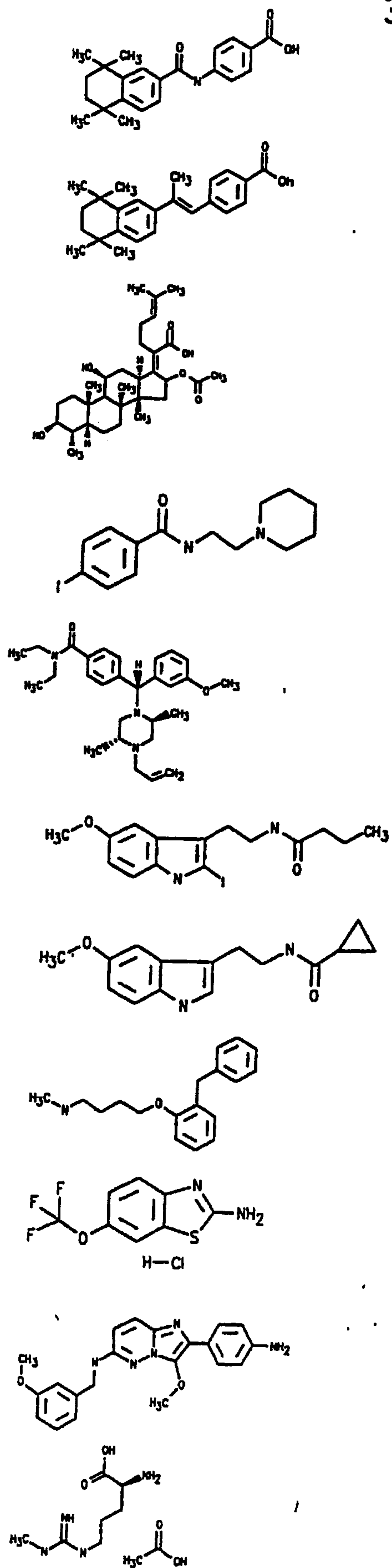


FIG. 17qq

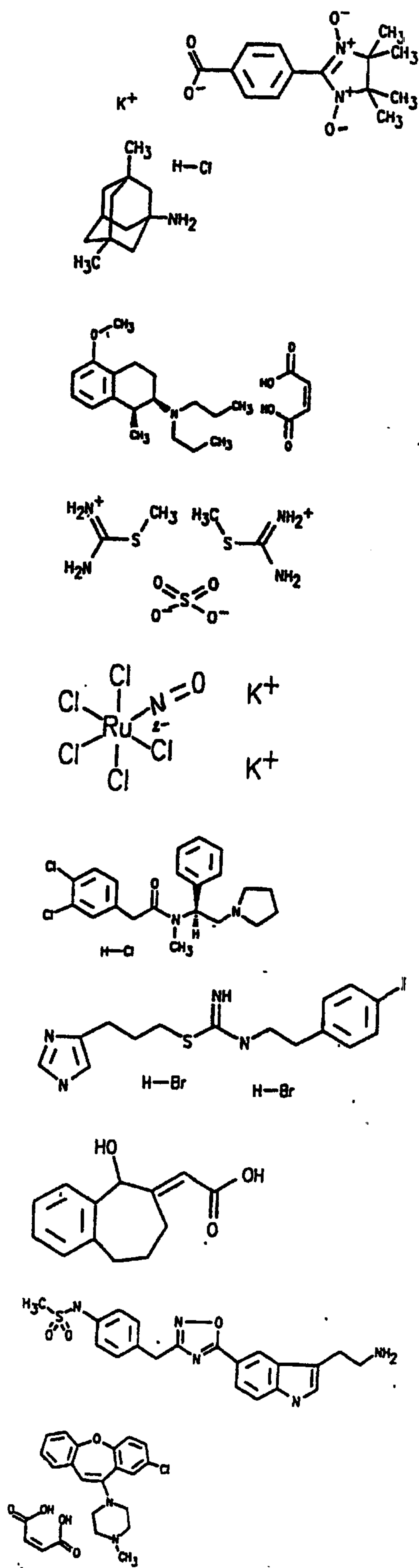


FIG. 17rr

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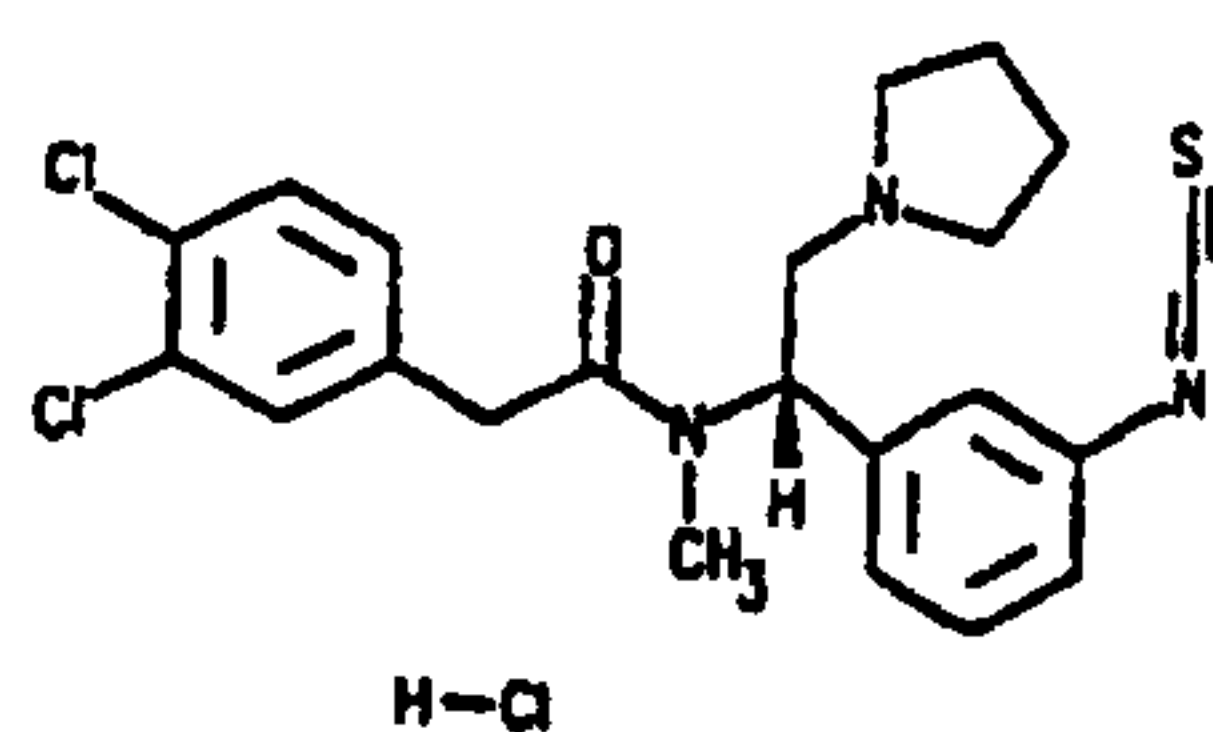
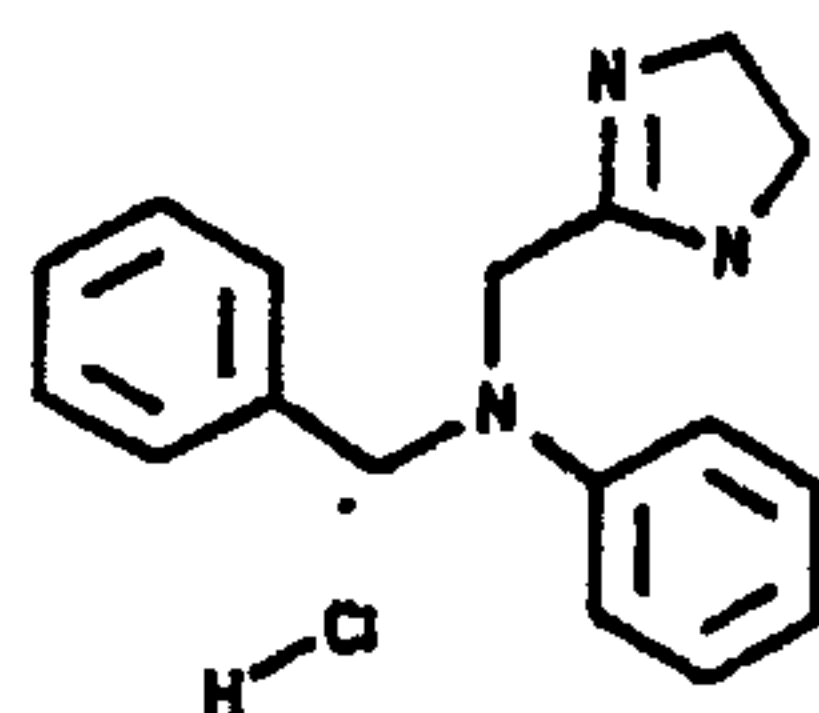
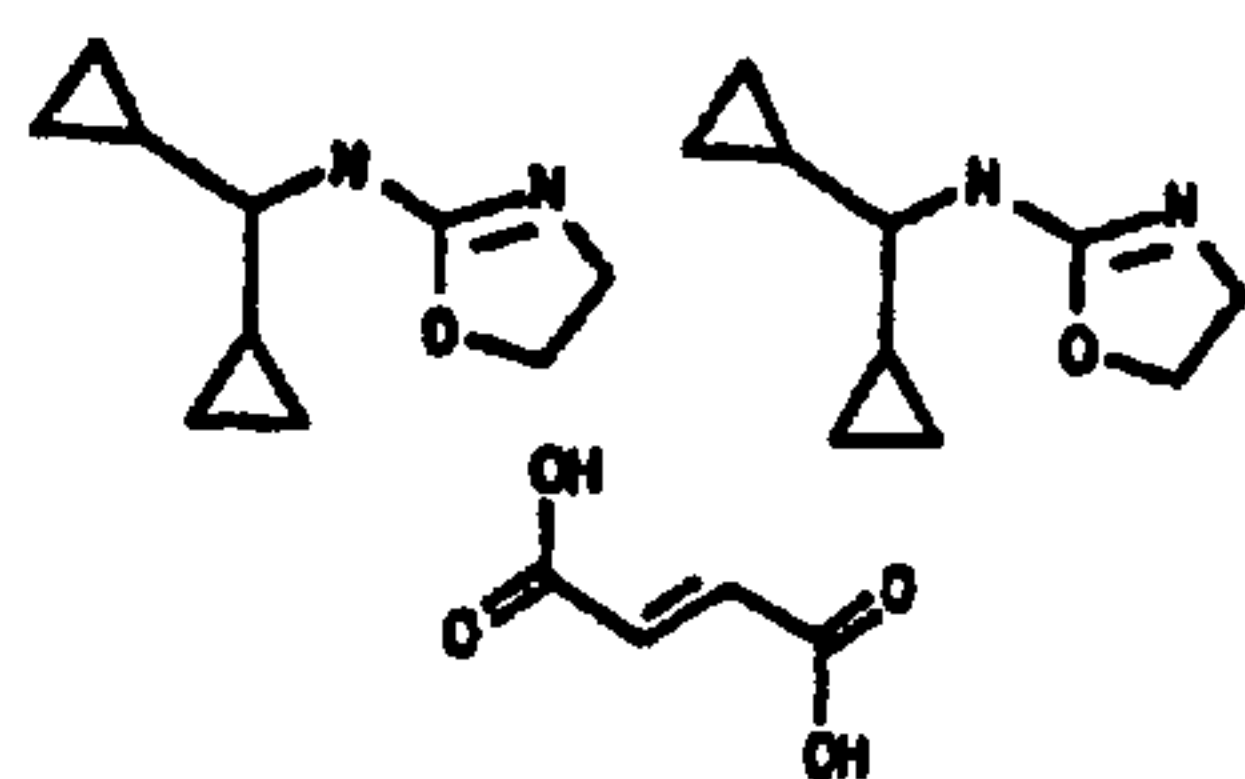
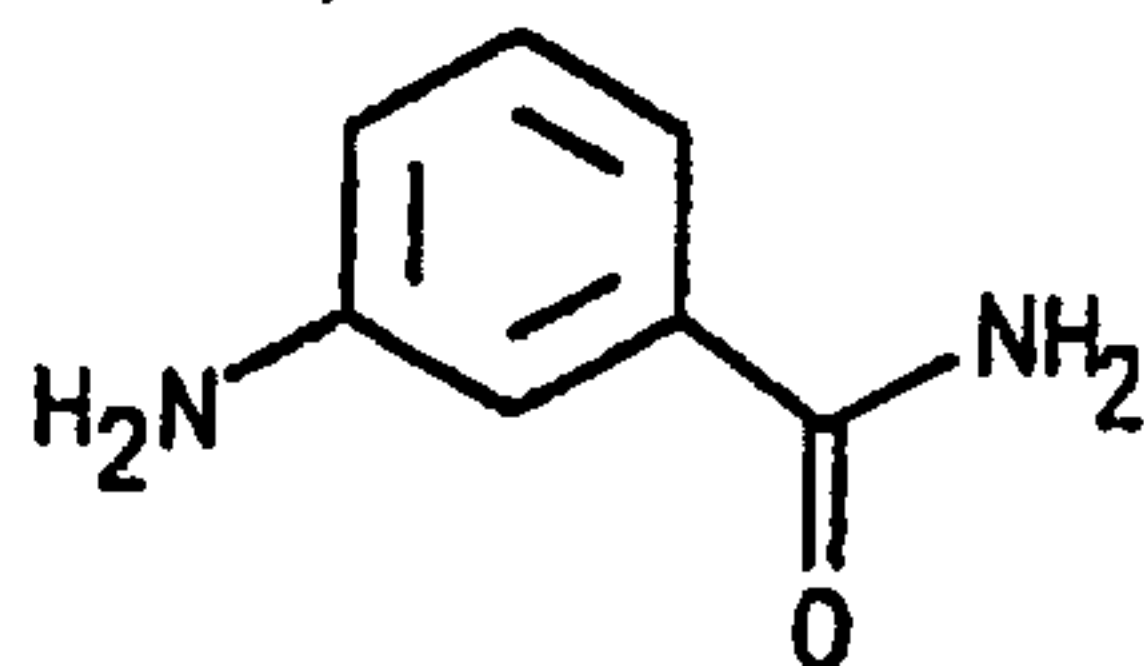
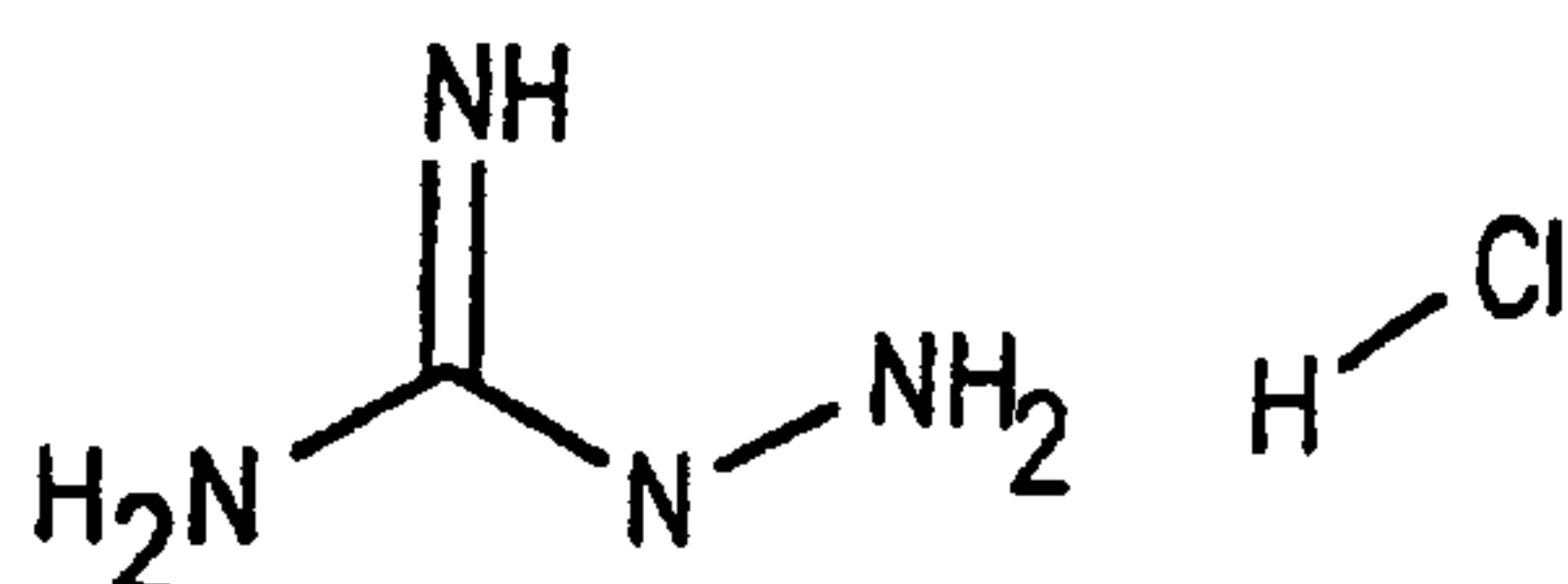
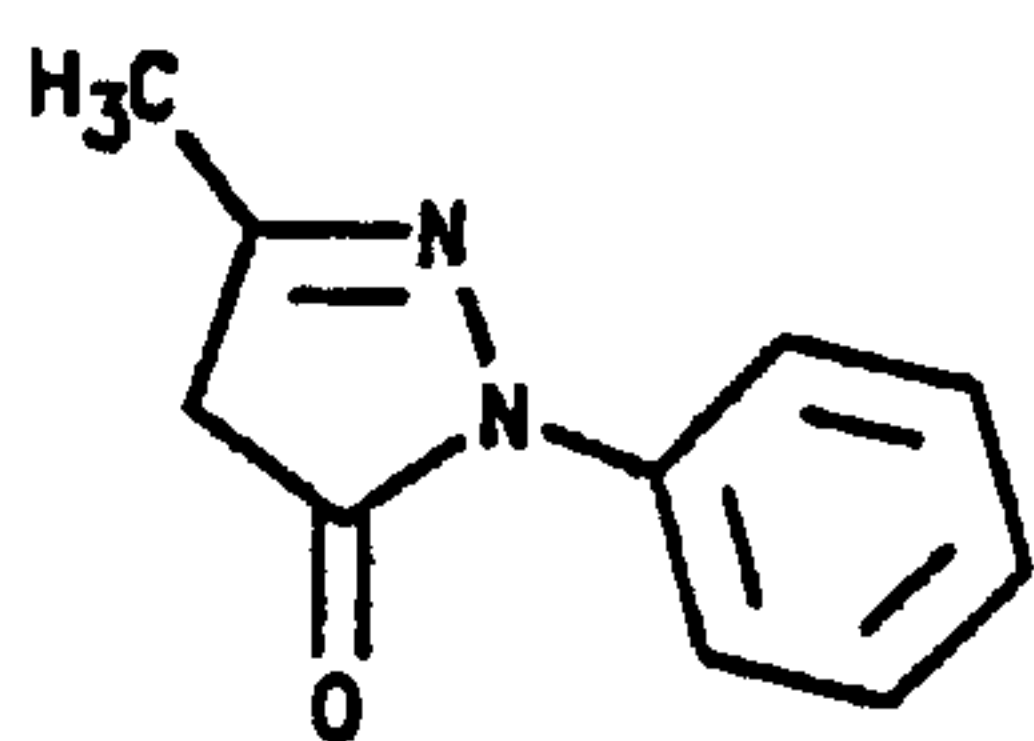
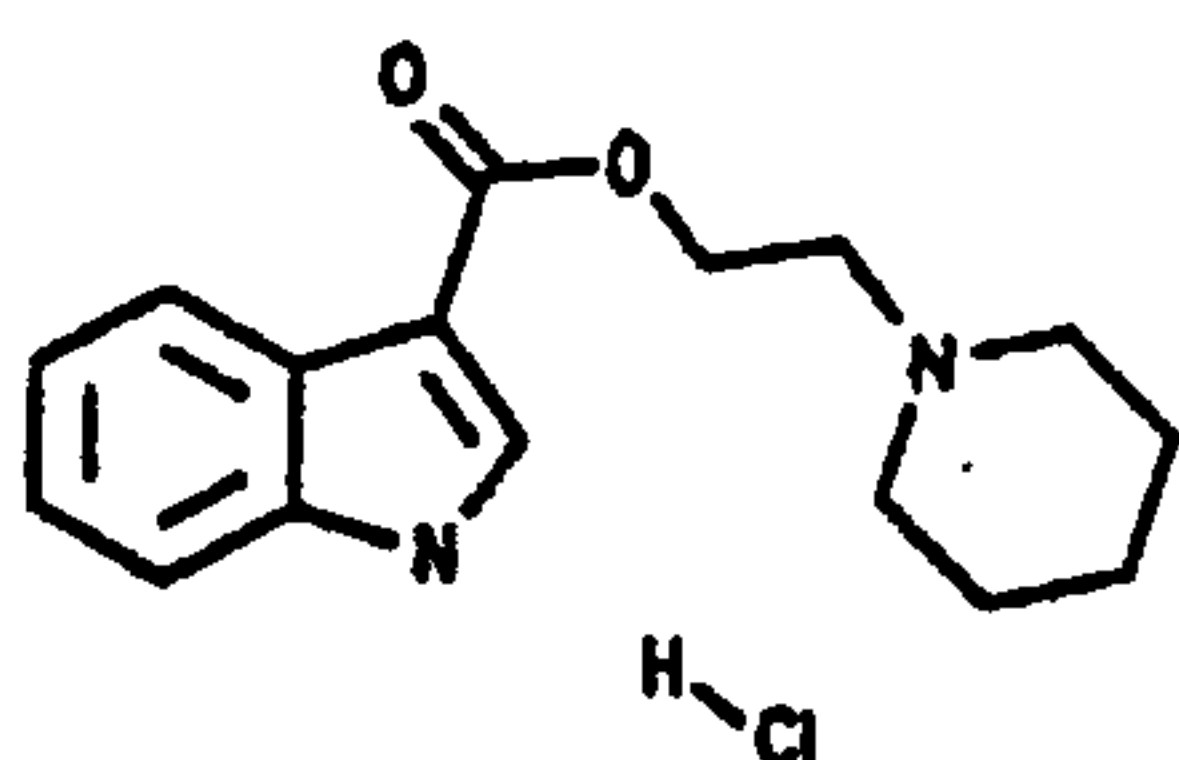
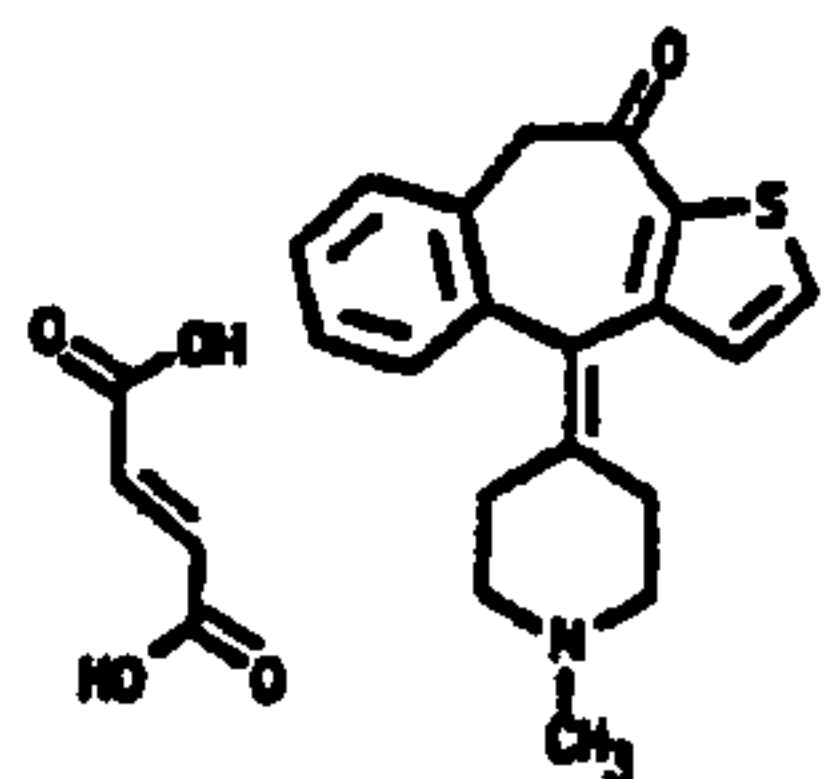
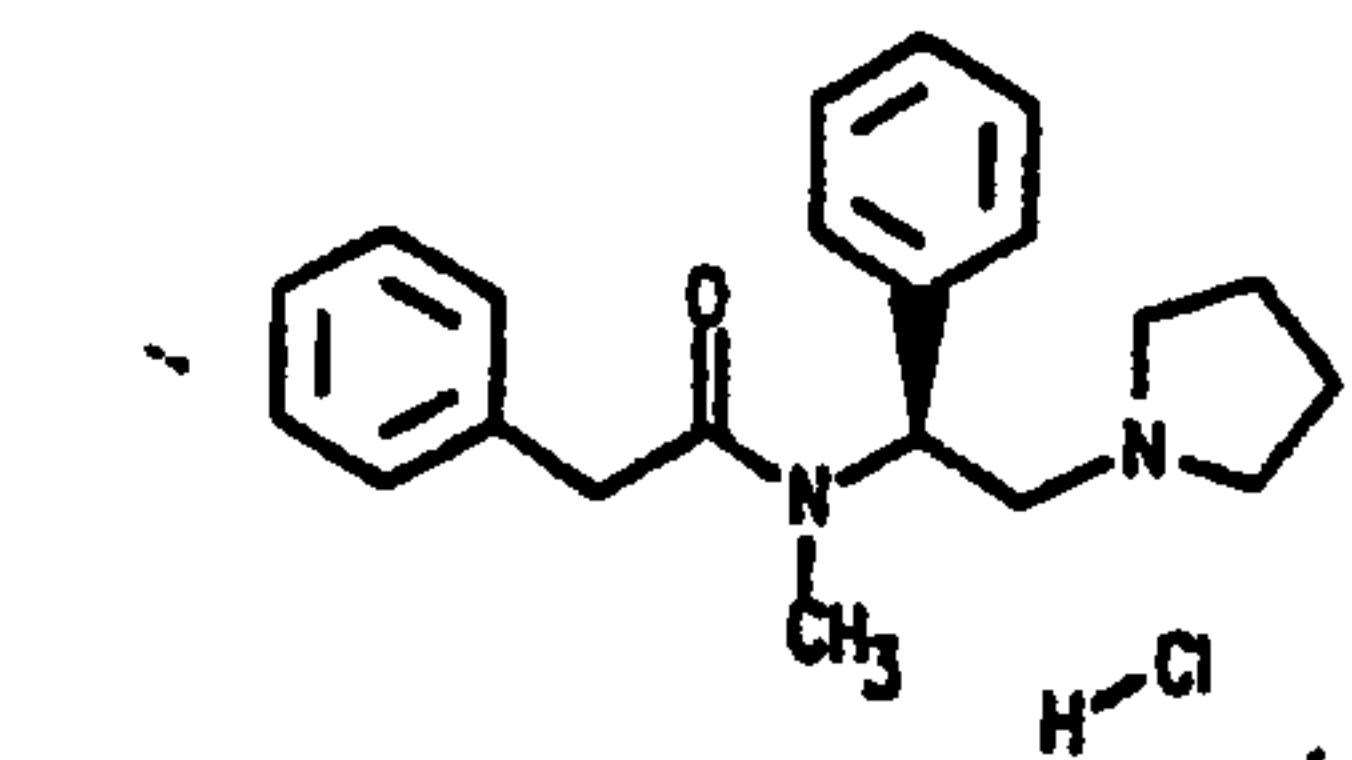


FIG. 17ss

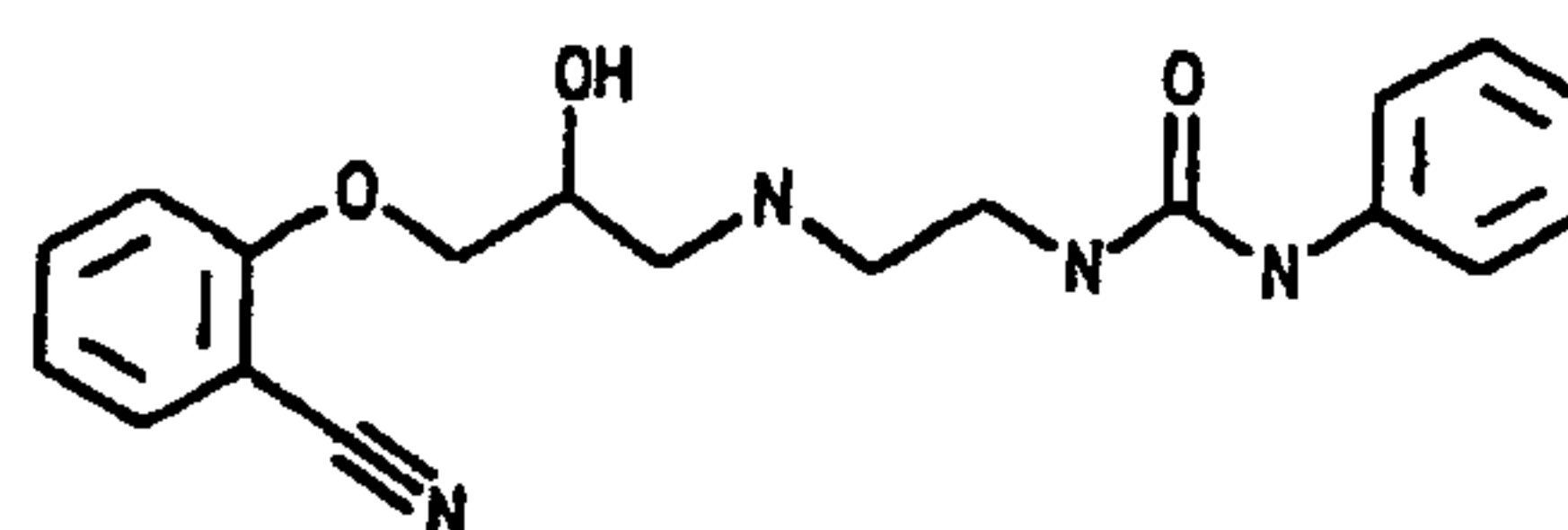
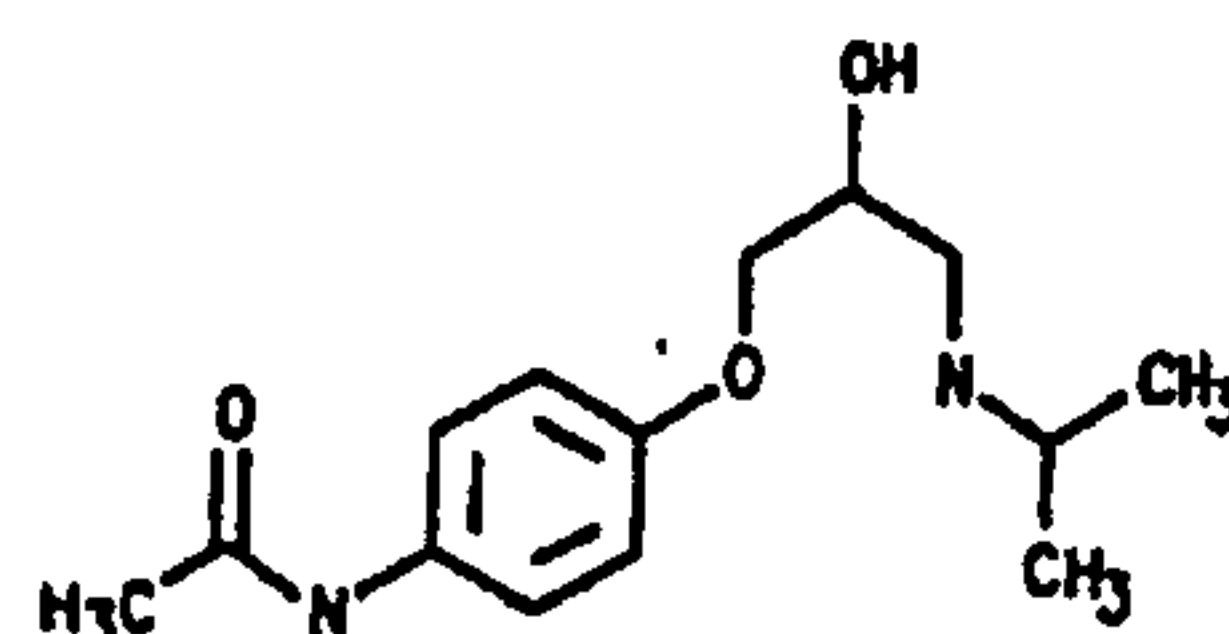
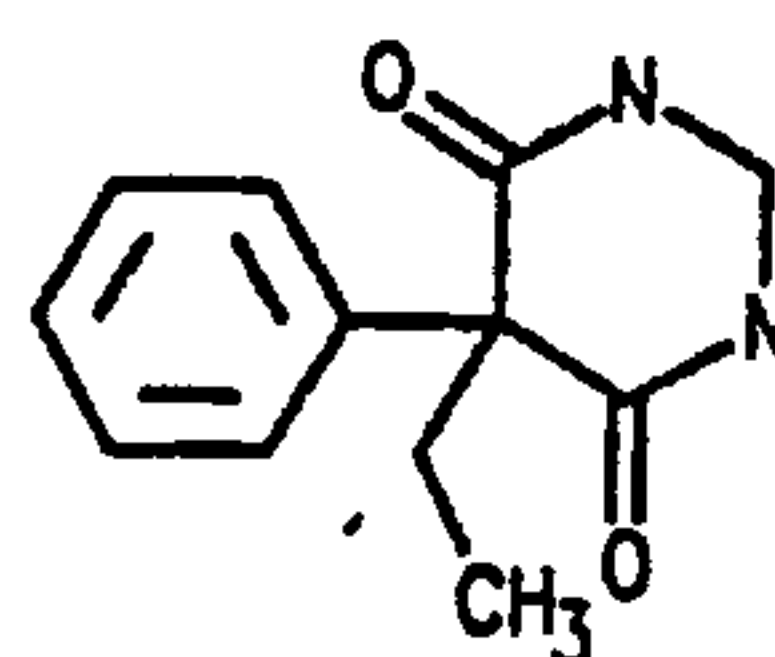
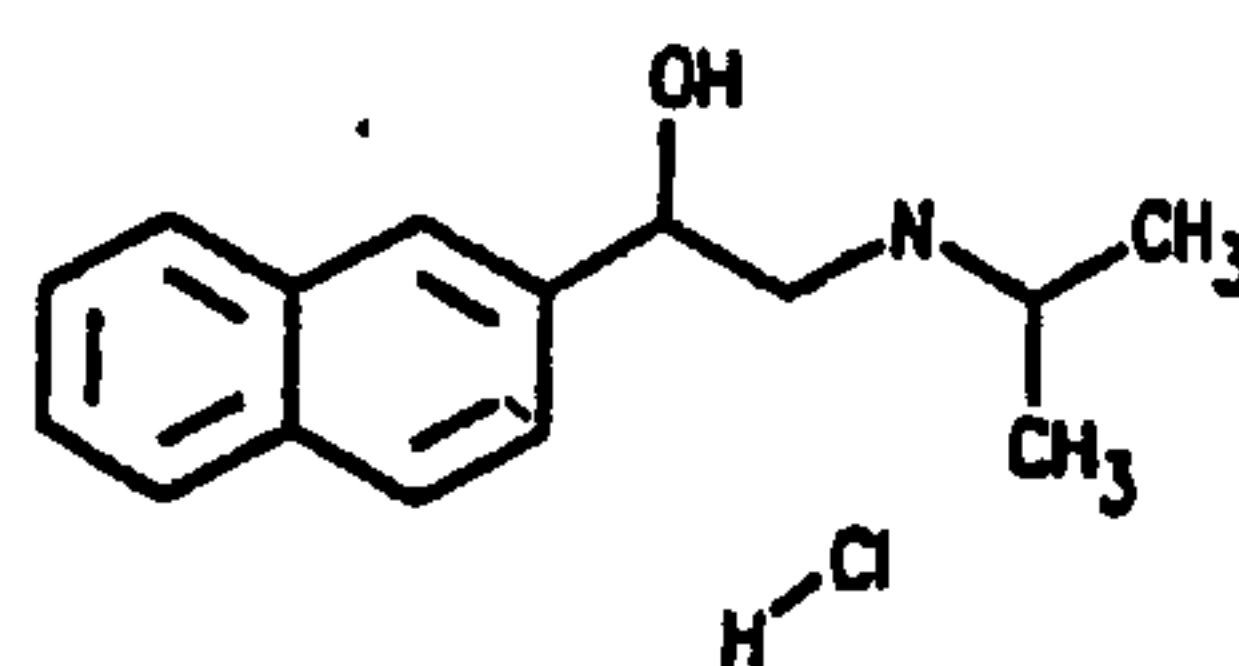
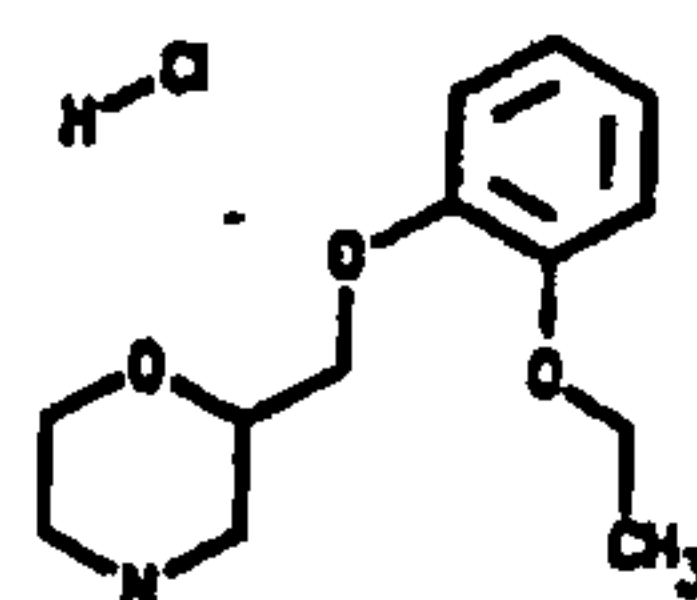
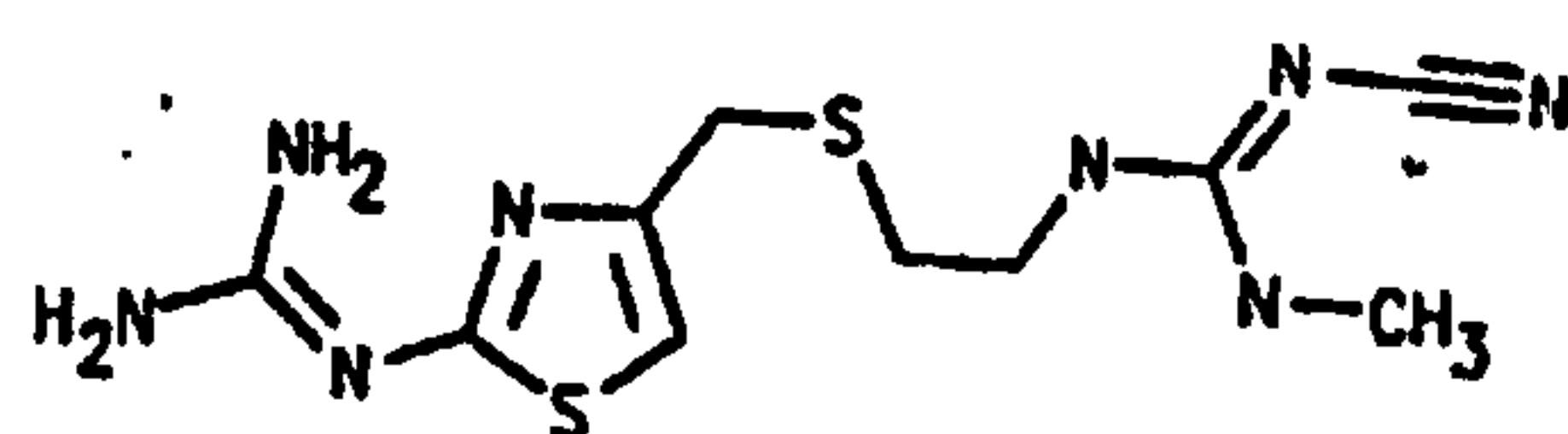
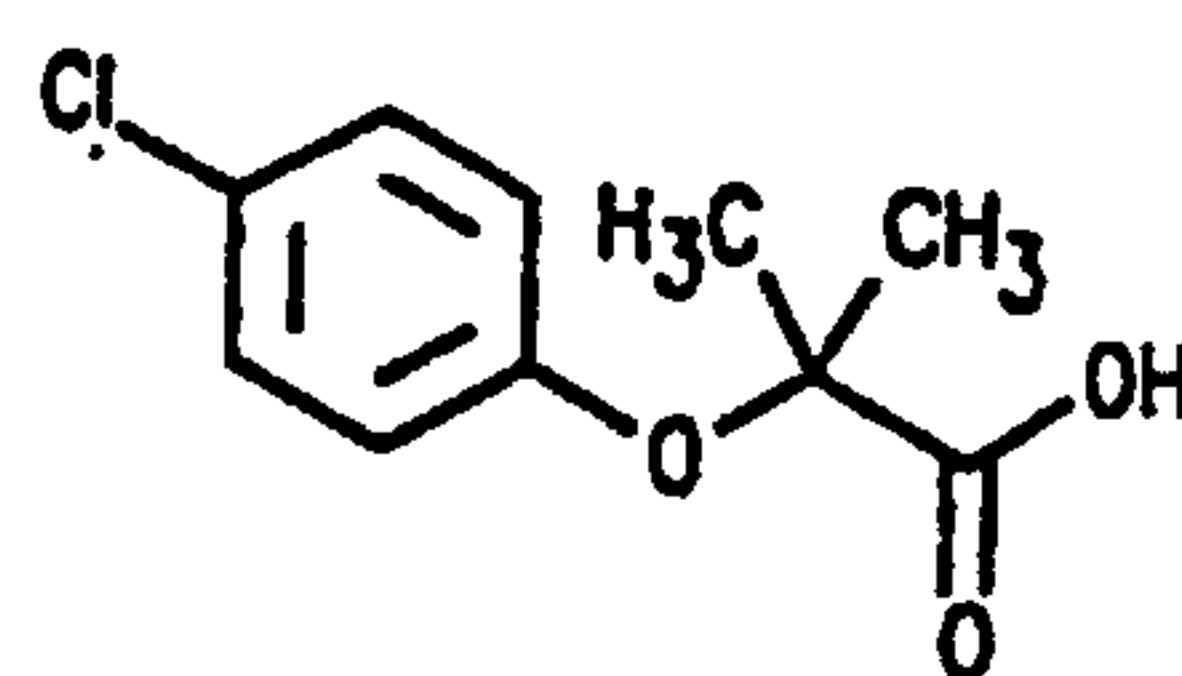
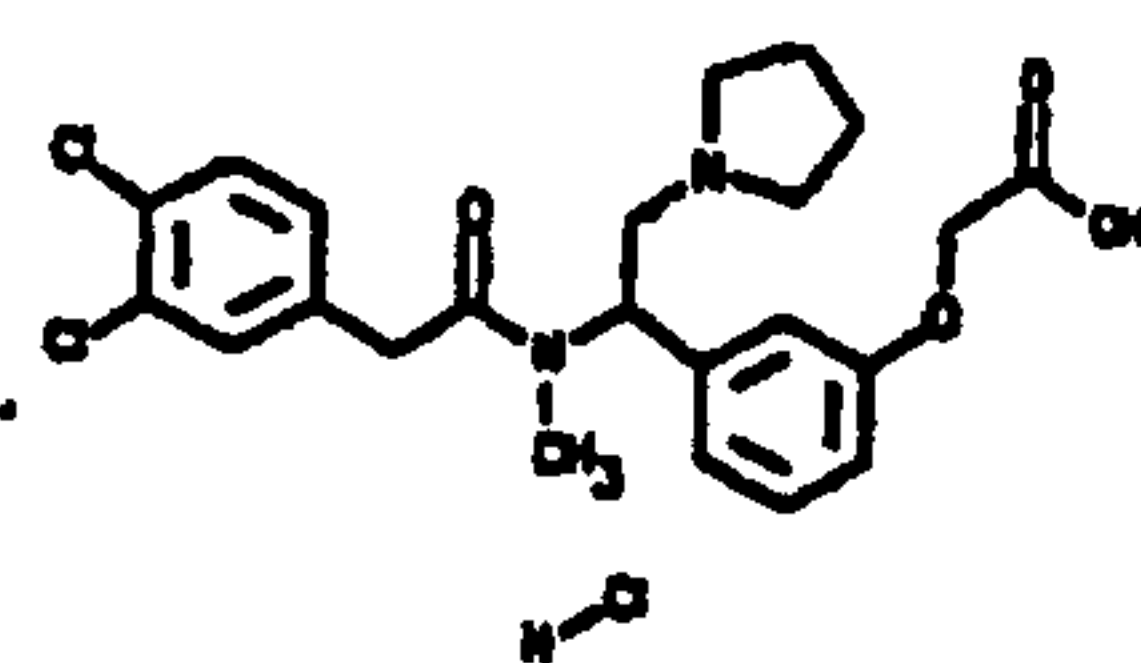
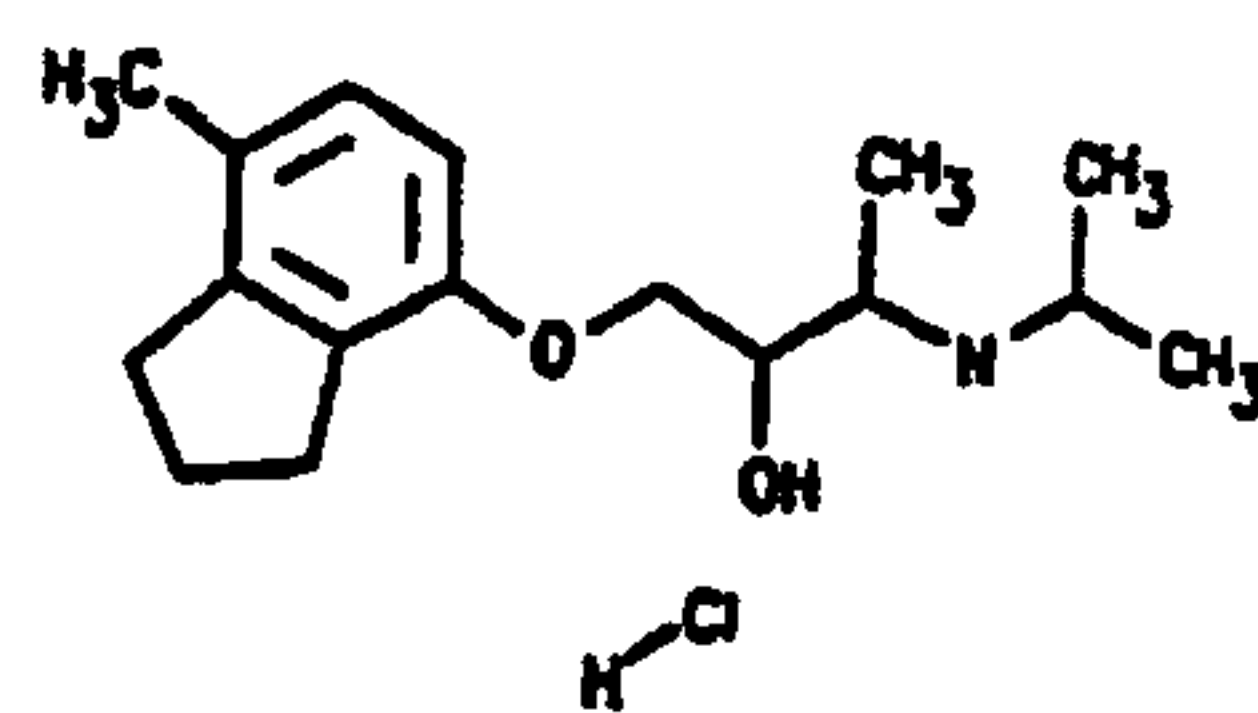
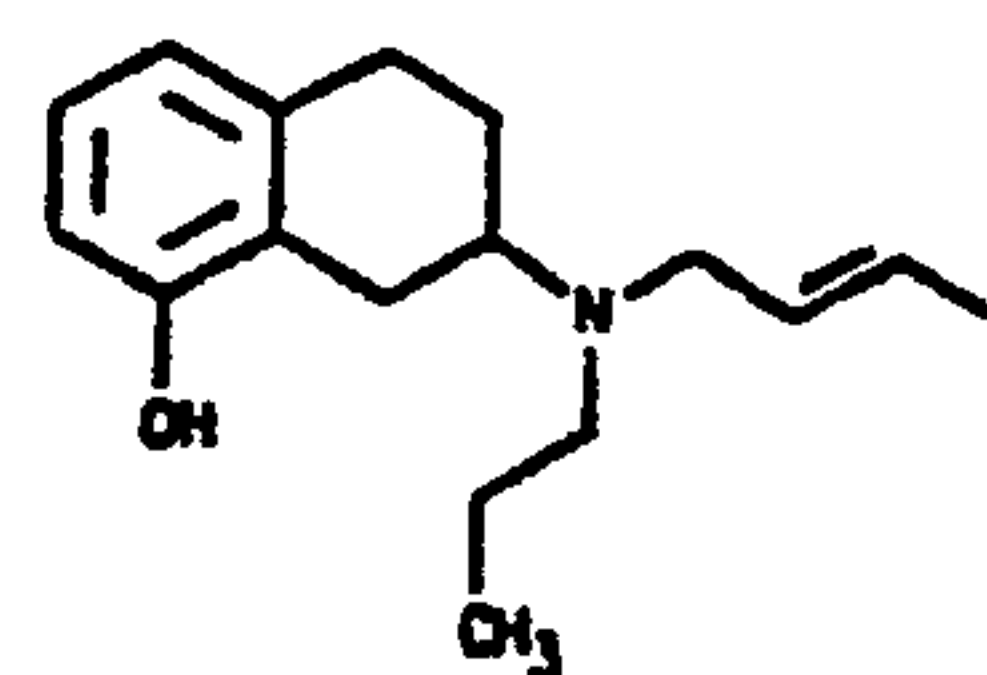


FIG. 17tt

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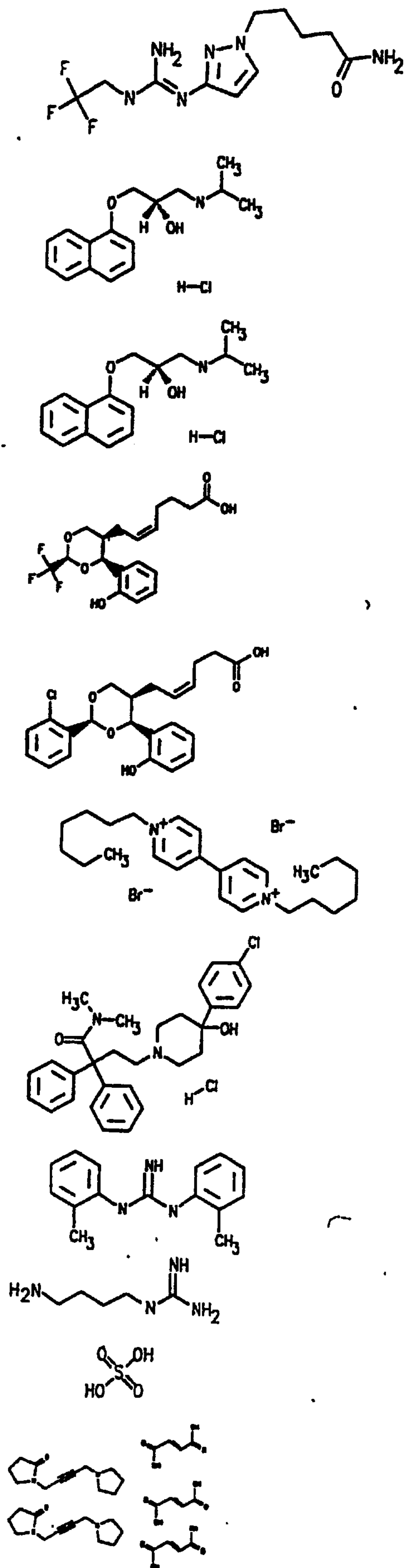


**FIG. 17uu**

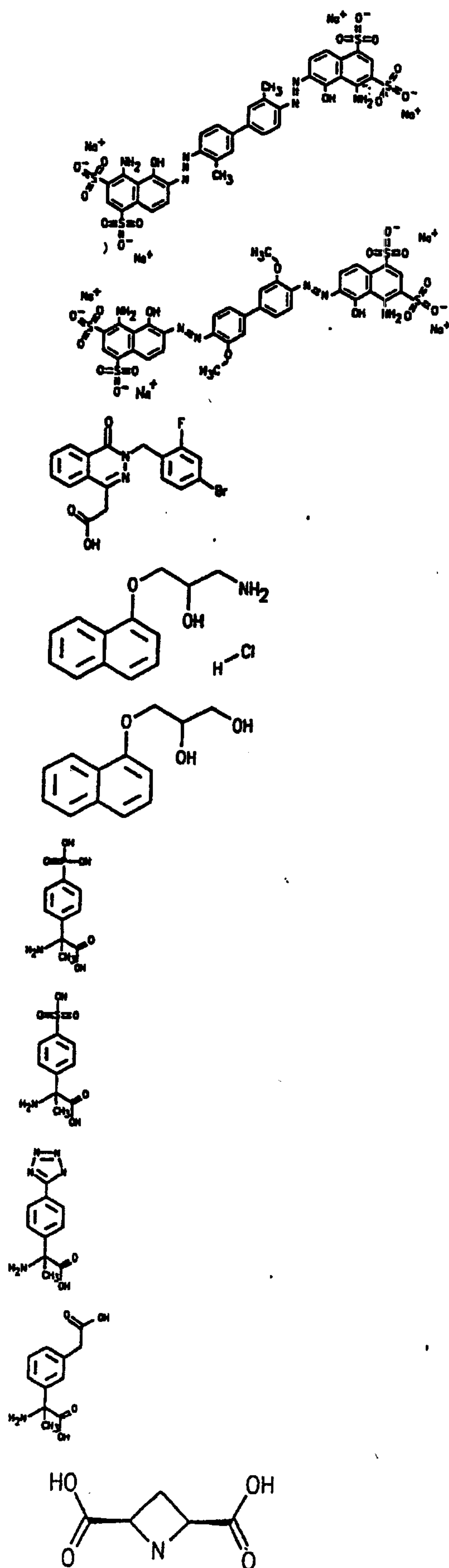


**FIG. 17vv**

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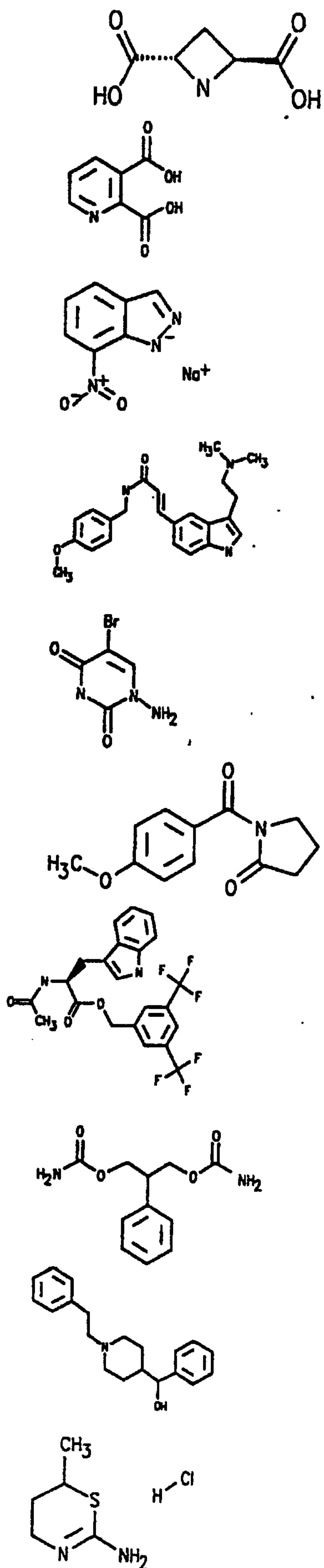


FIG. 17ww

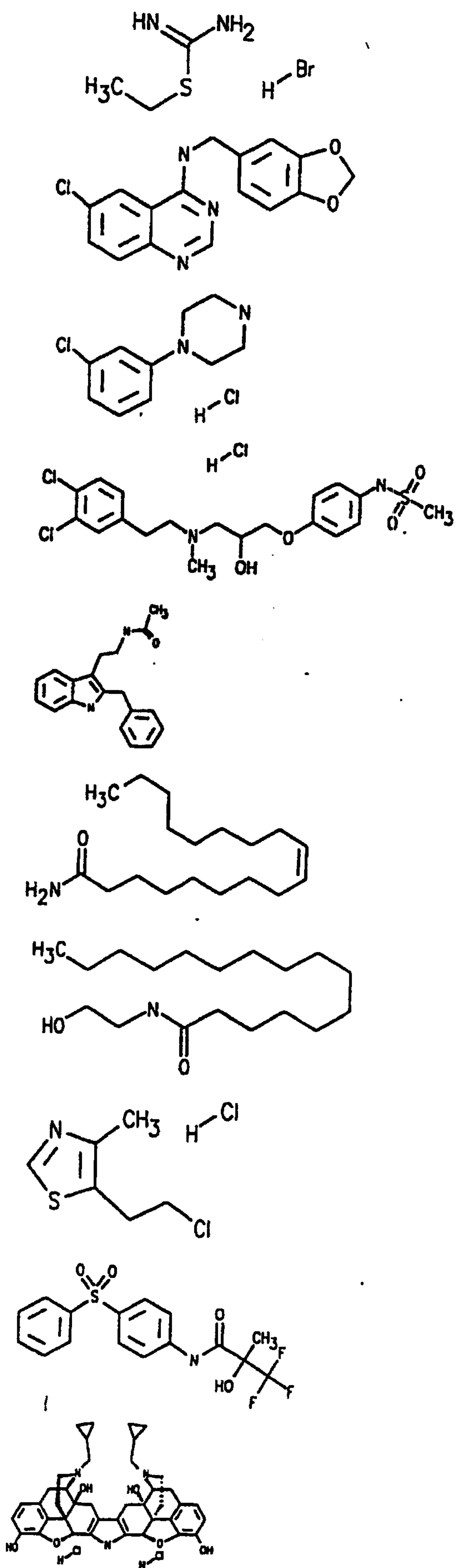


FIG. 17xx

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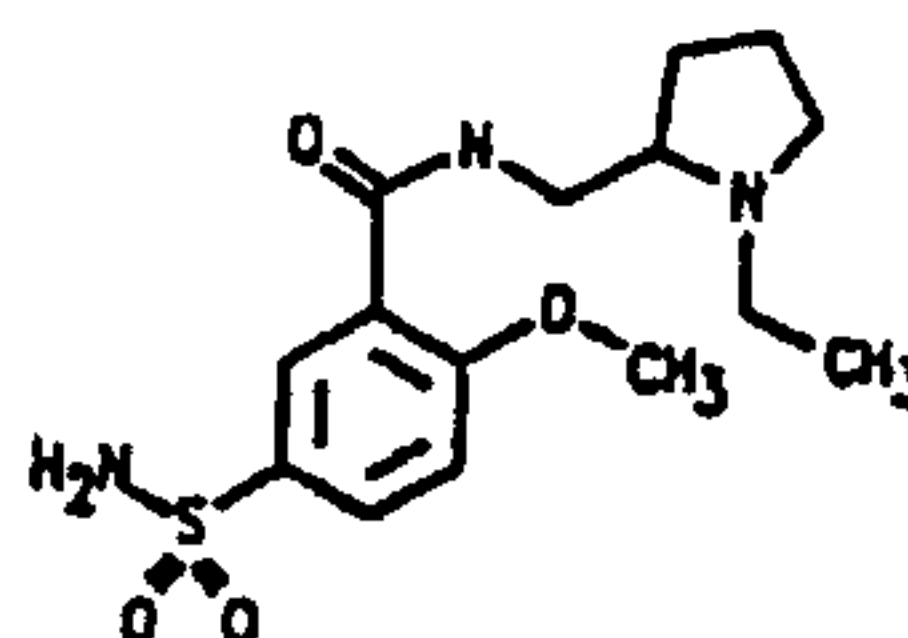
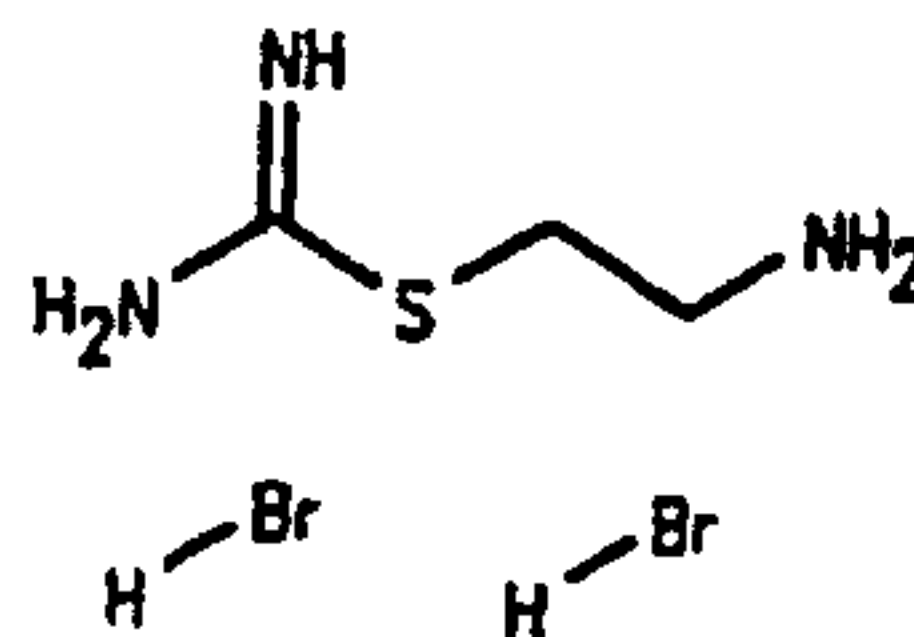
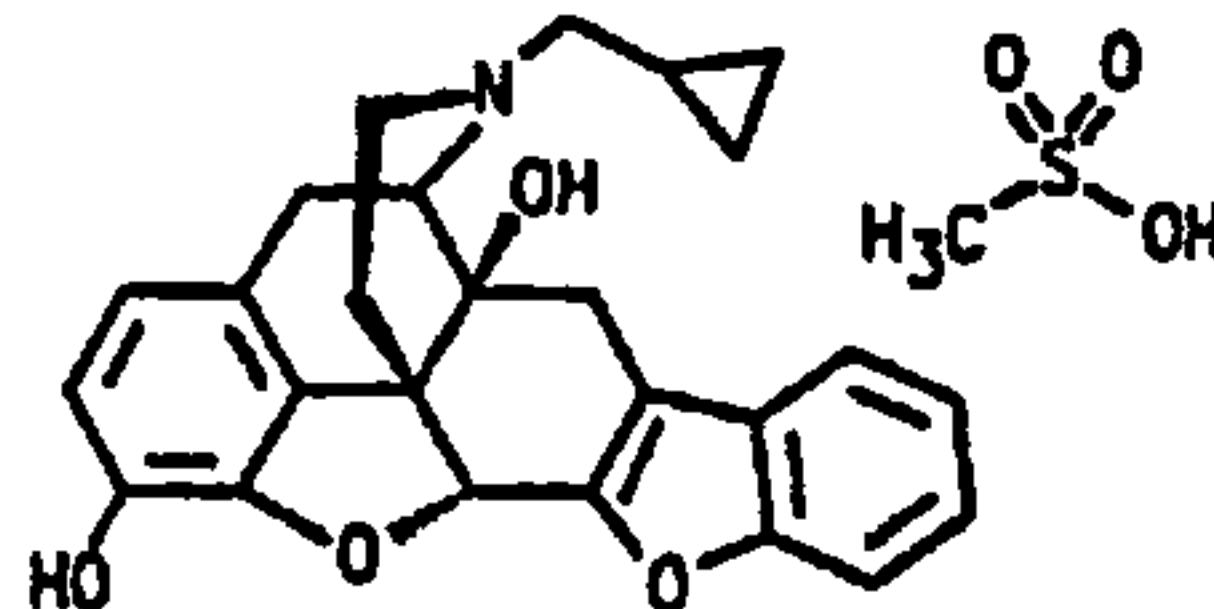
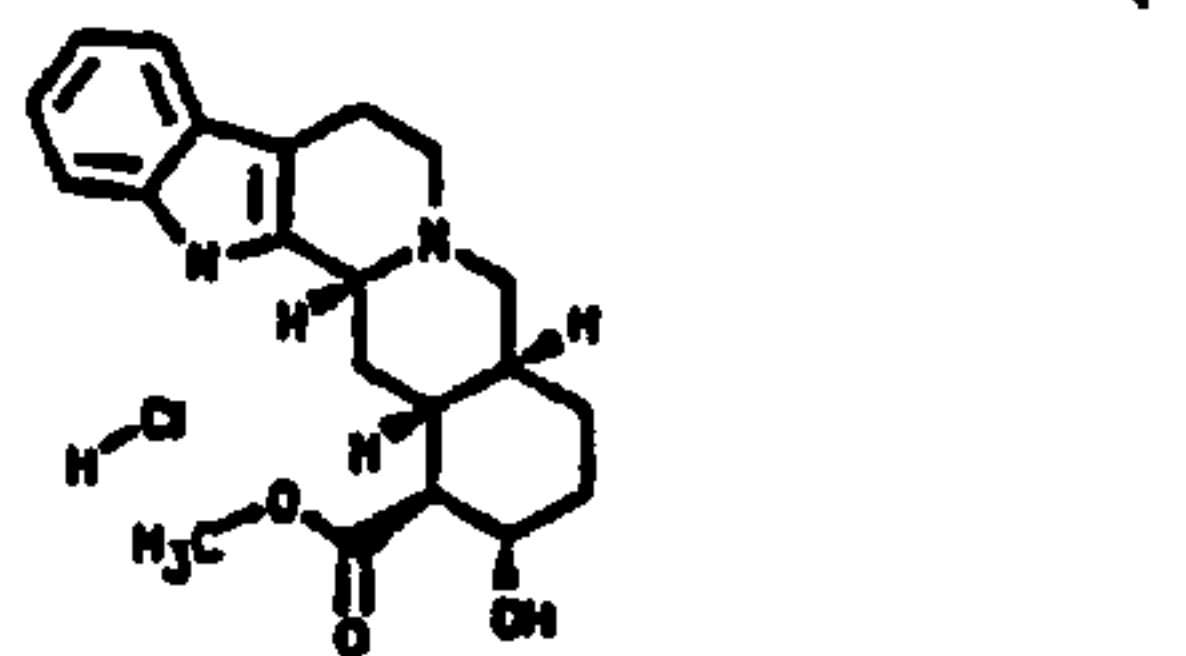
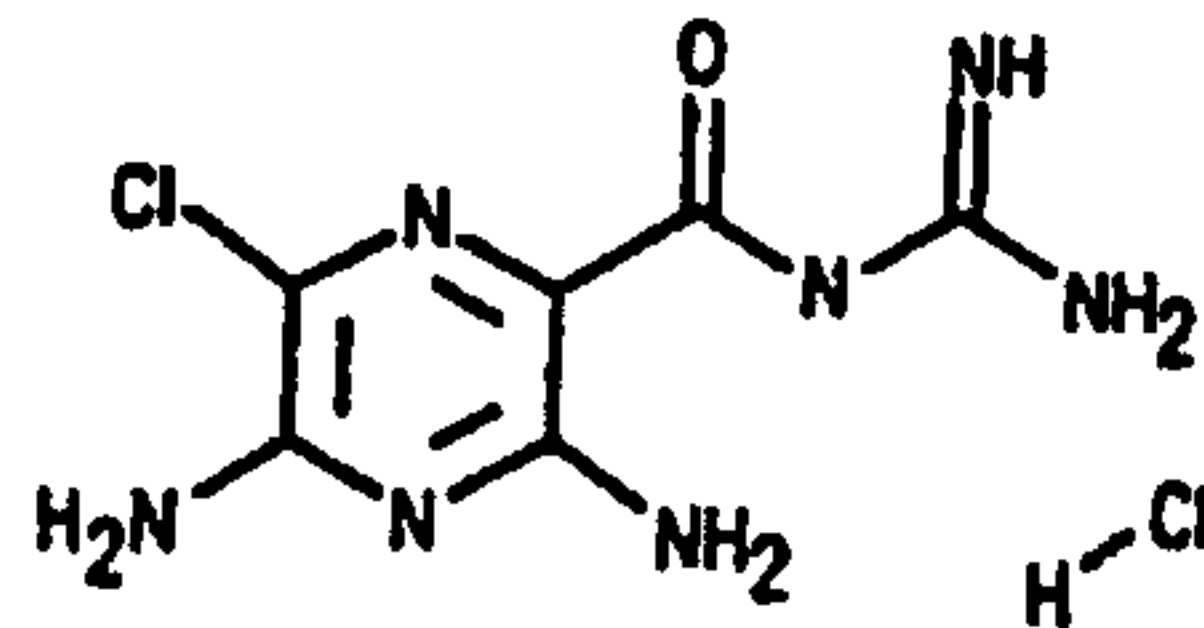
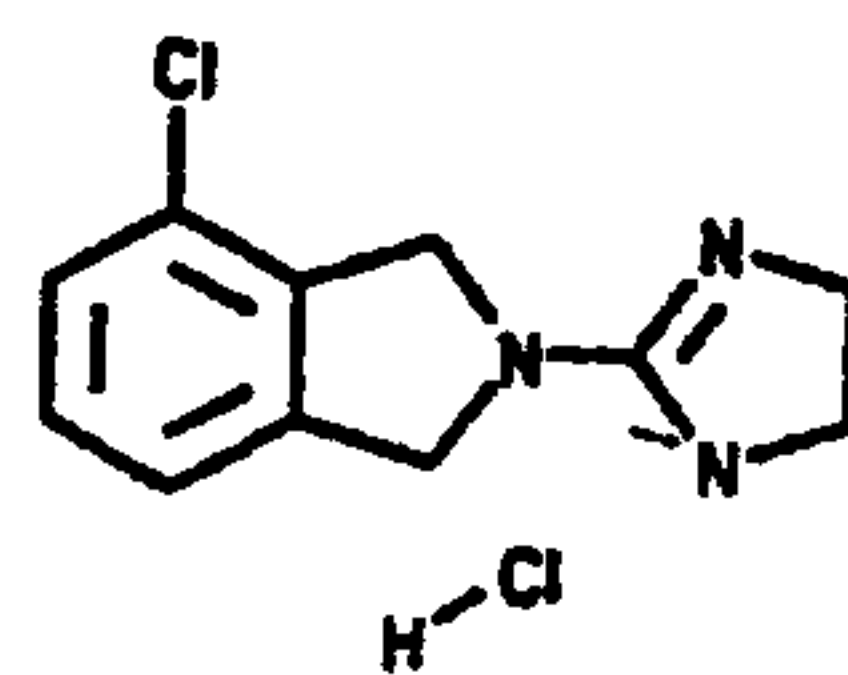
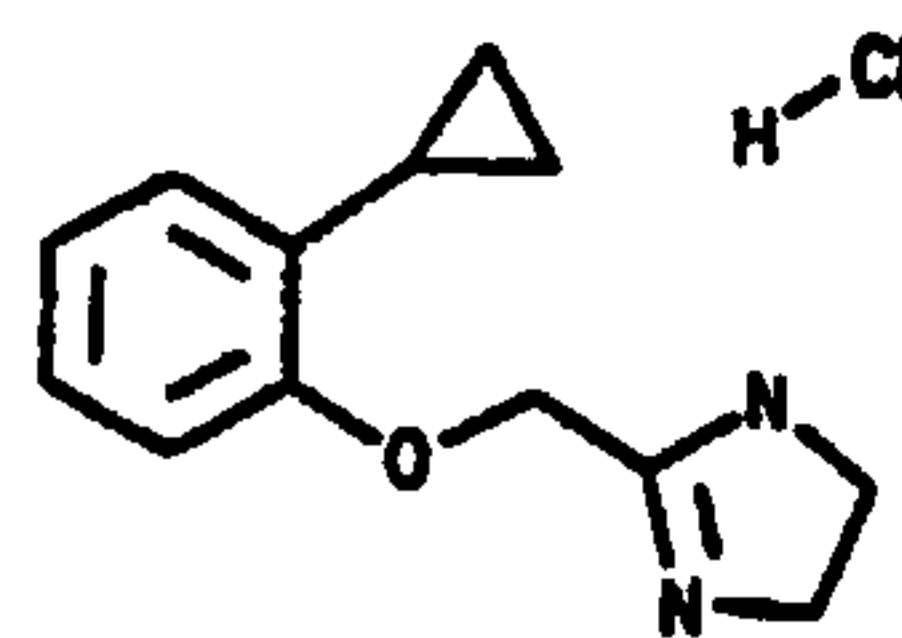
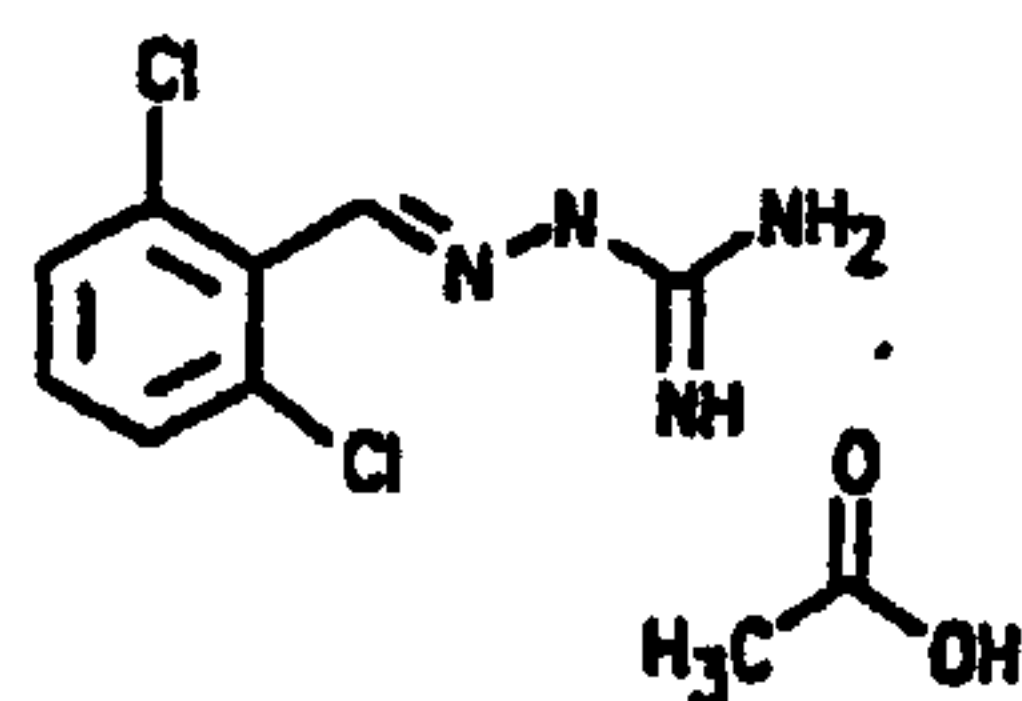
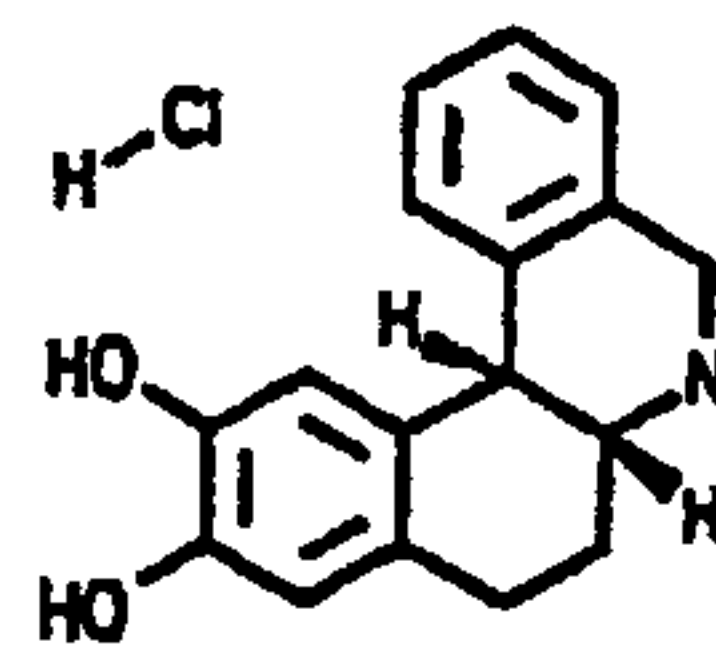
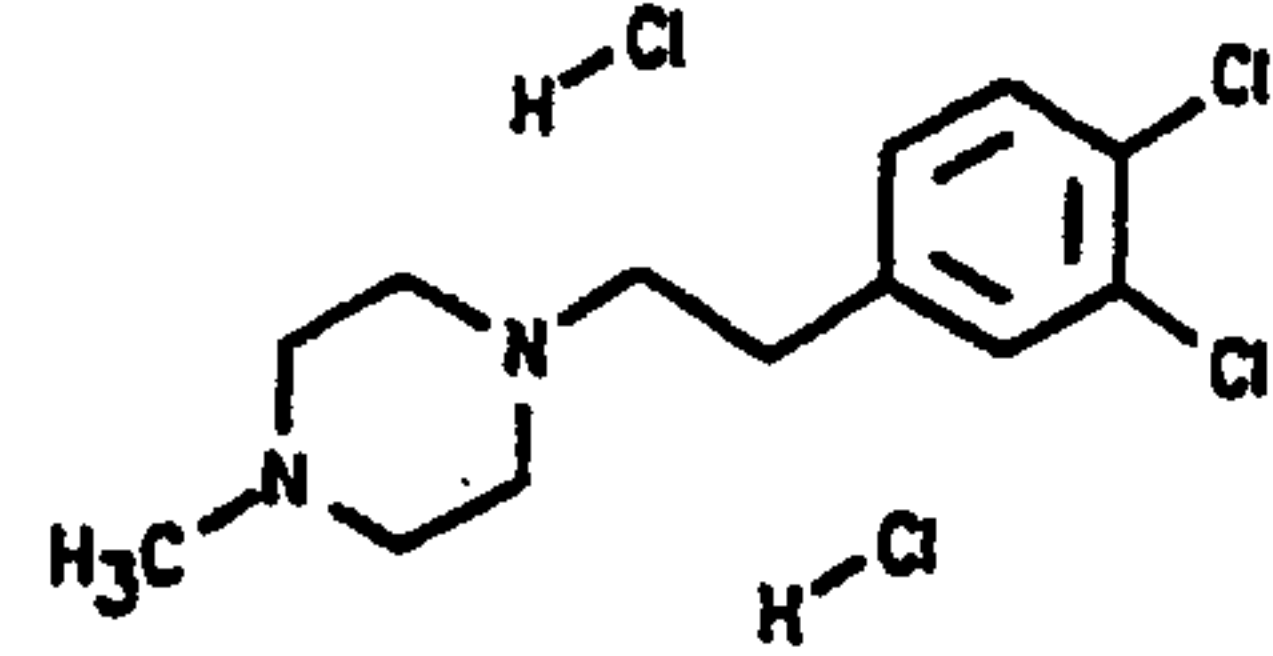
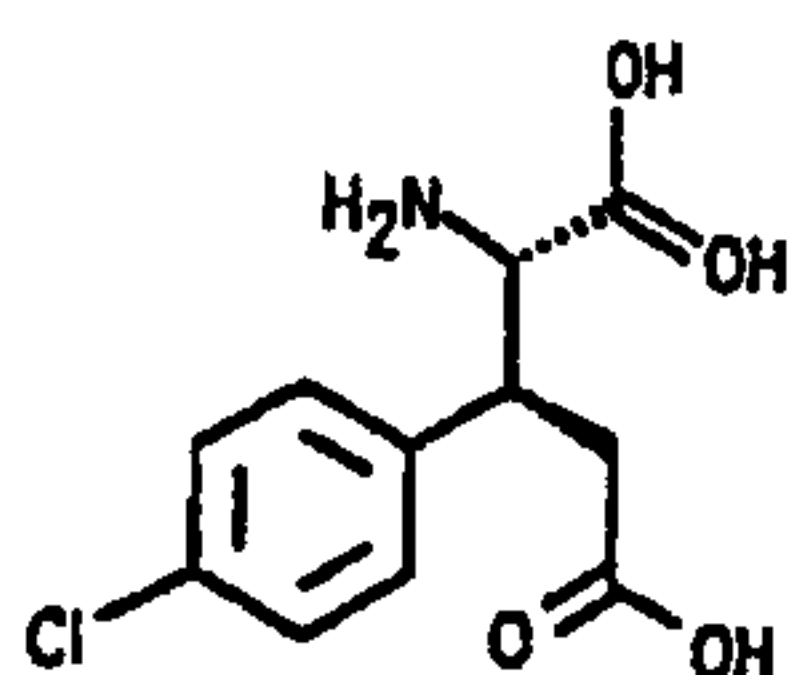
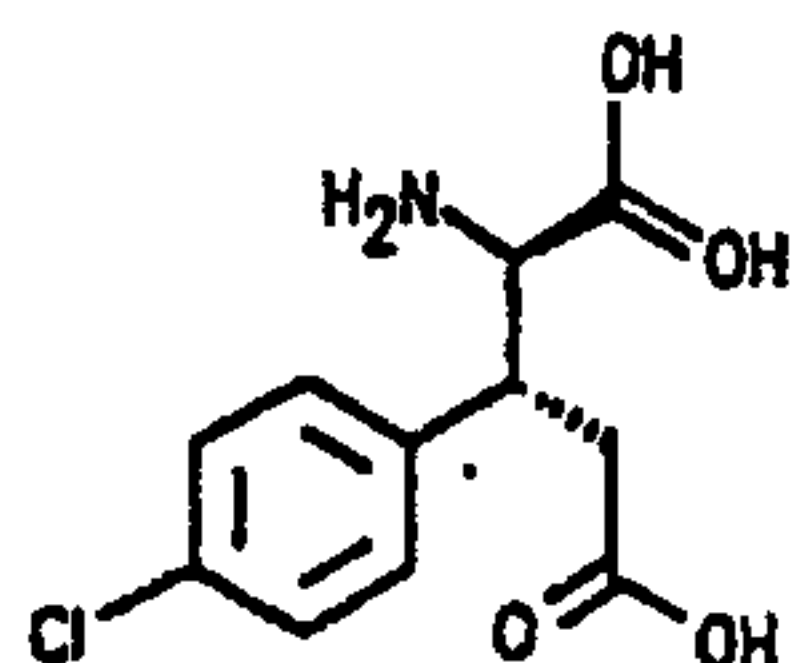
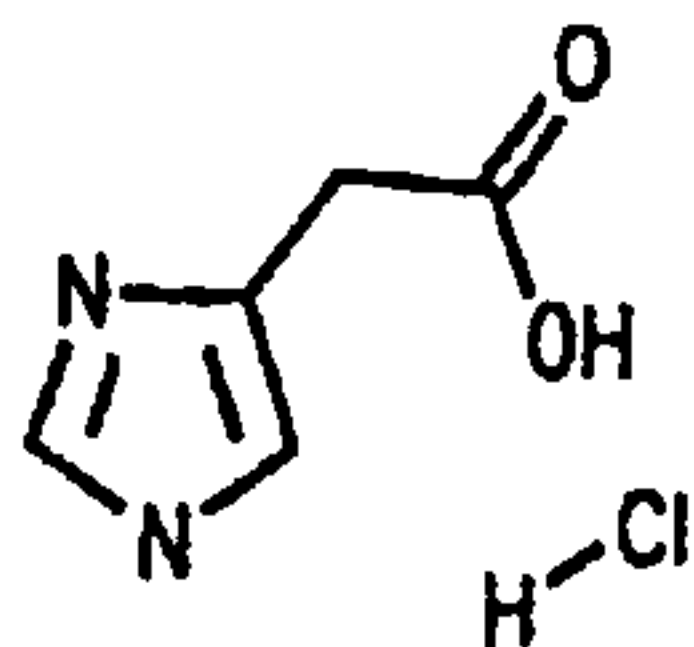
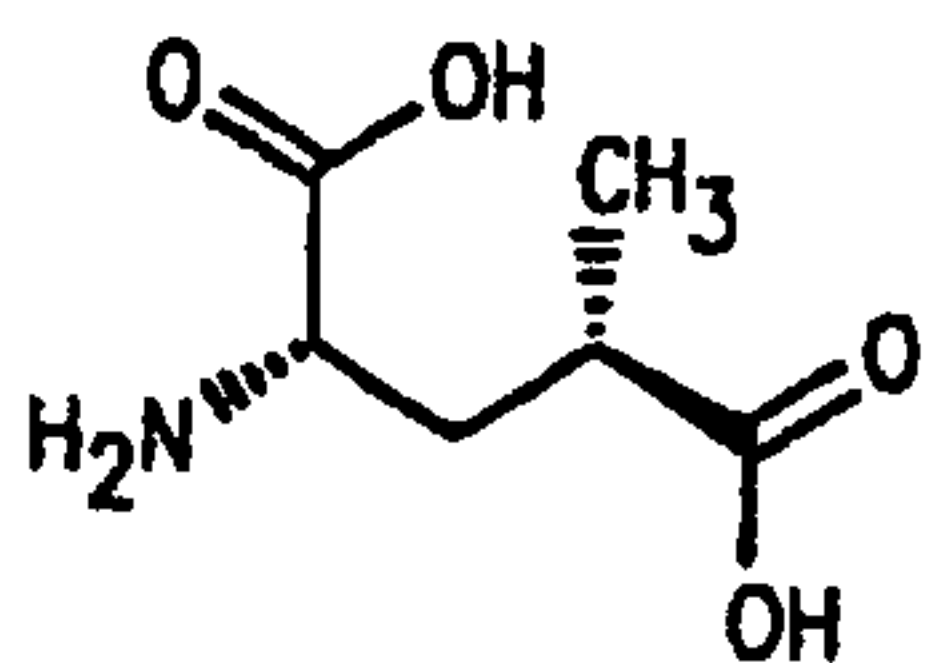
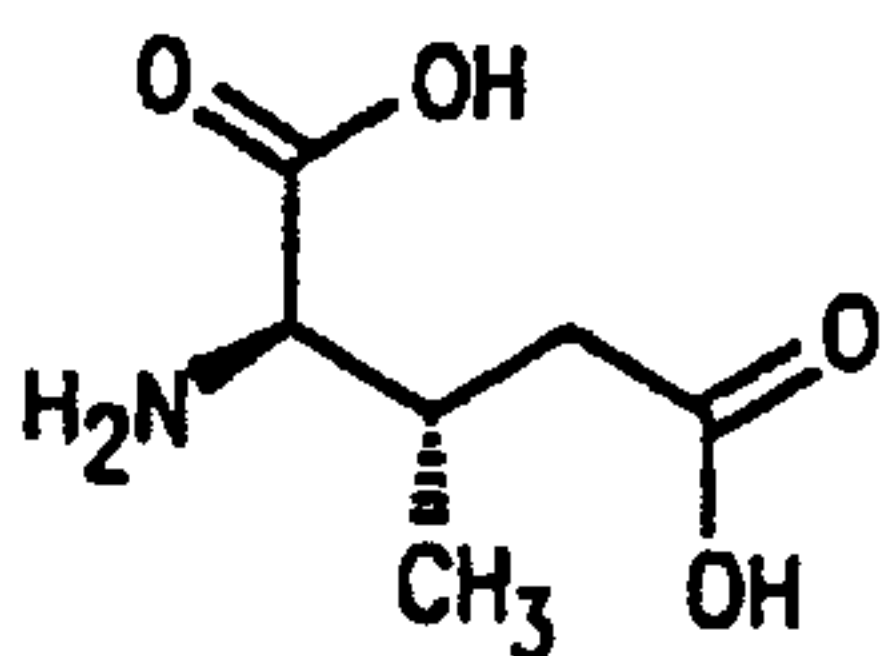
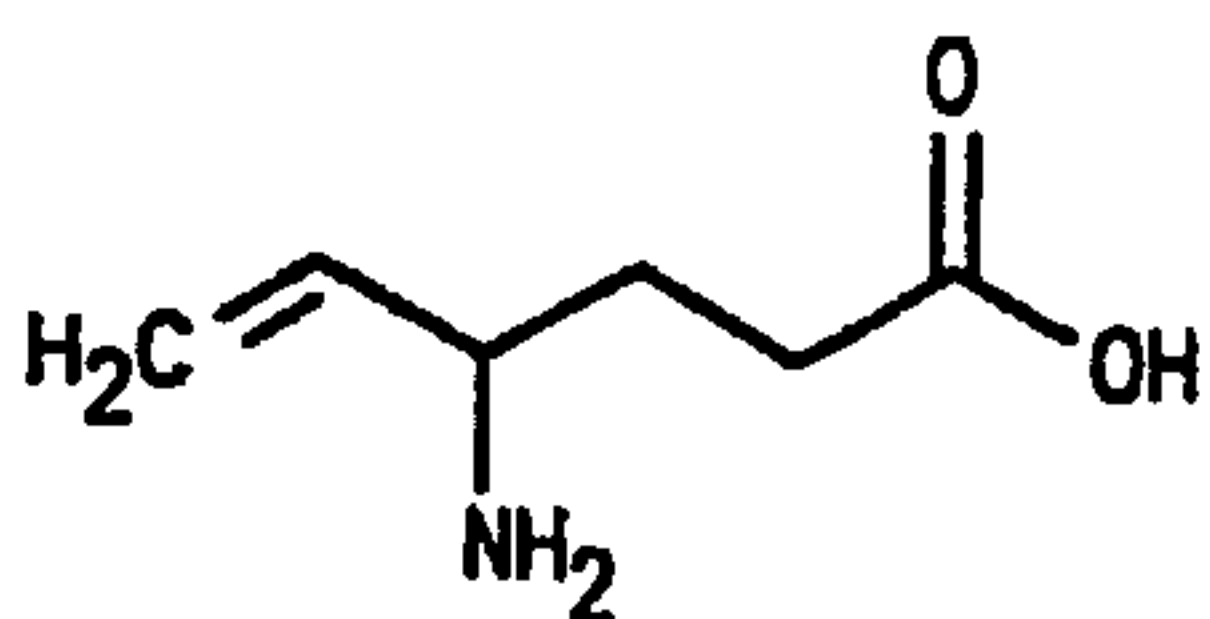
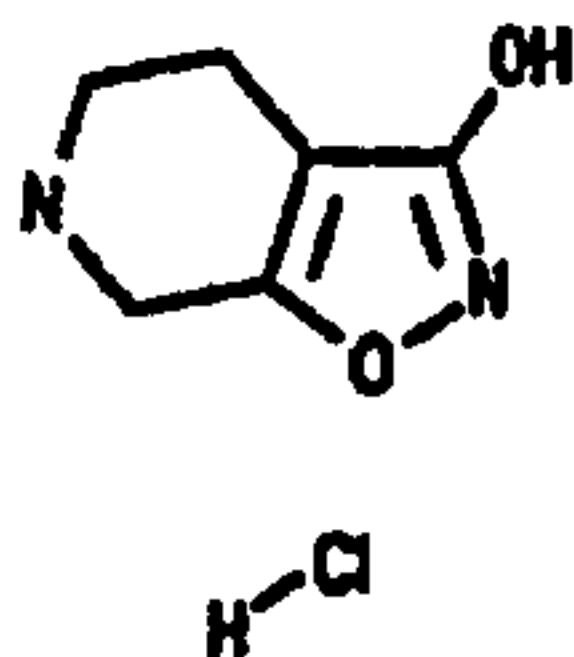
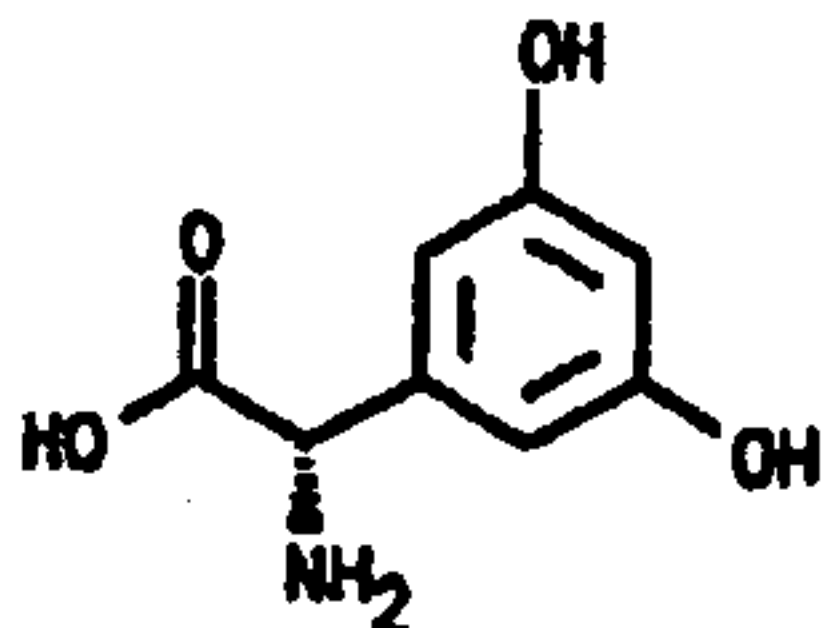
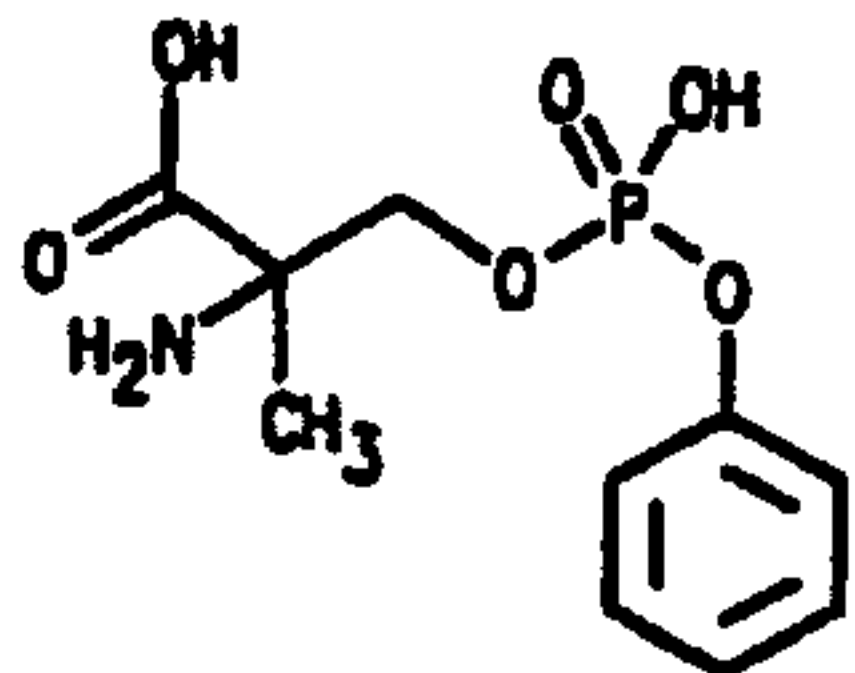
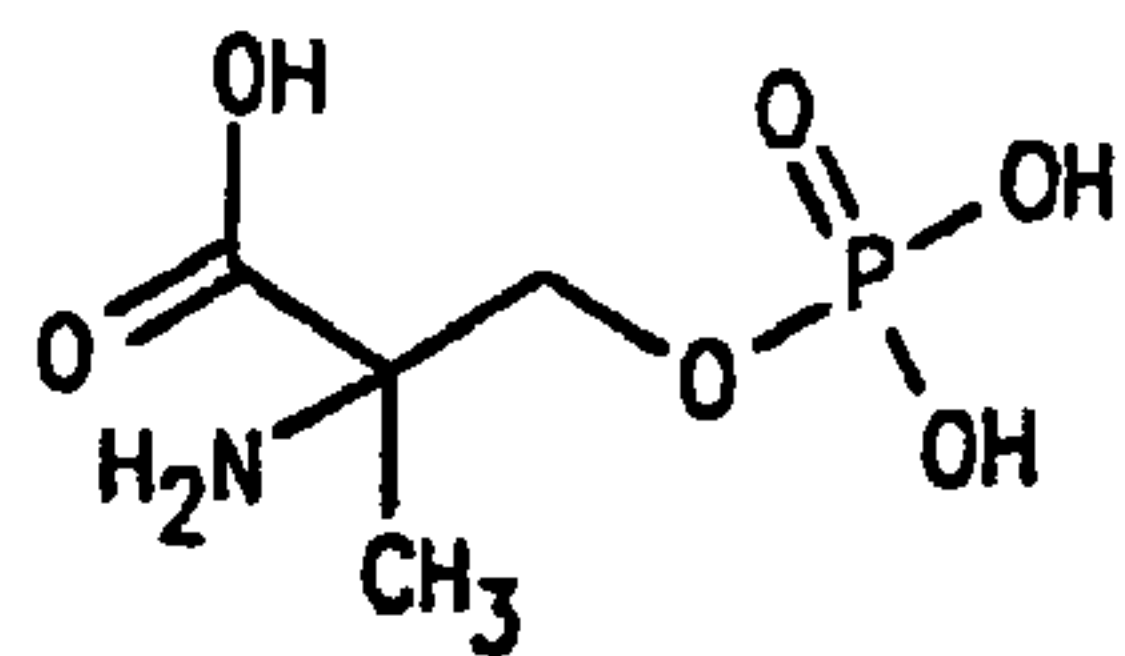


FIG. 17yy

**FIG. 17zz**

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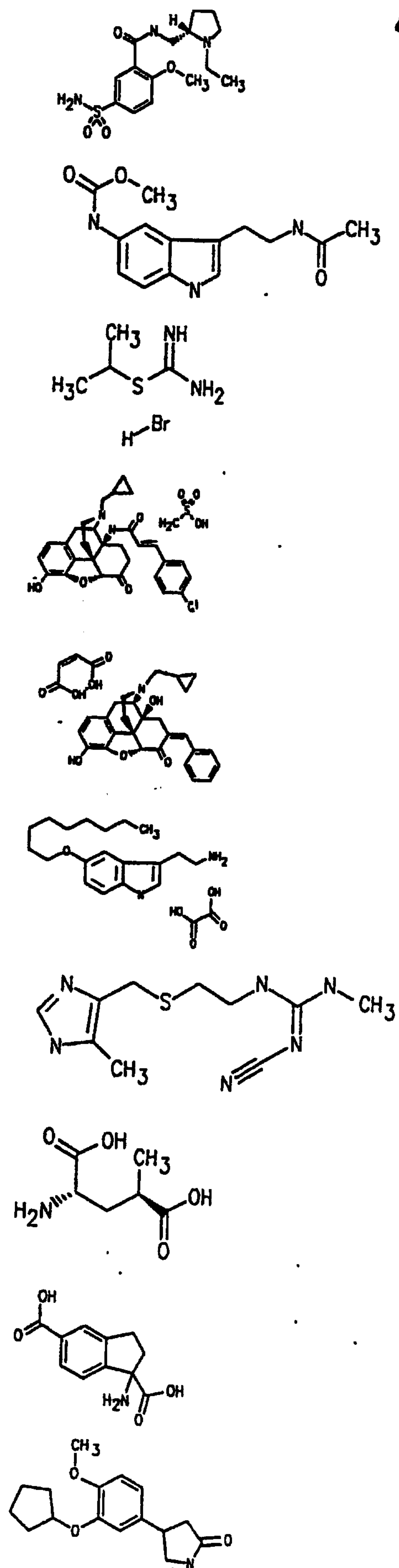


FIG. 17aaa

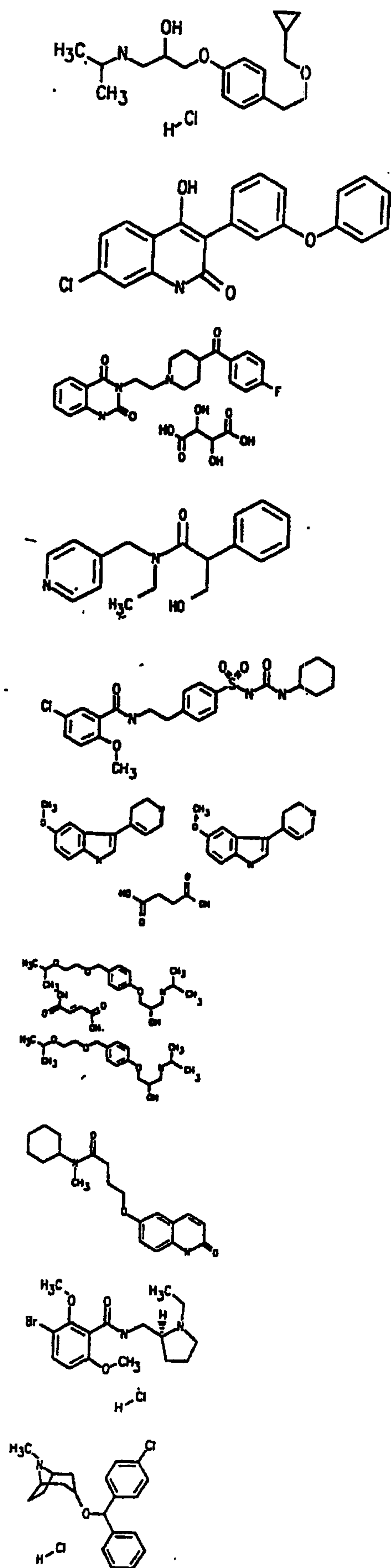


FIG. 17bbb

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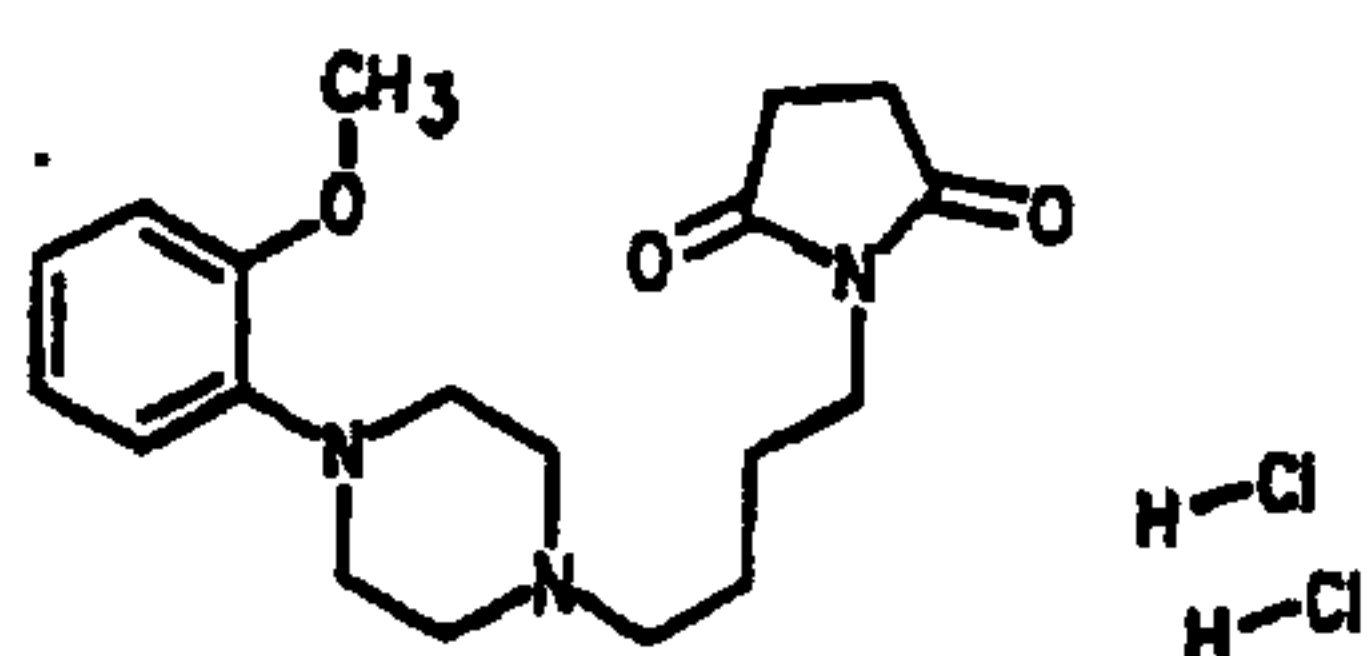
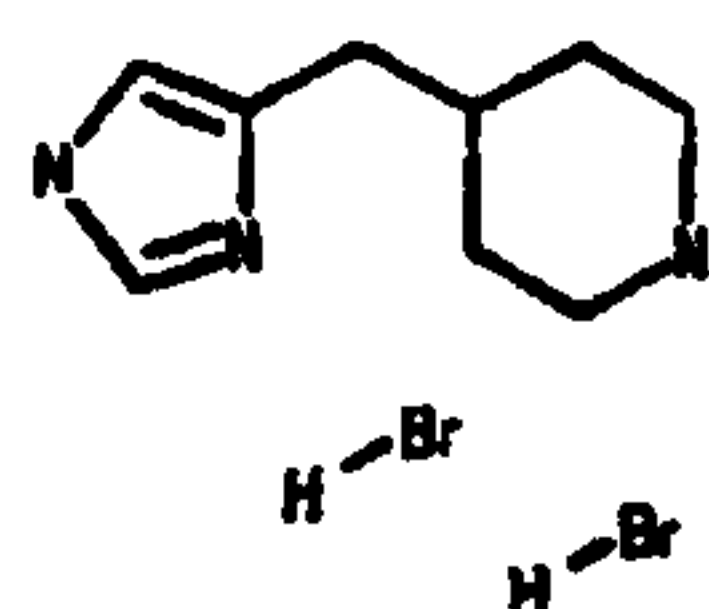
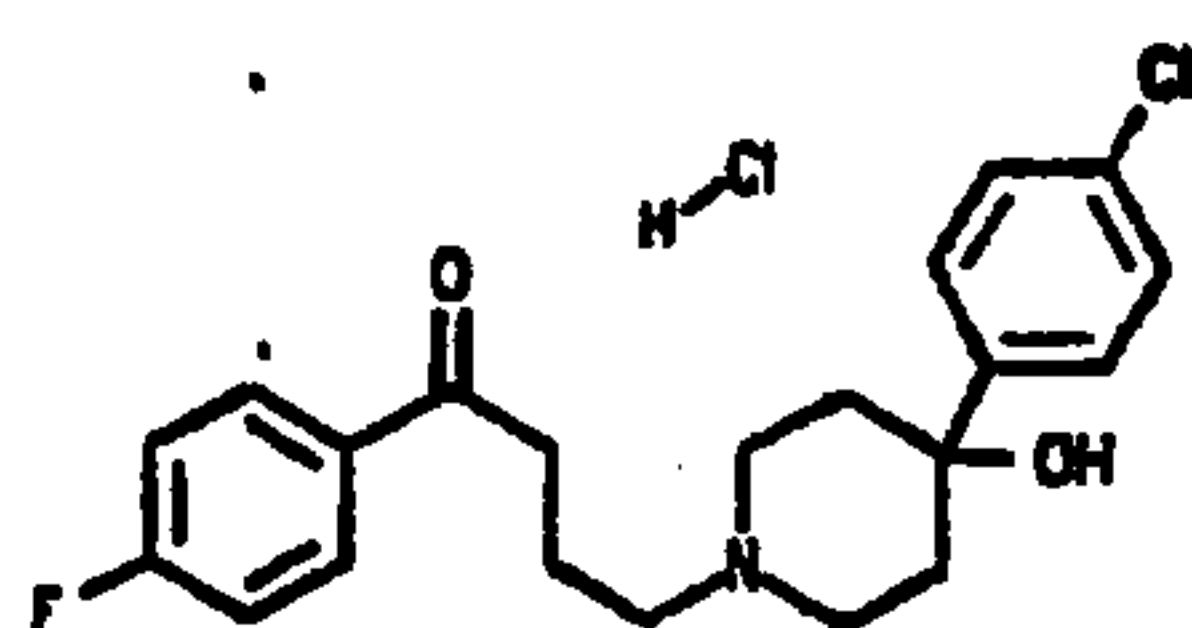
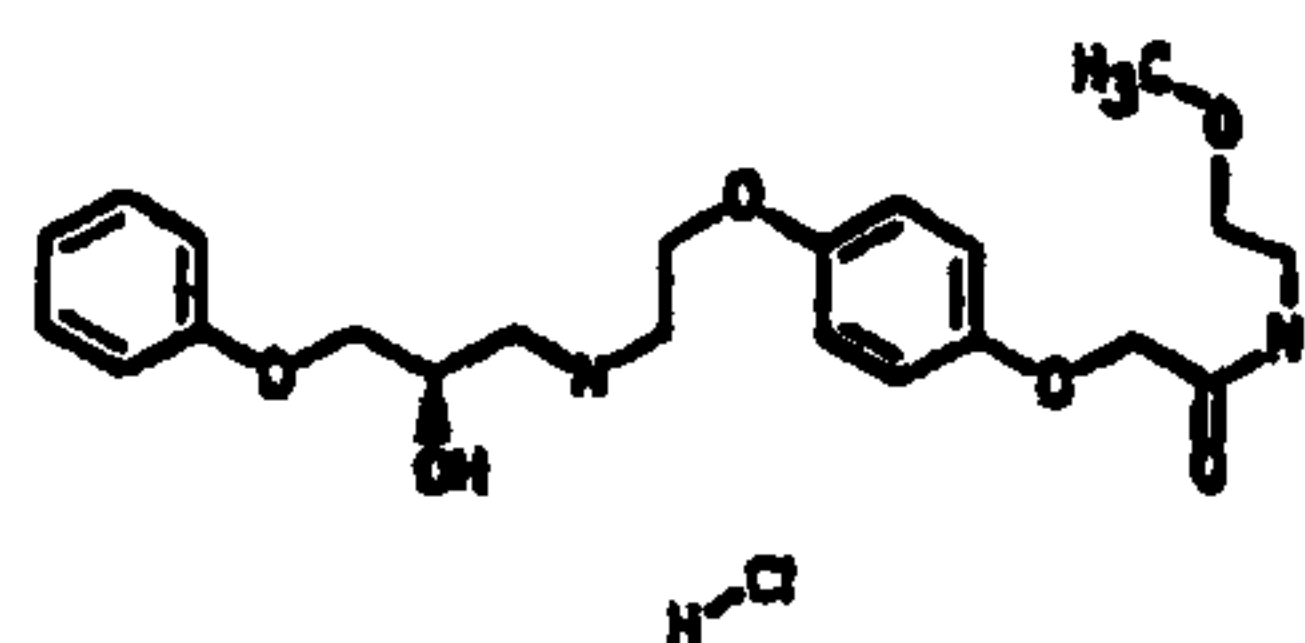
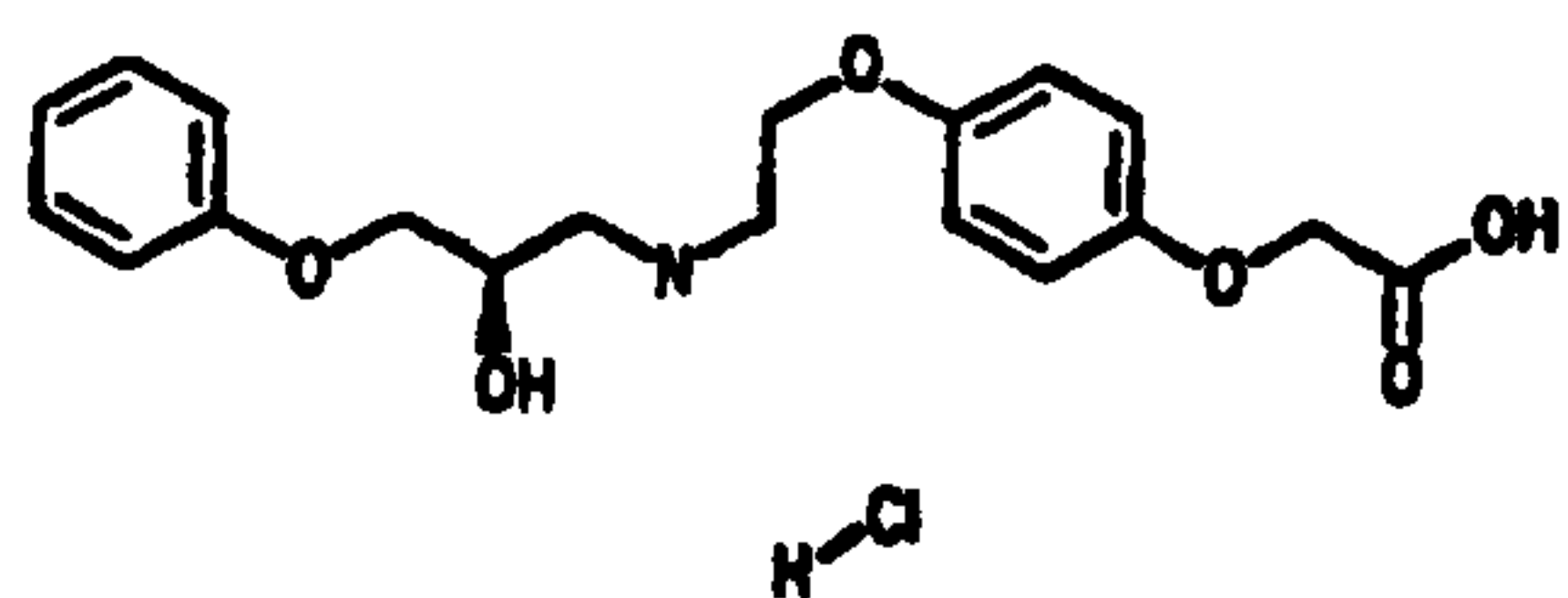
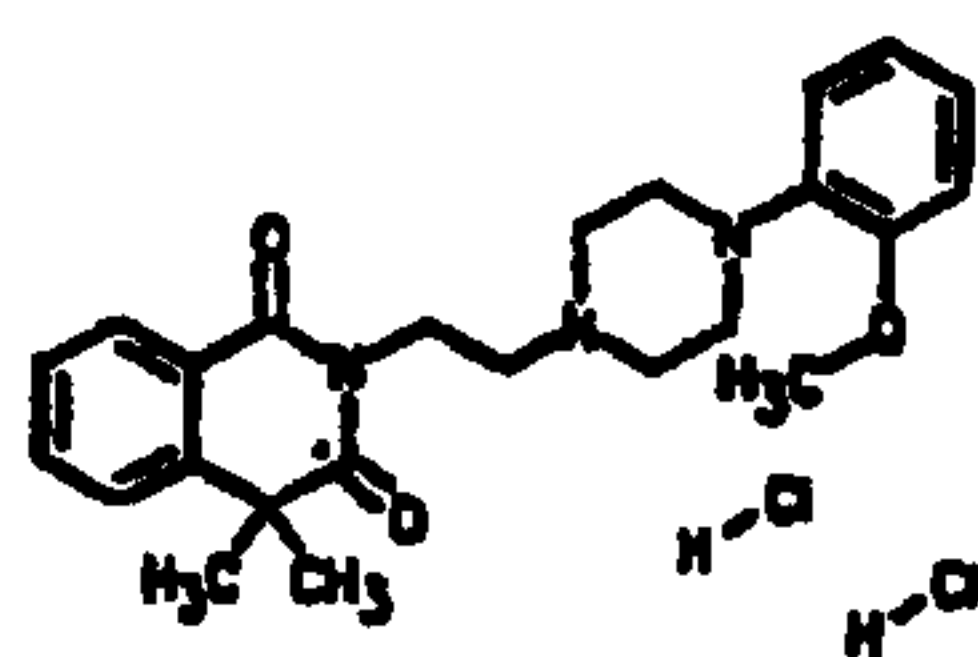
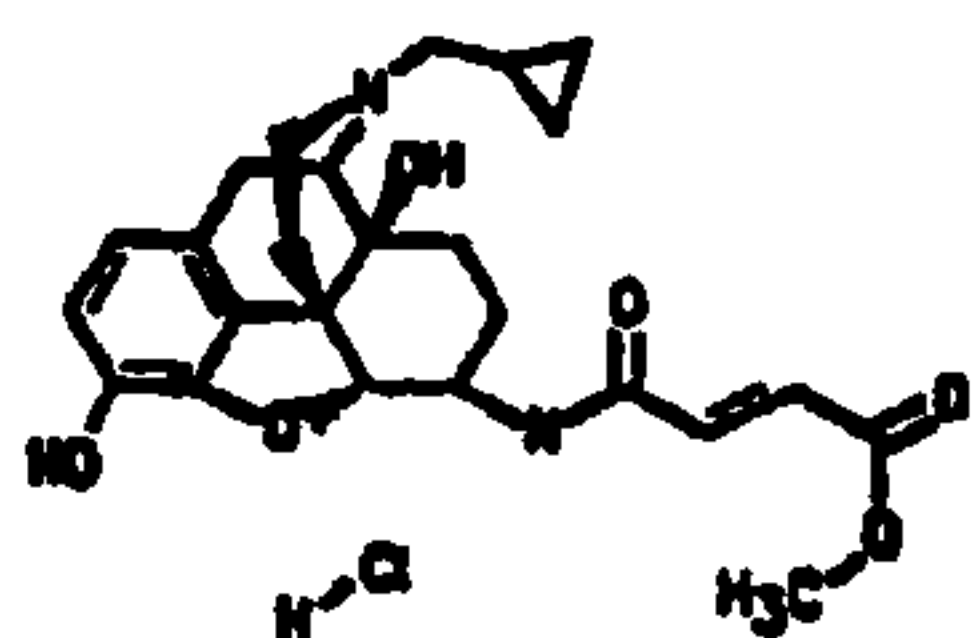
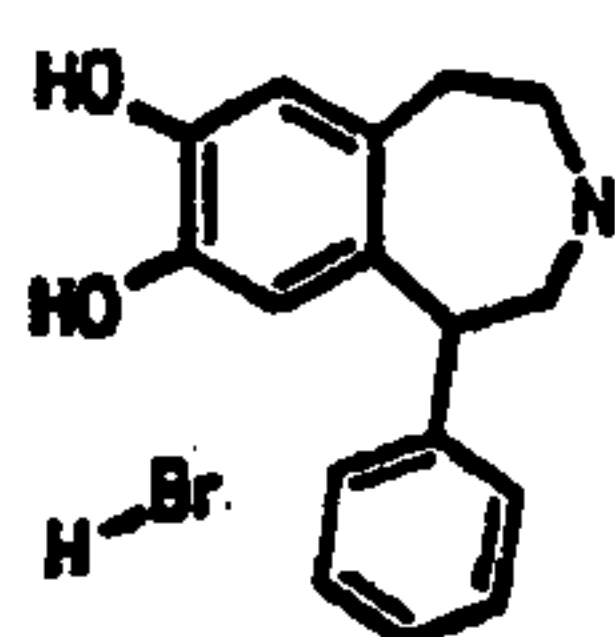
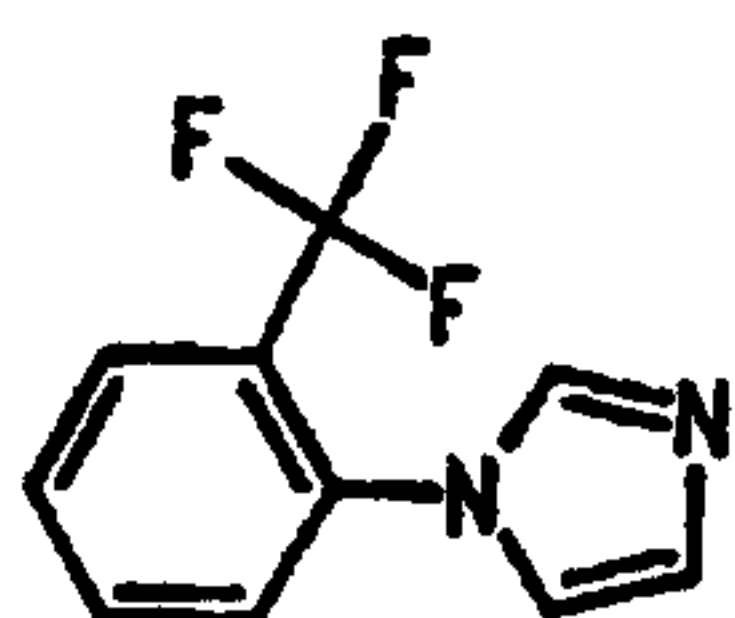
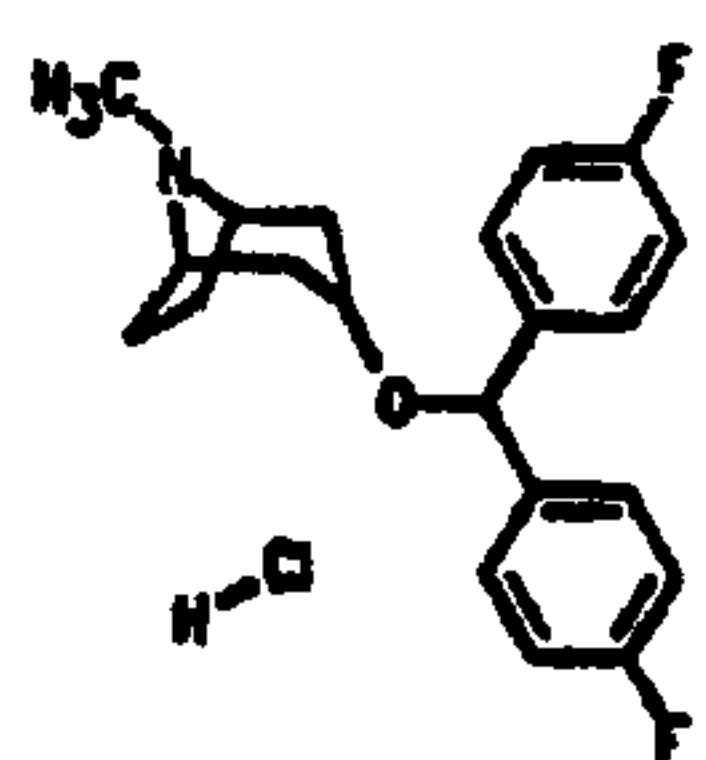


FIG. 17ccc

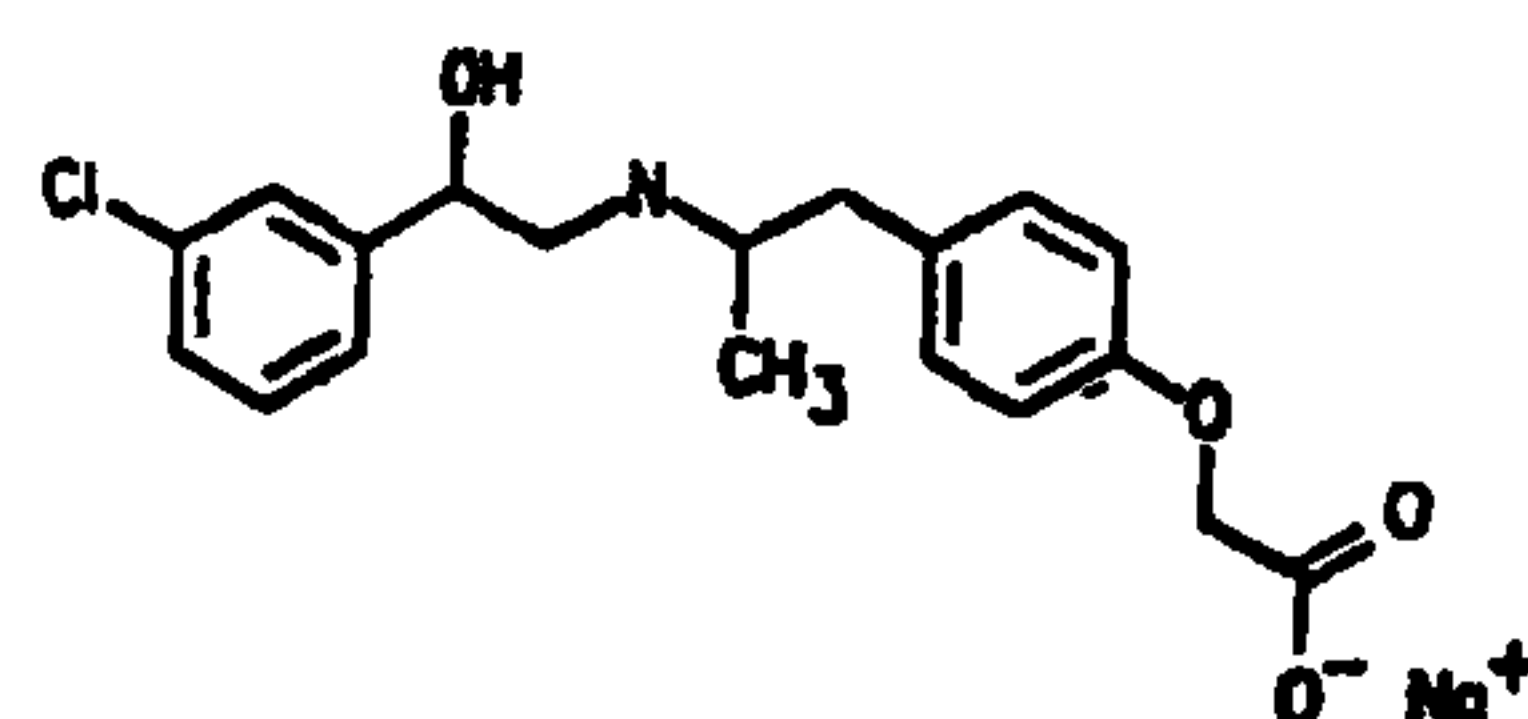
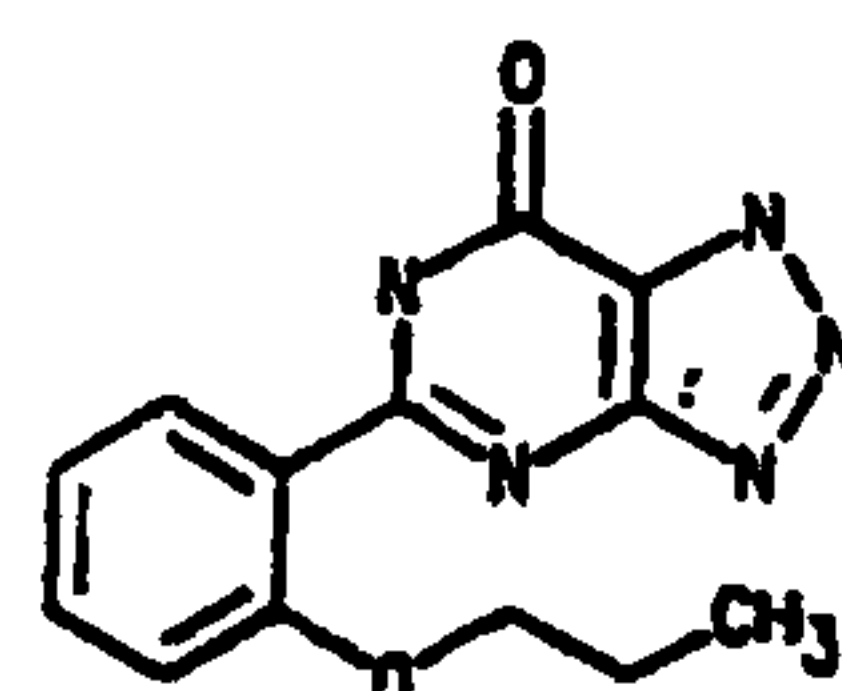
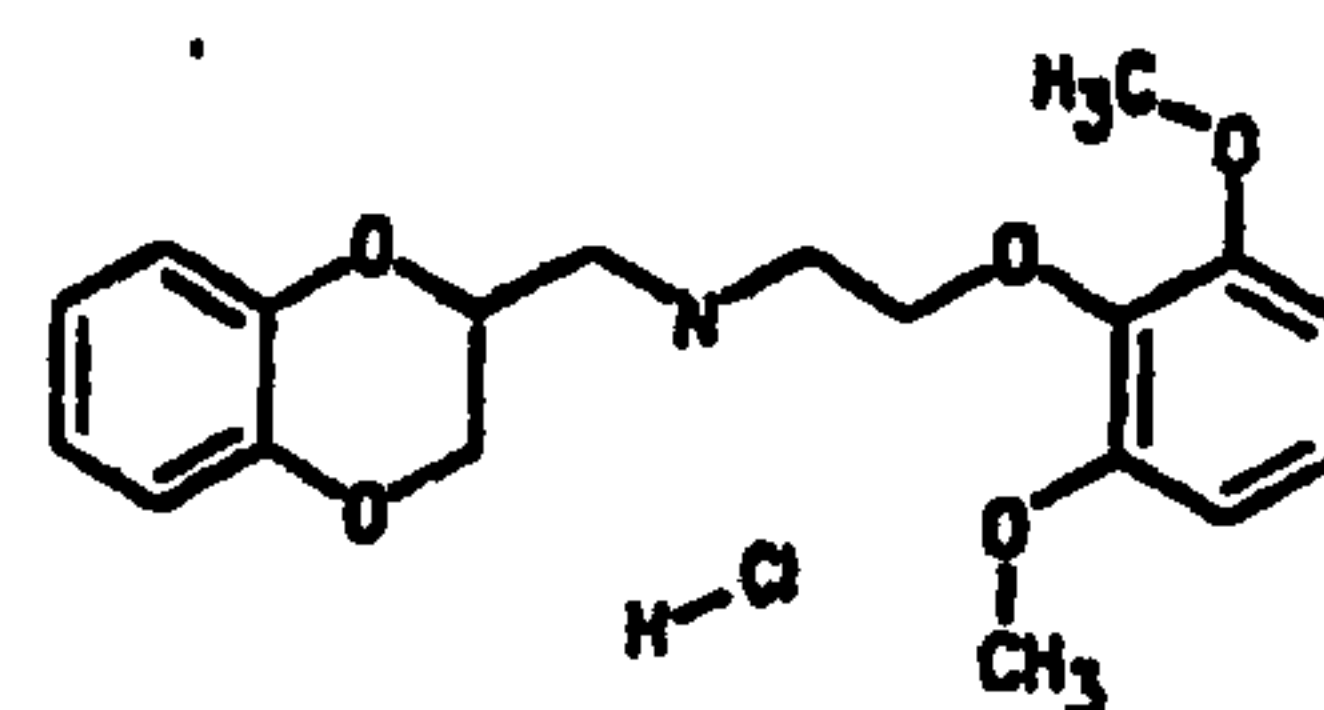
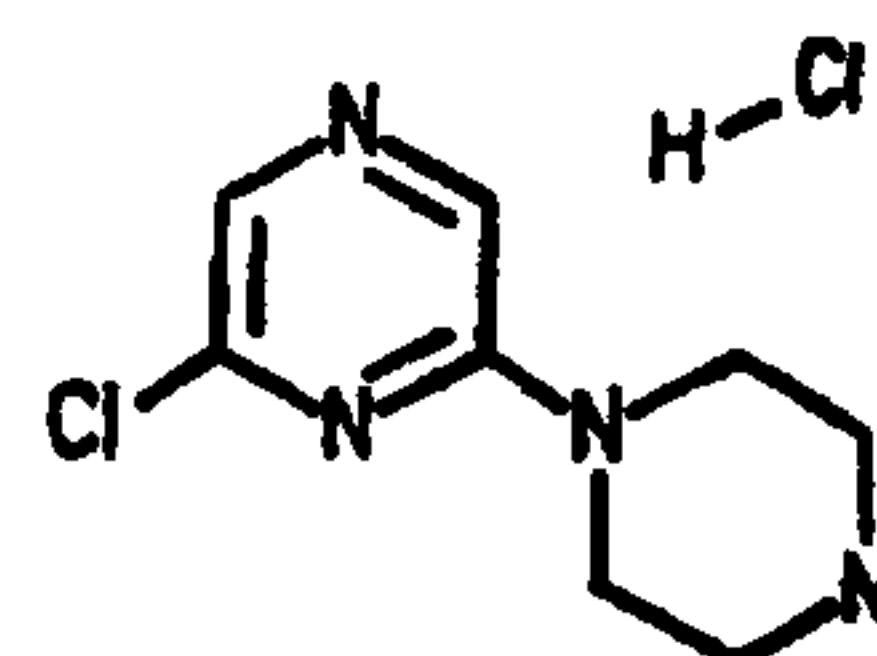
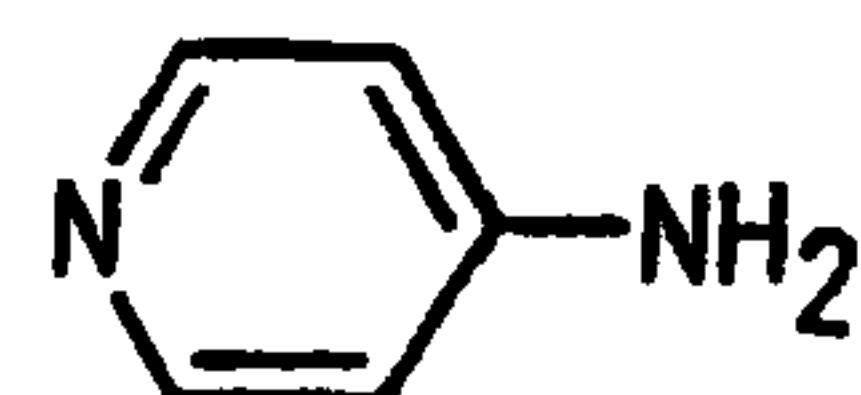
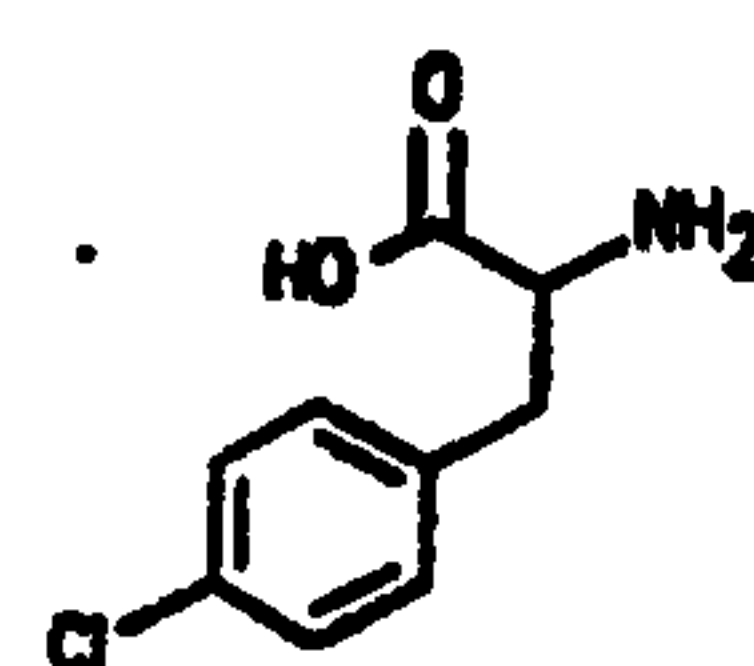
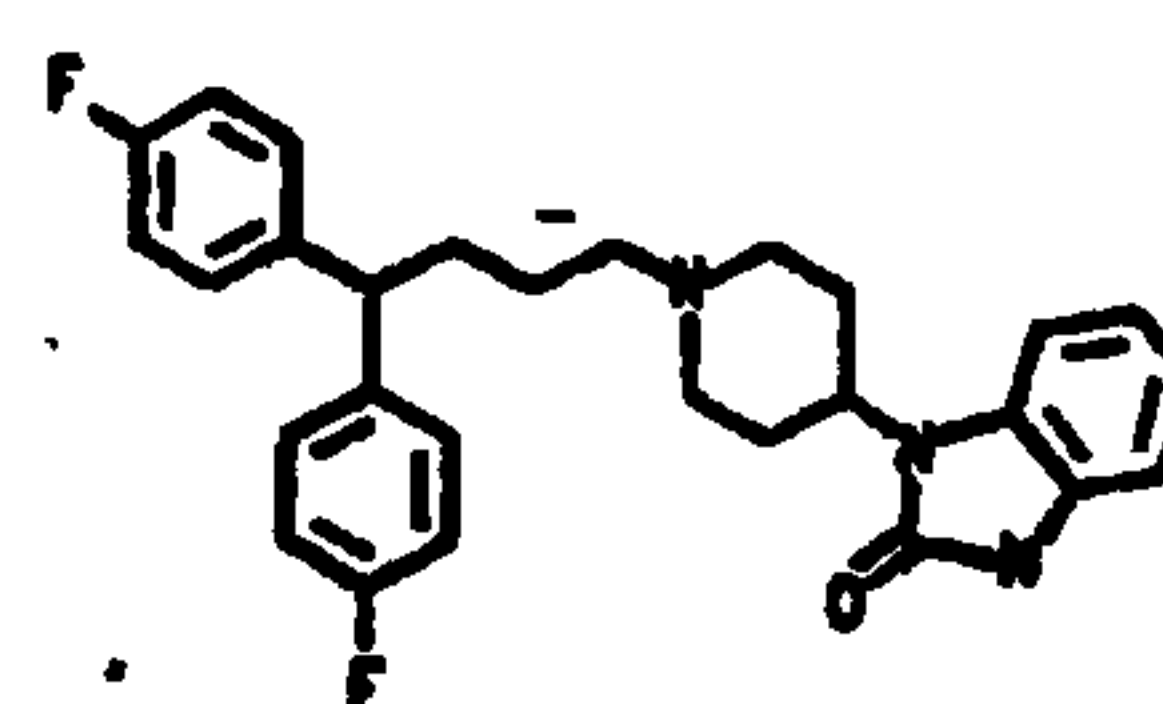
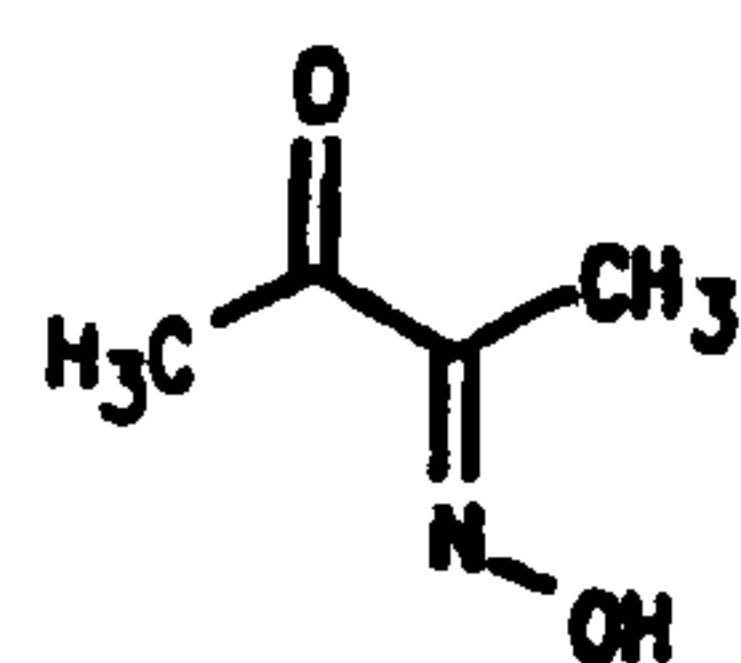
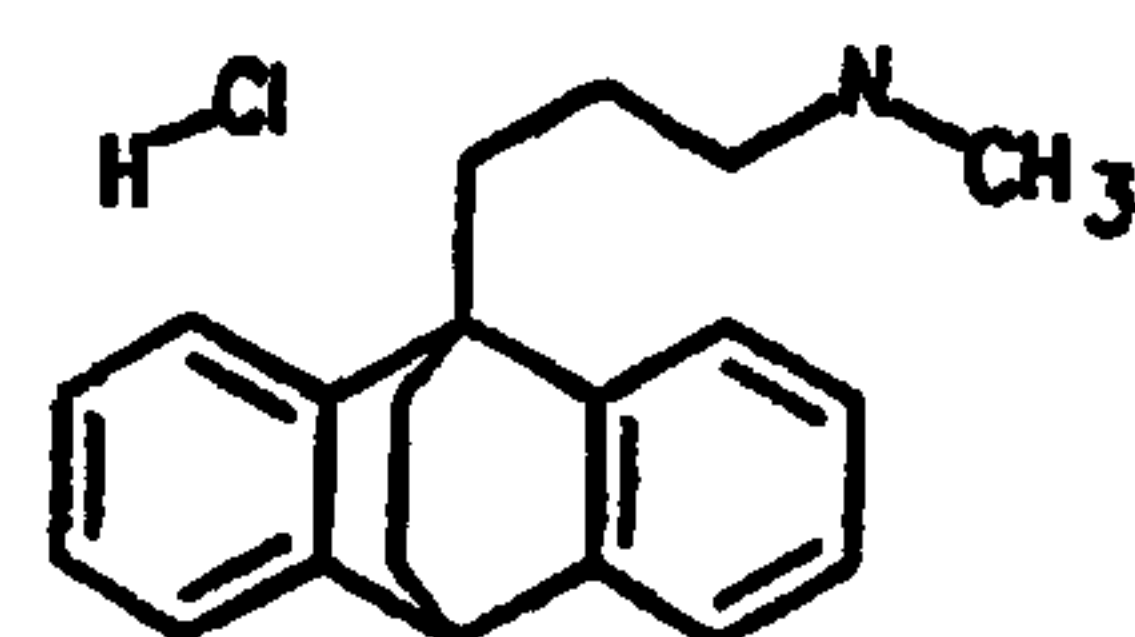
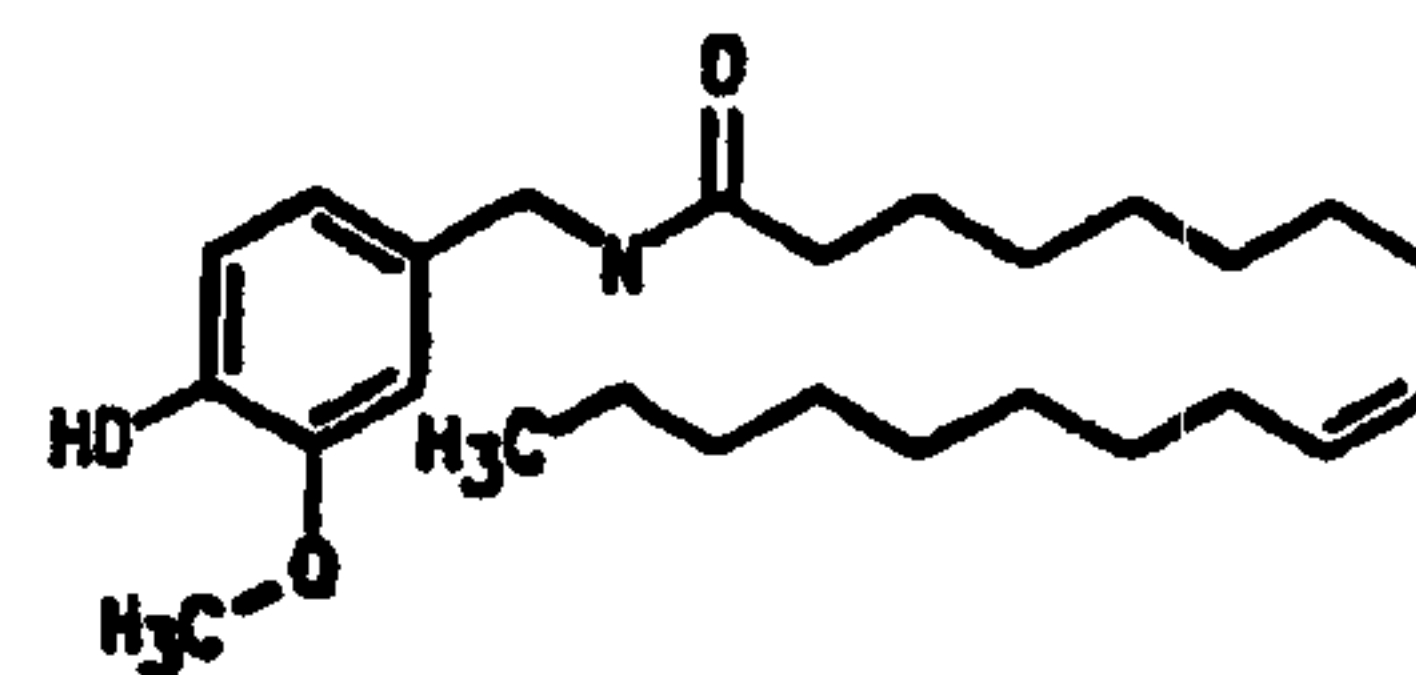


FIG. 17ddd

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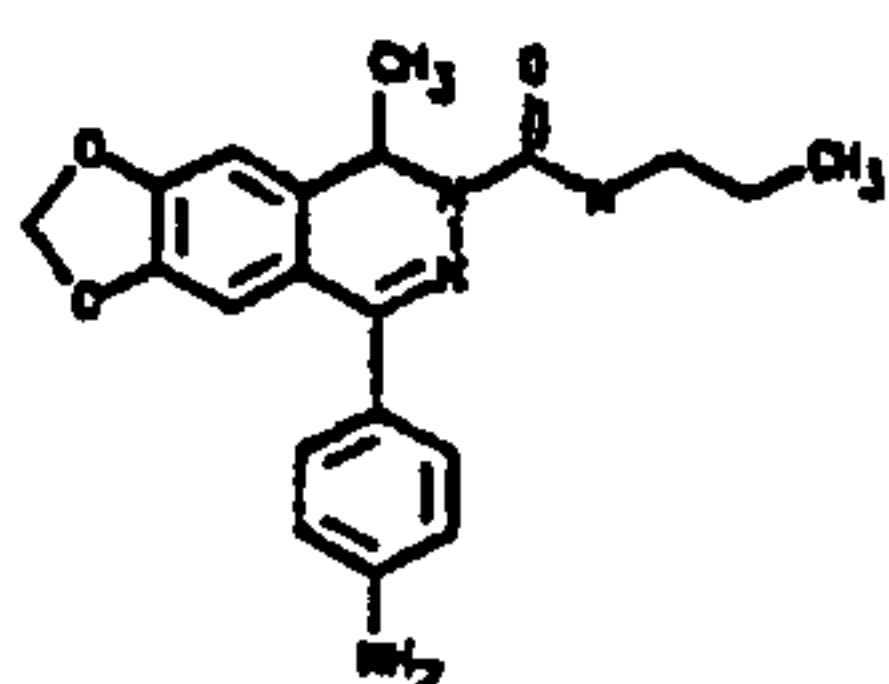
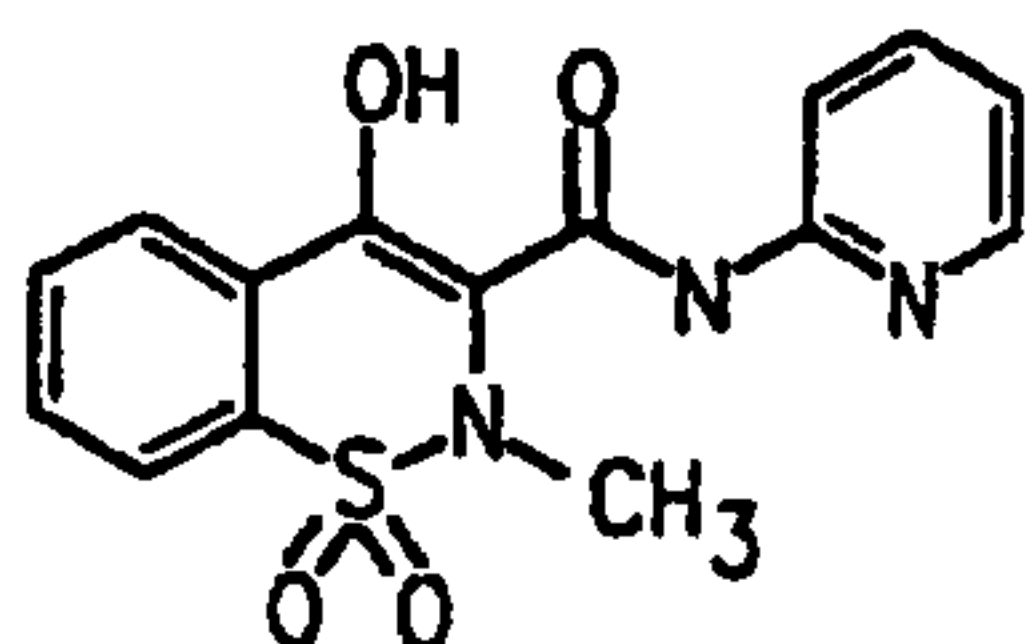
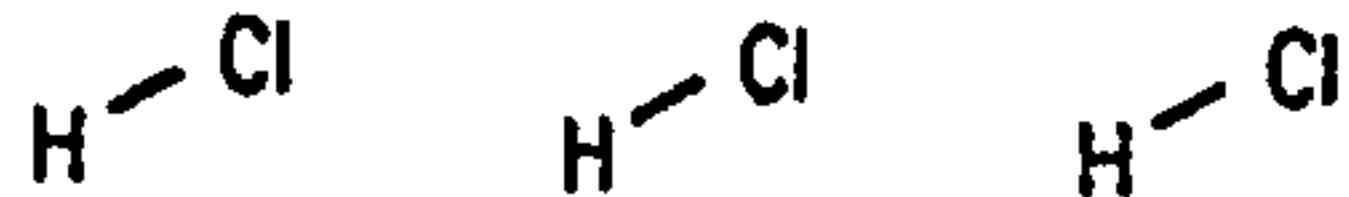
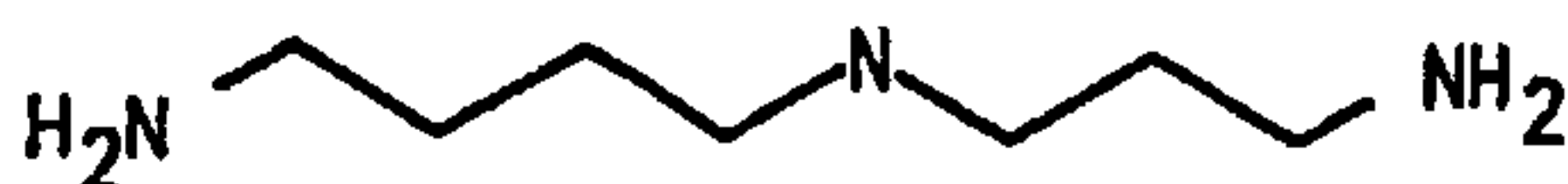
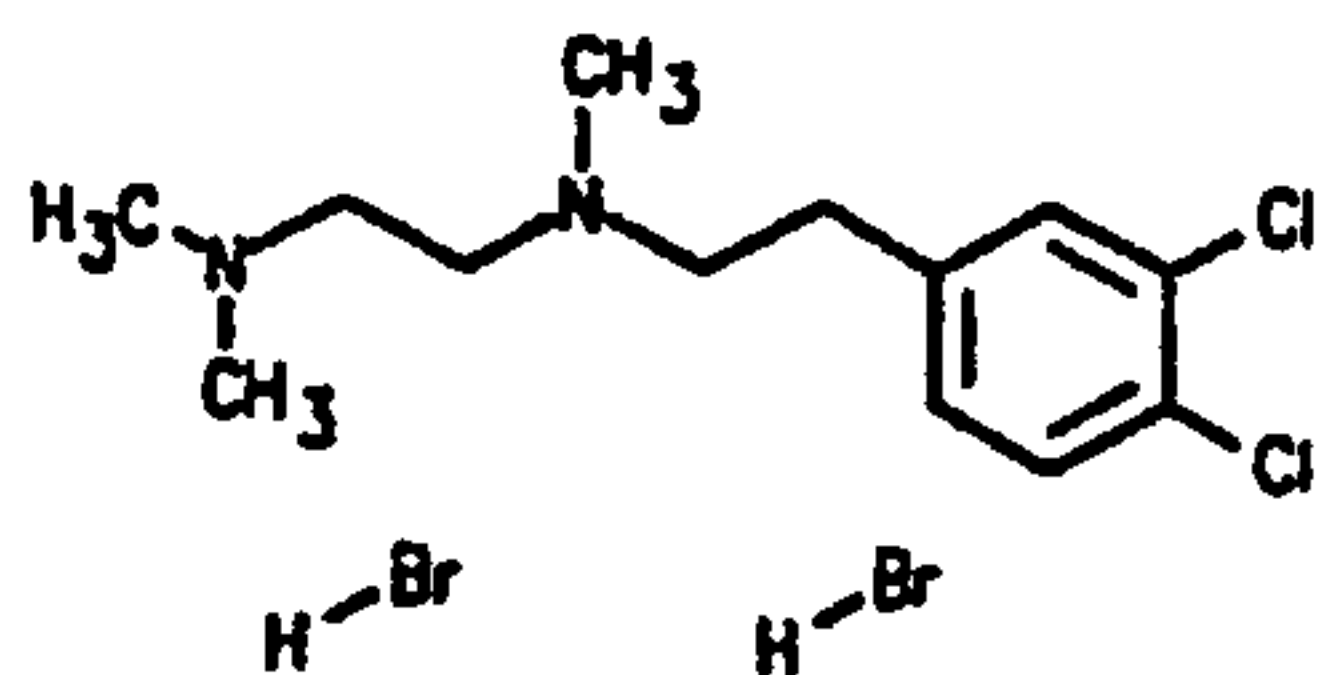
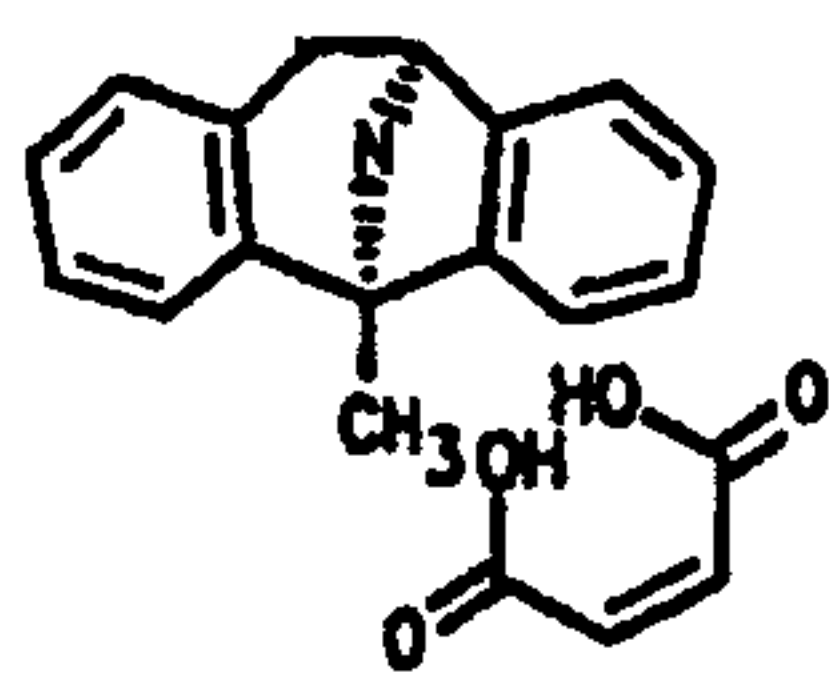
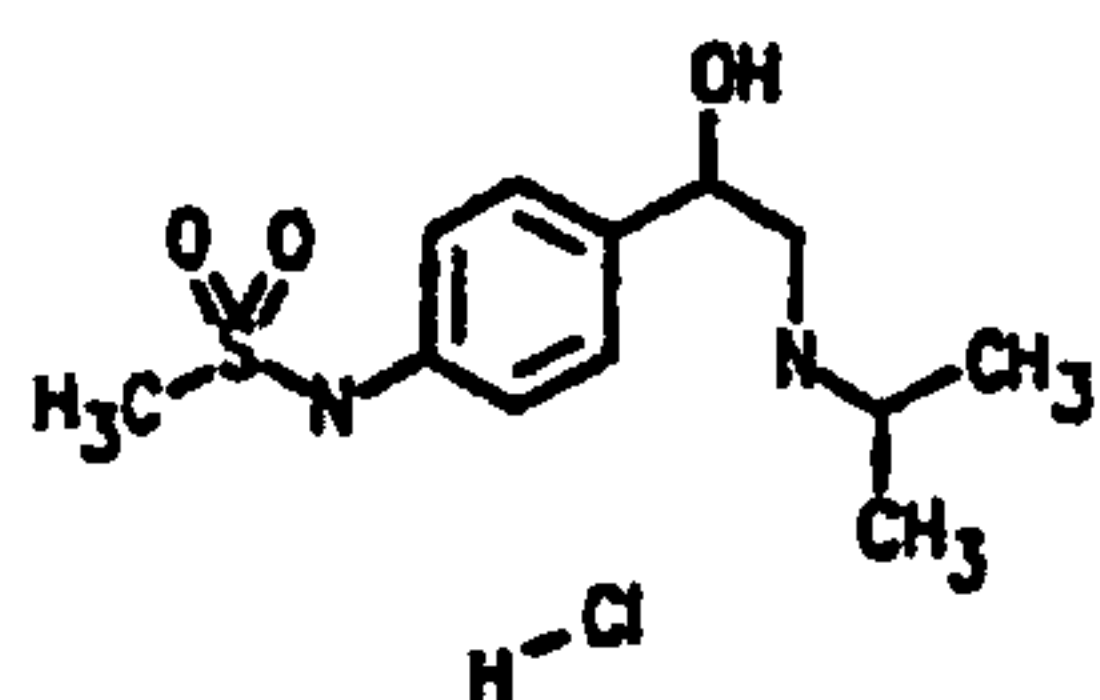
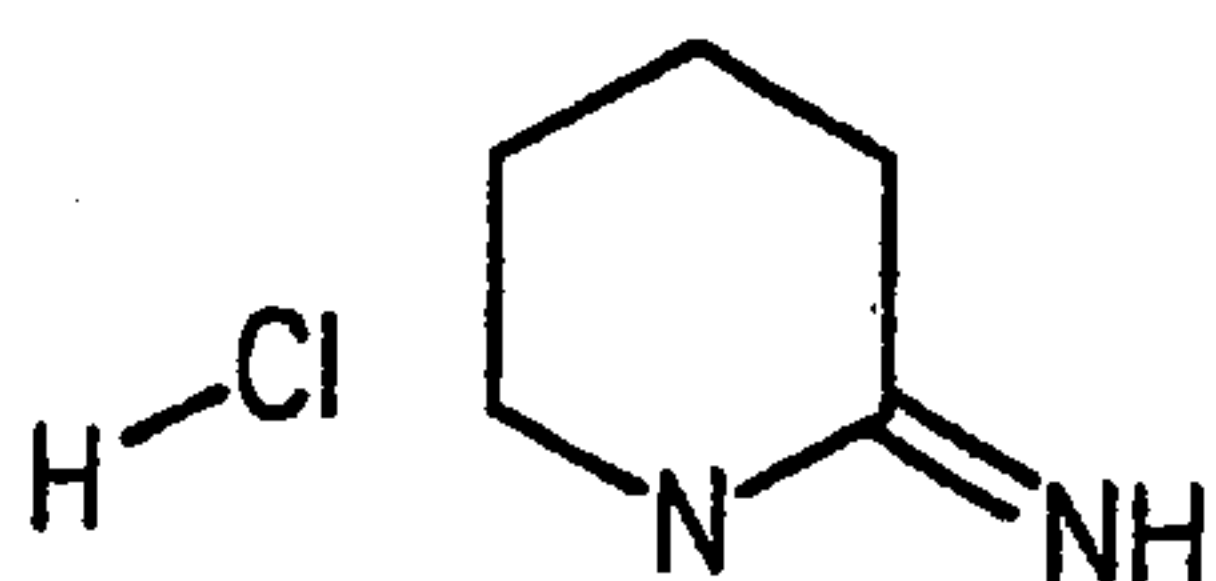
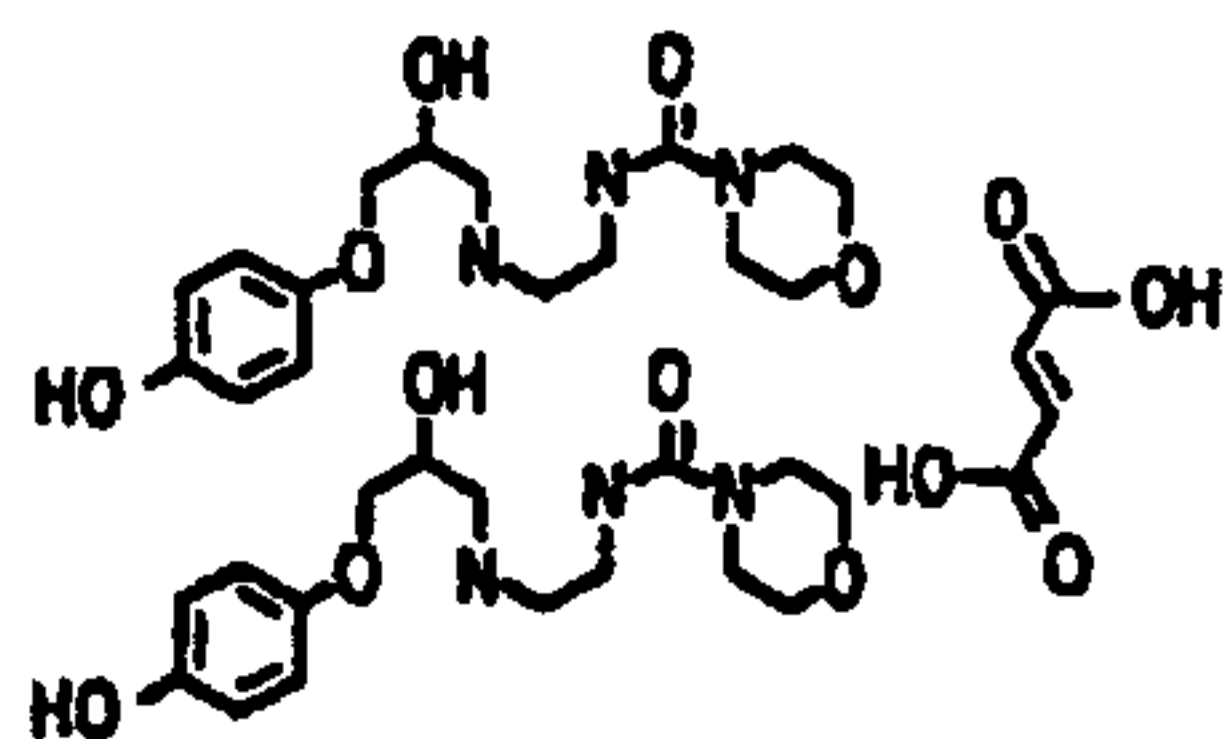
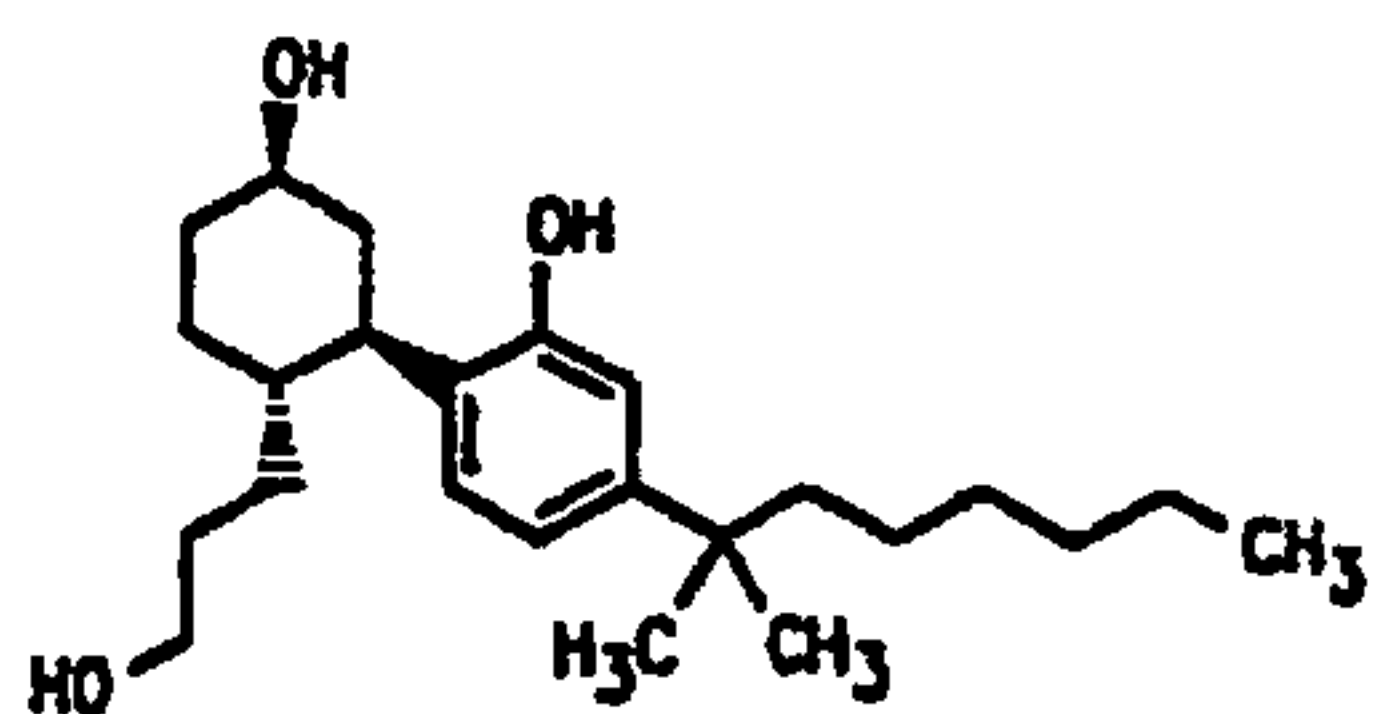


**FIG. 17eee**

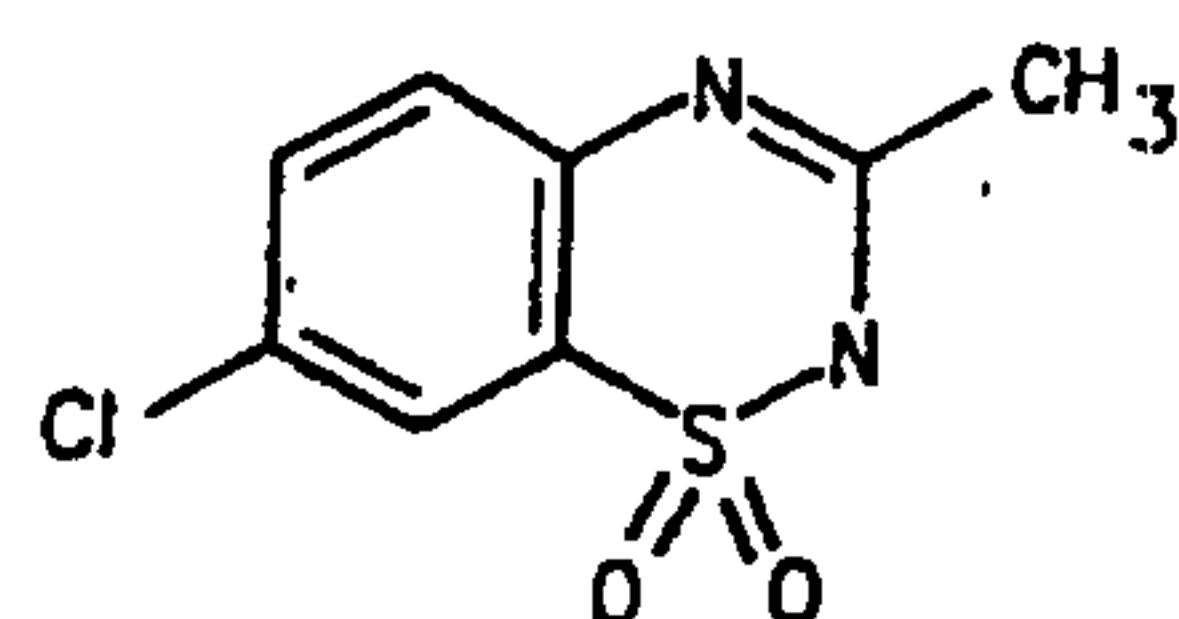
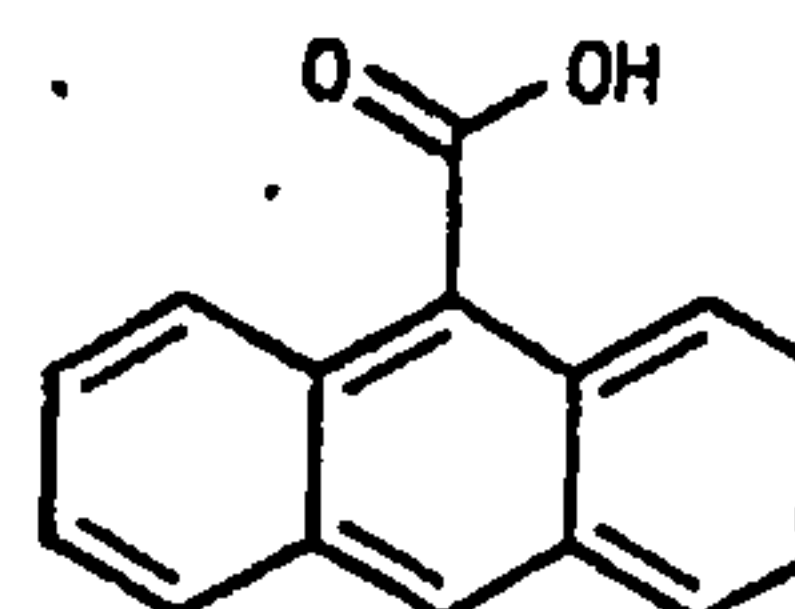
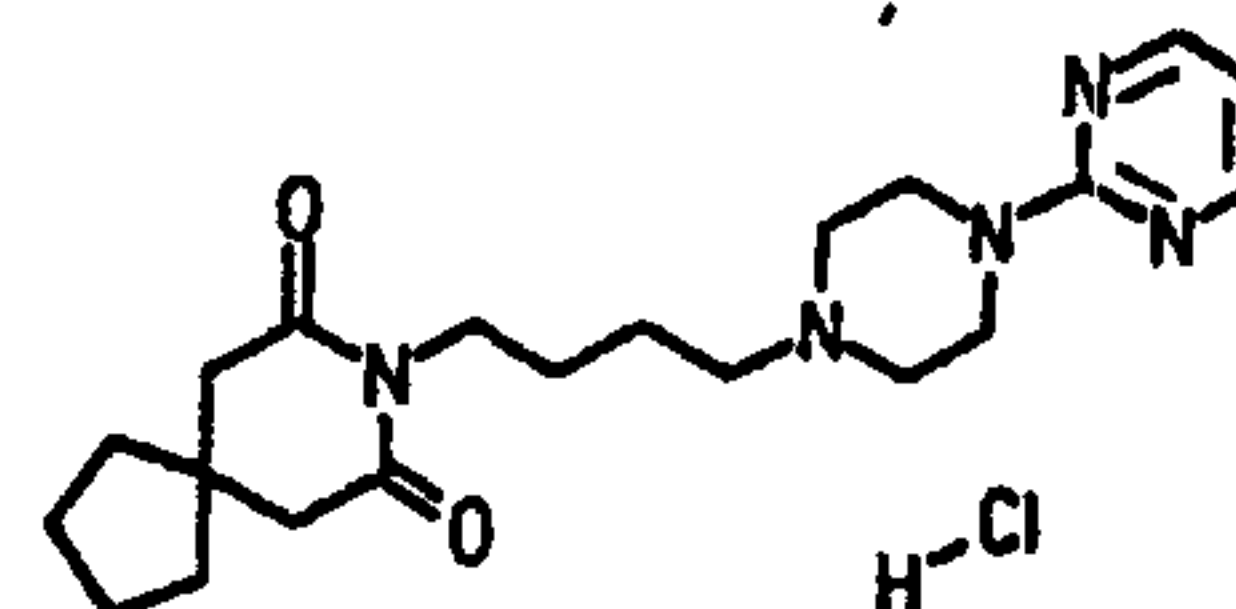
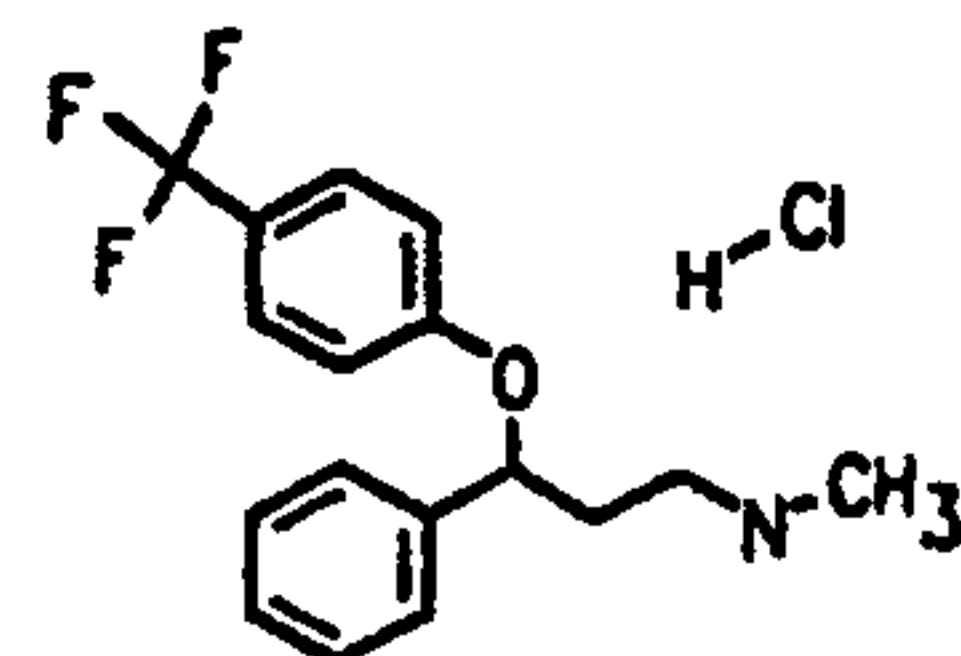
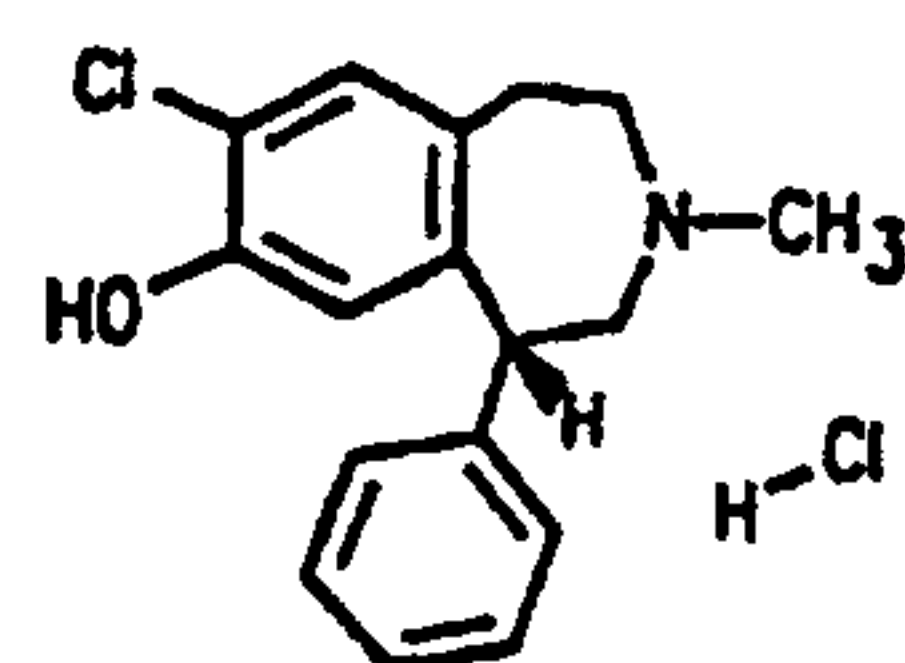
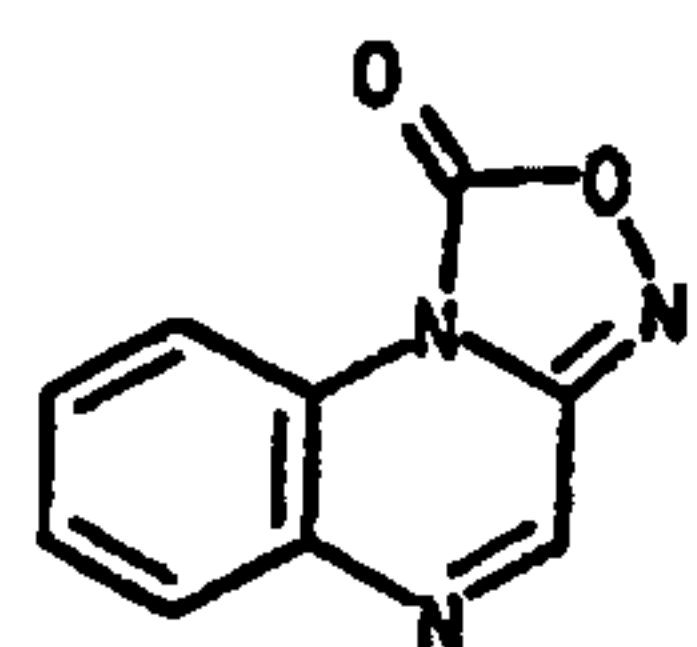
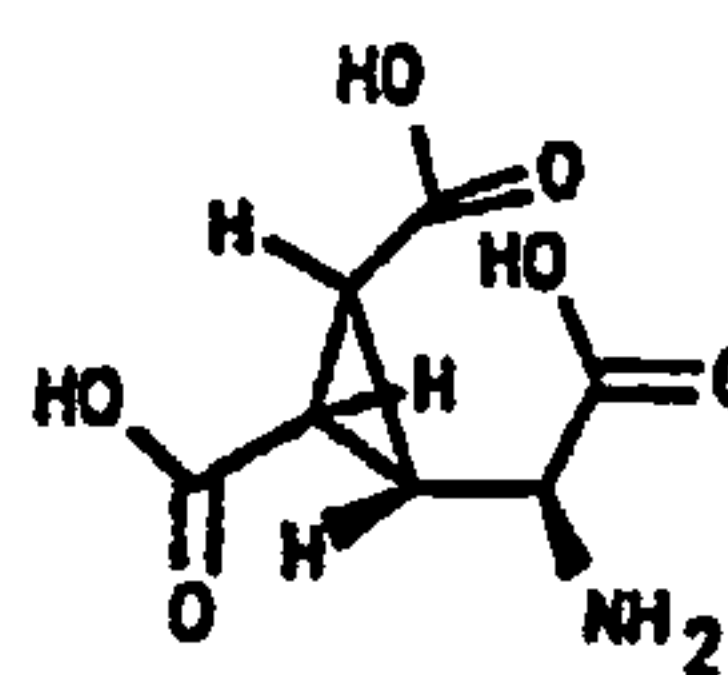
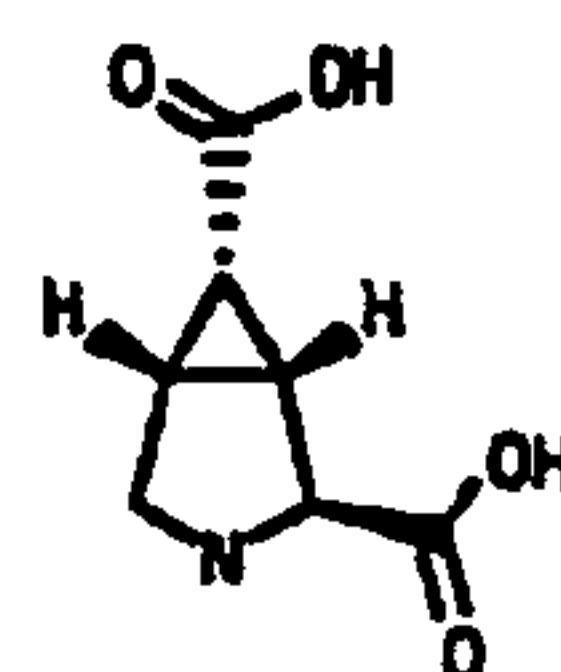
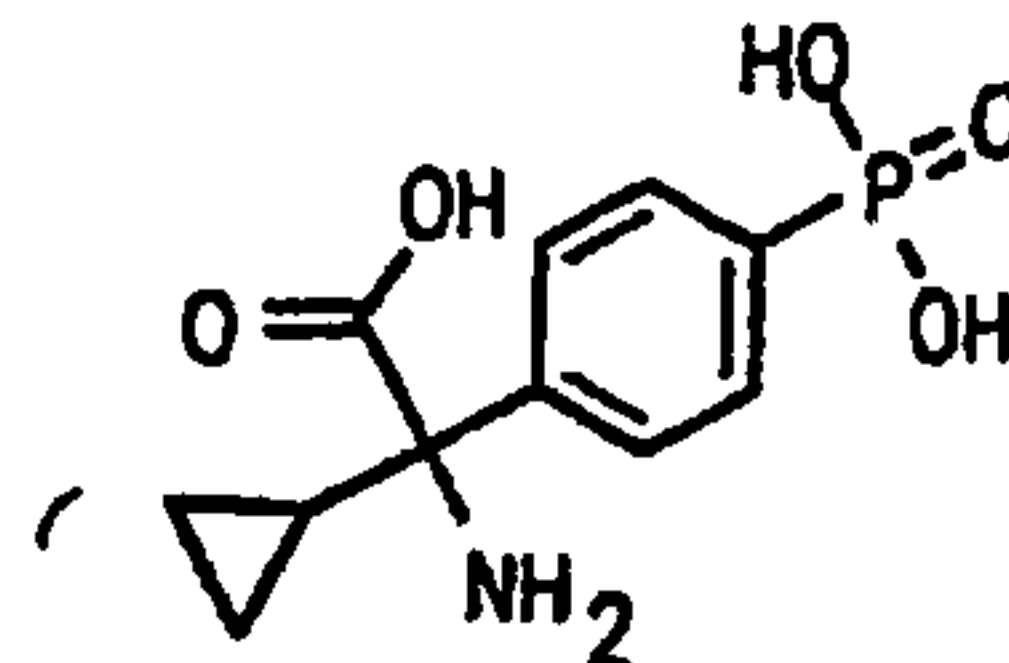
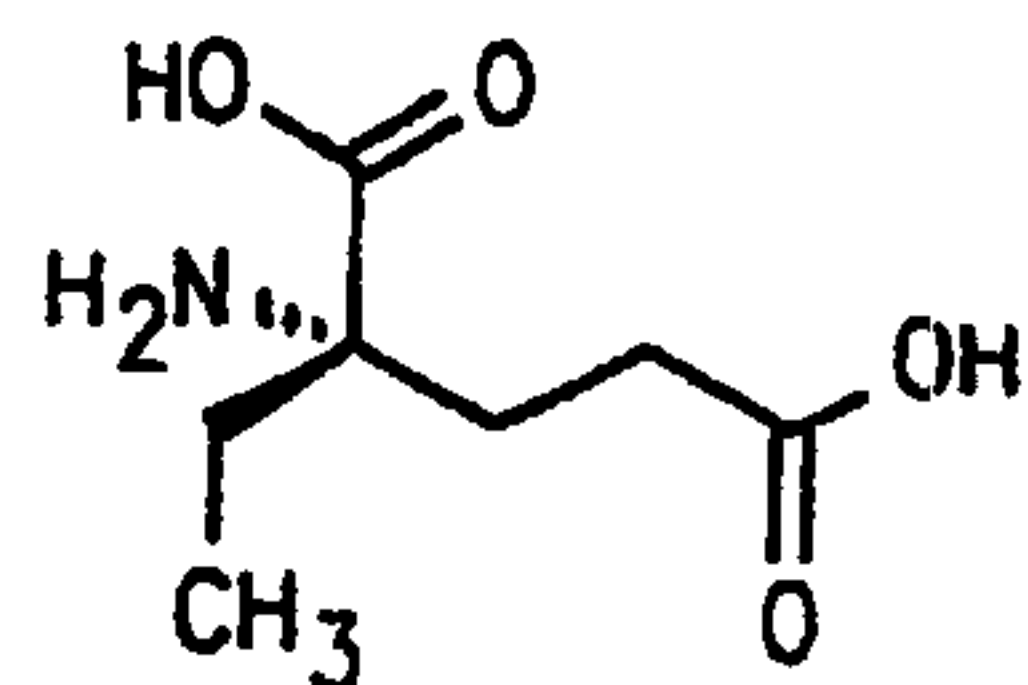


FIG. 17fff

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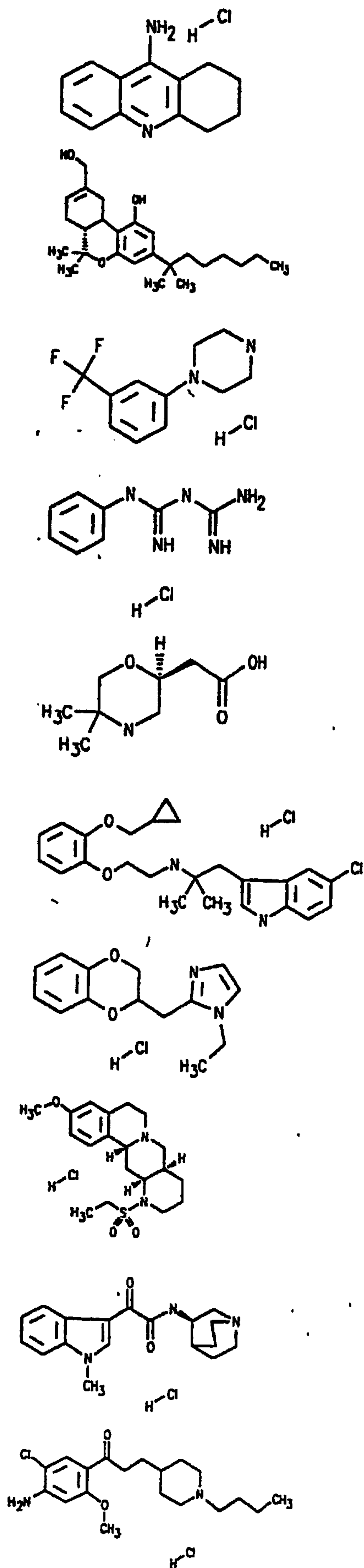


FIG. 17ggg

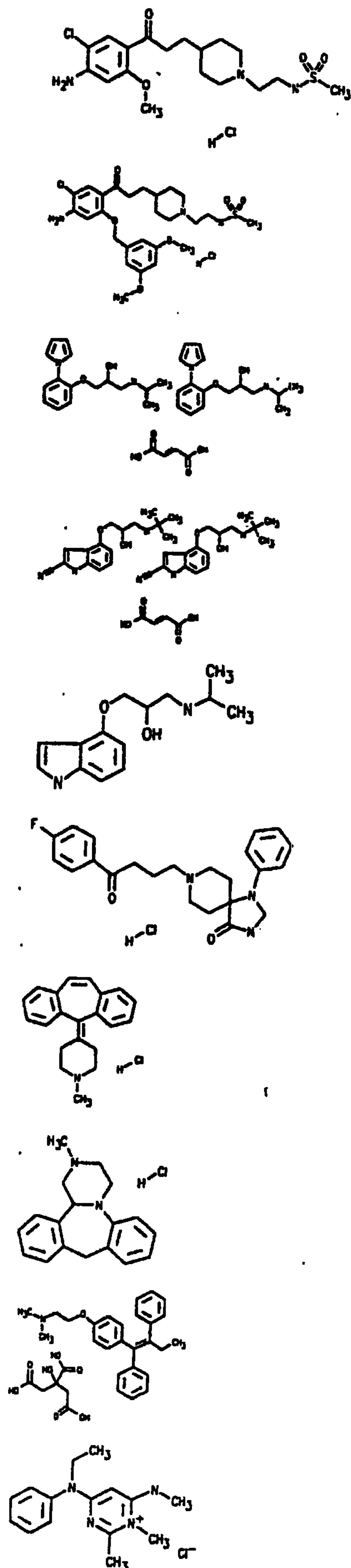


FIG. 17hhh

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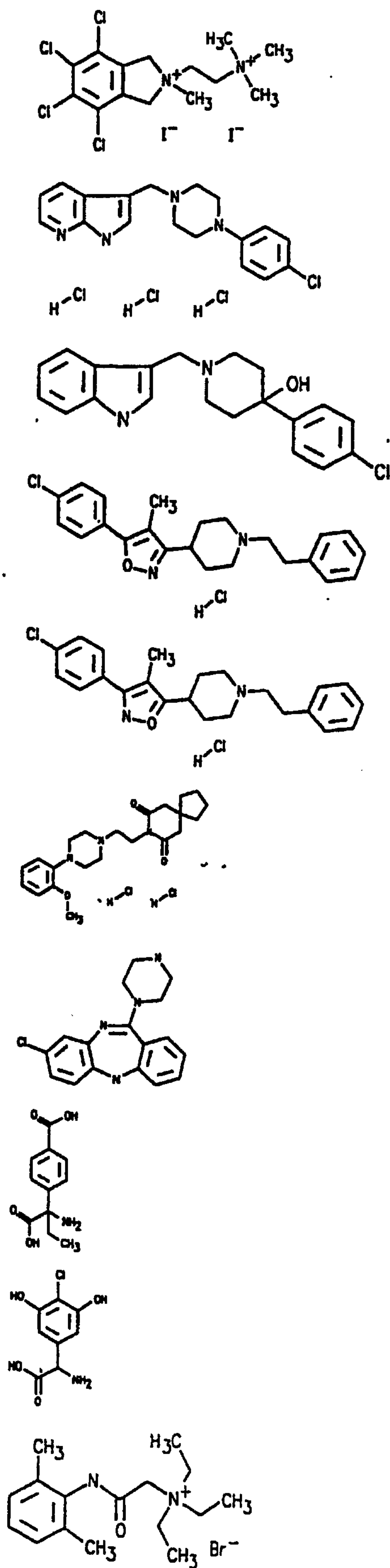


FIG. 17iii

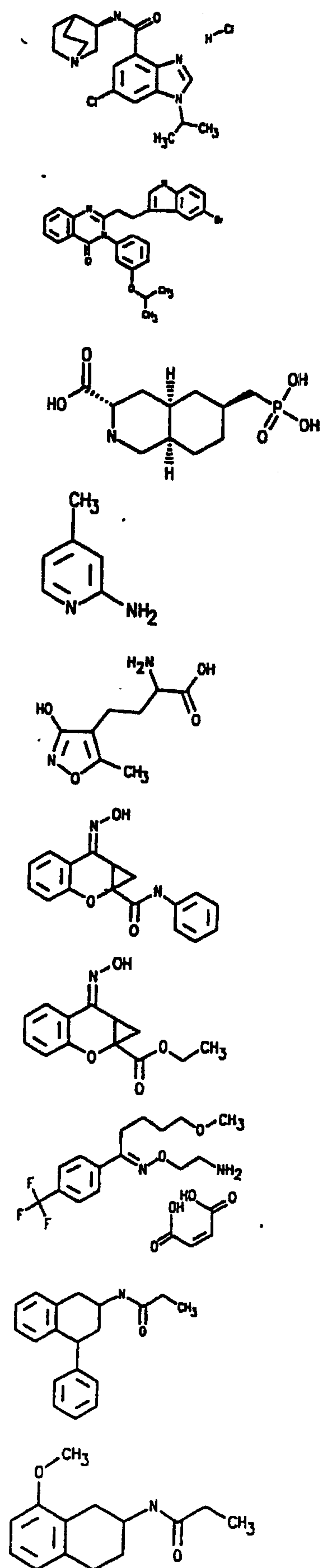


FIG. 17iii

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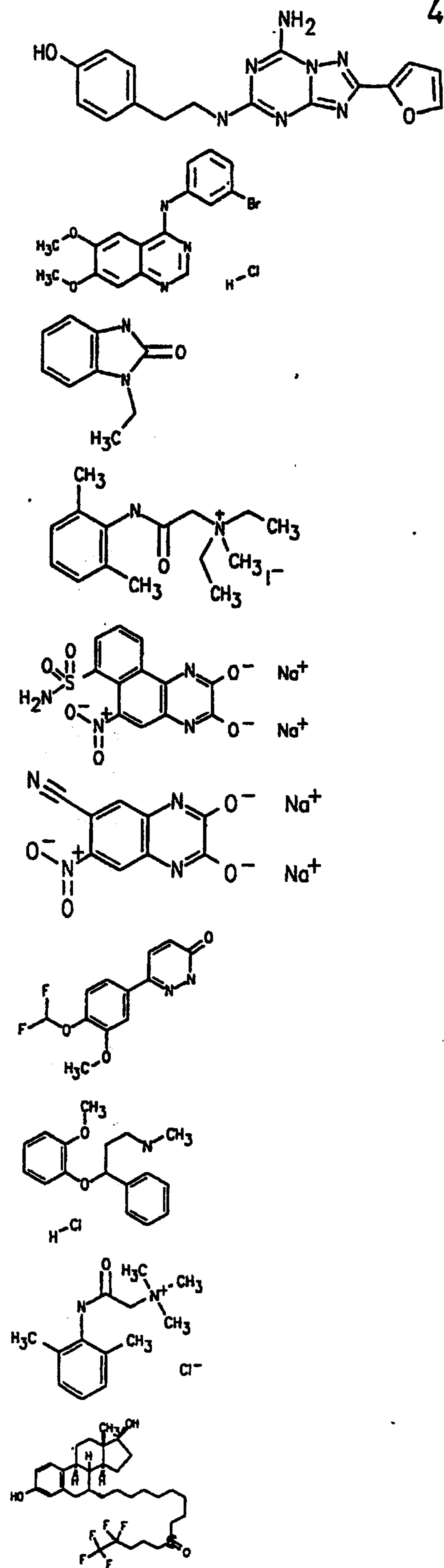


FIG. 17kkk

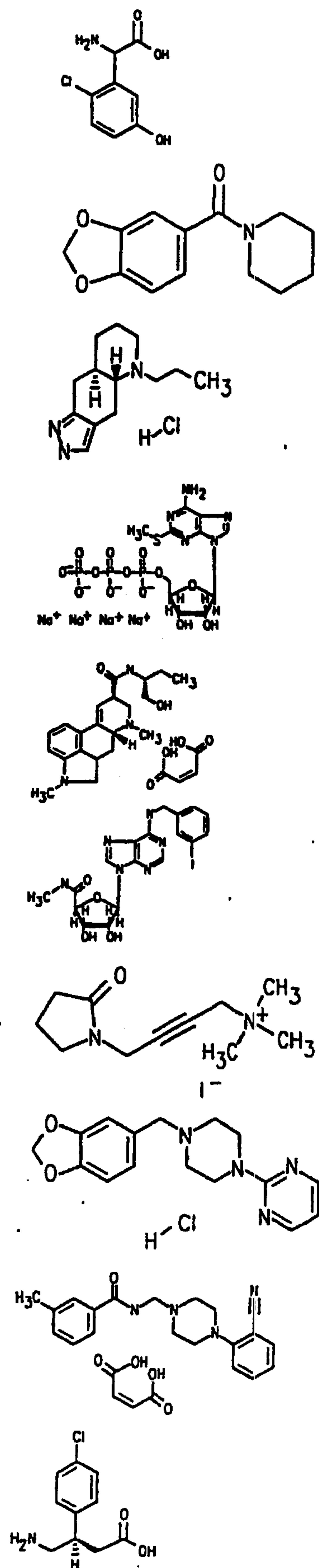


FIG. 17III

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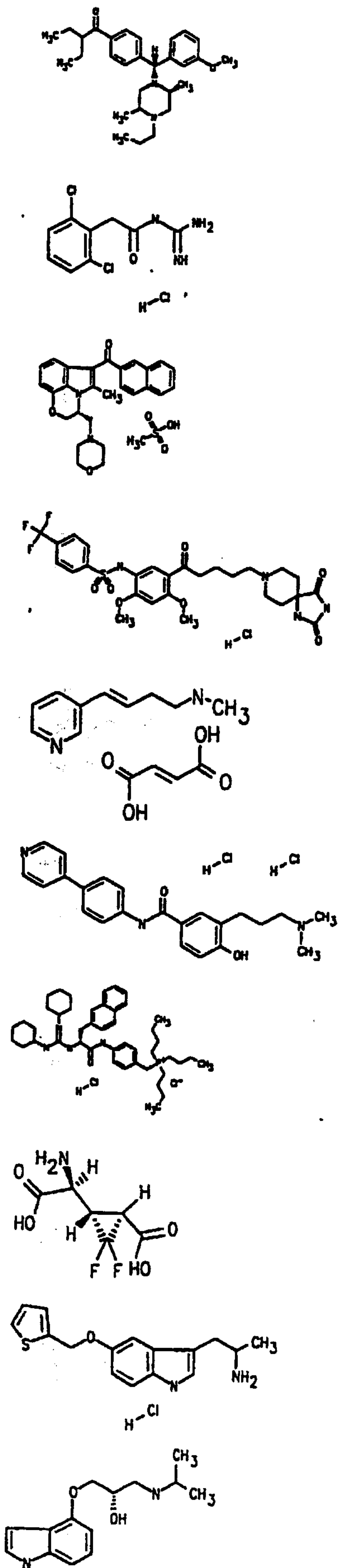


FIG. 17mmm

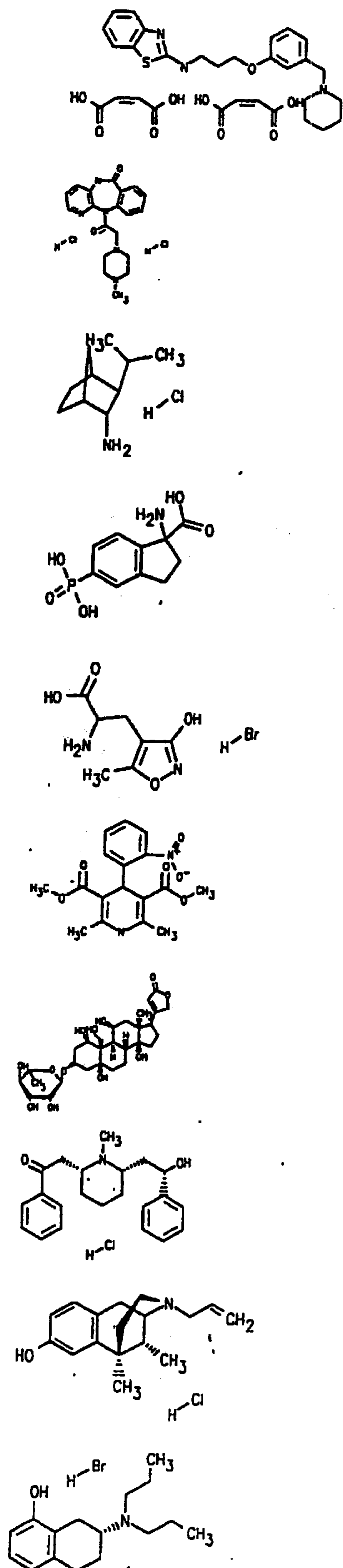


FIG. 17nnn

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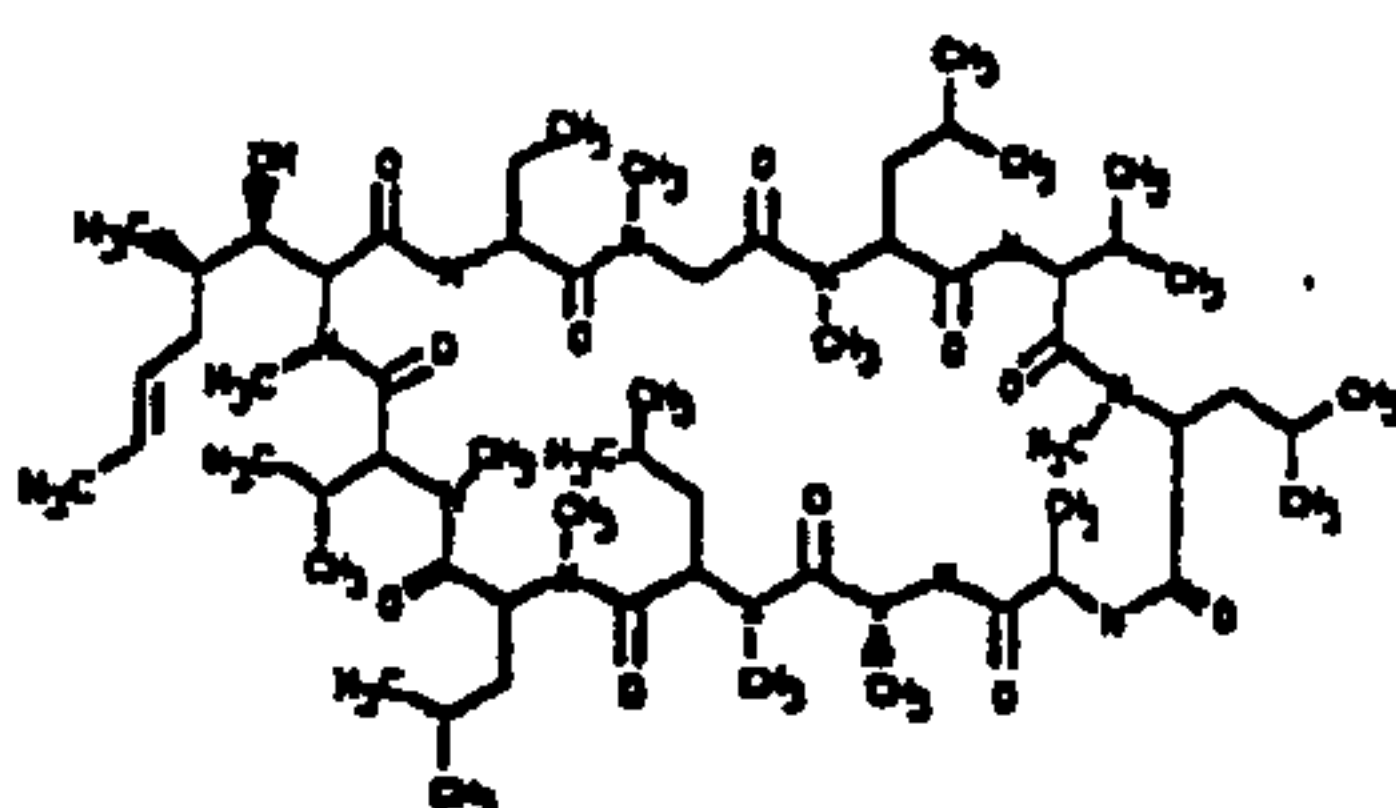
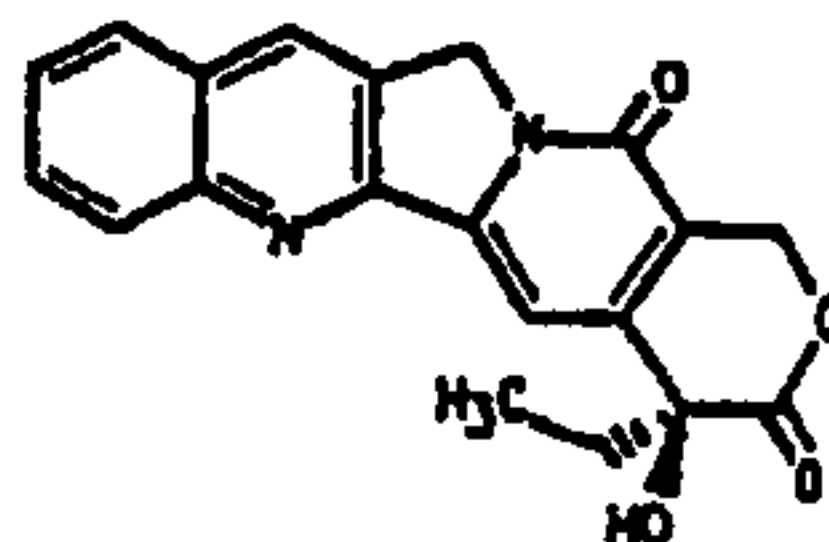
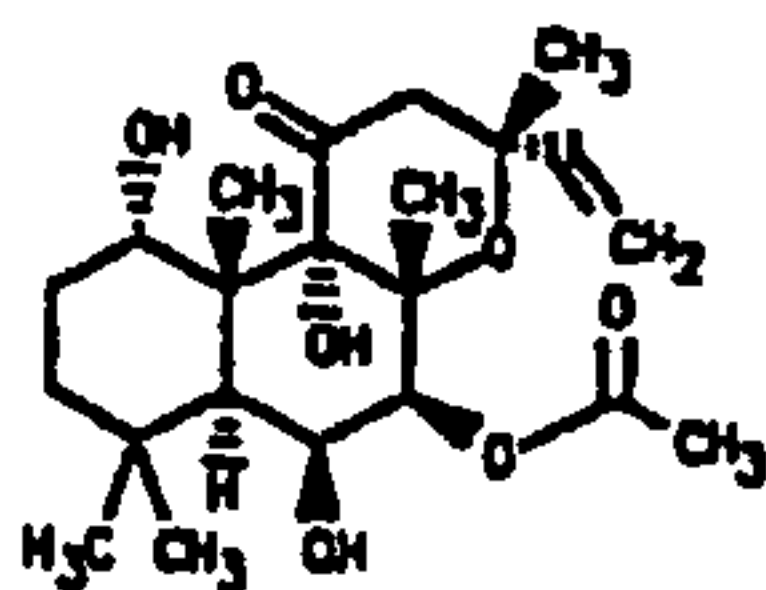
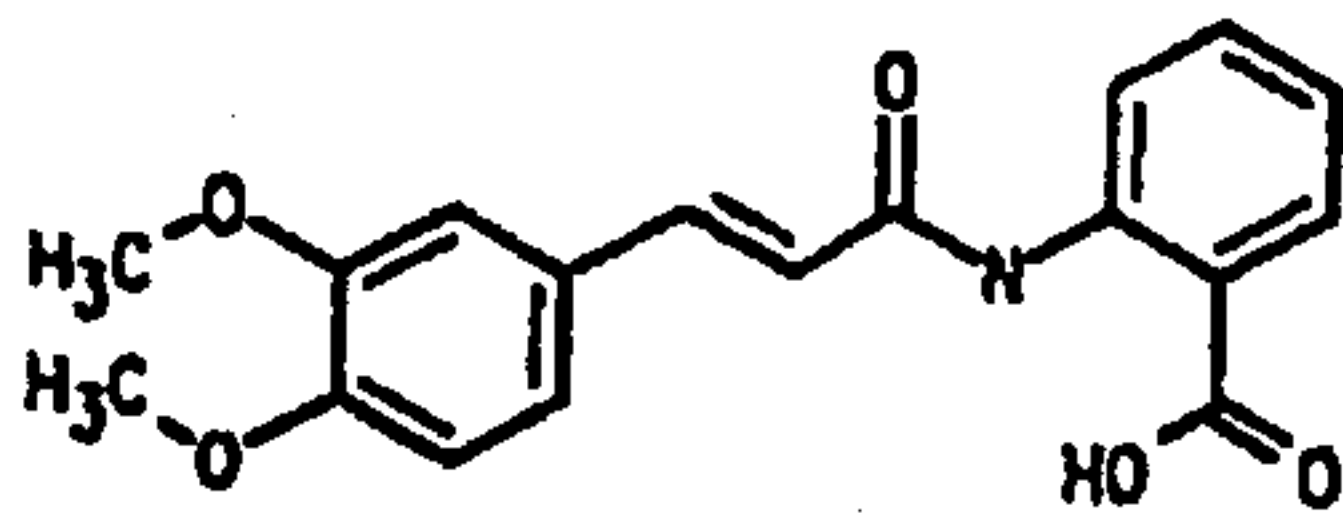
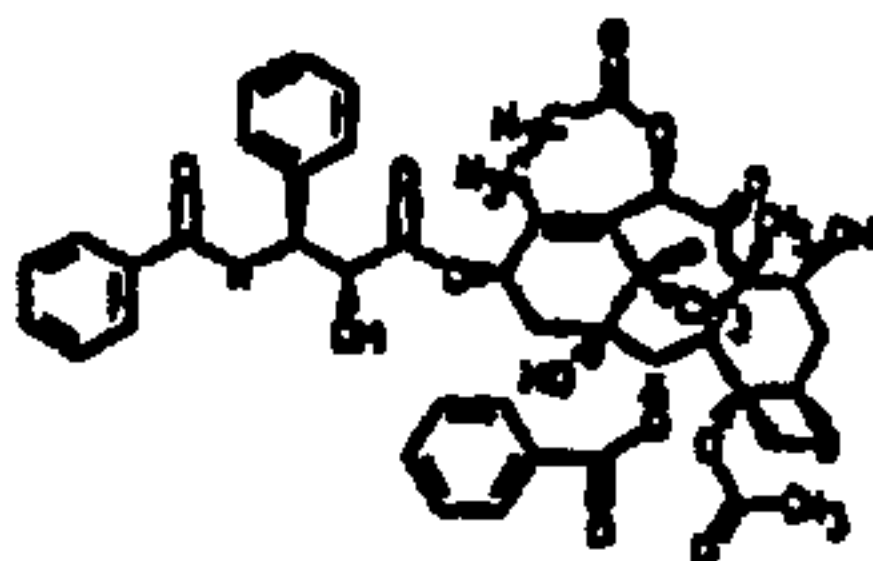
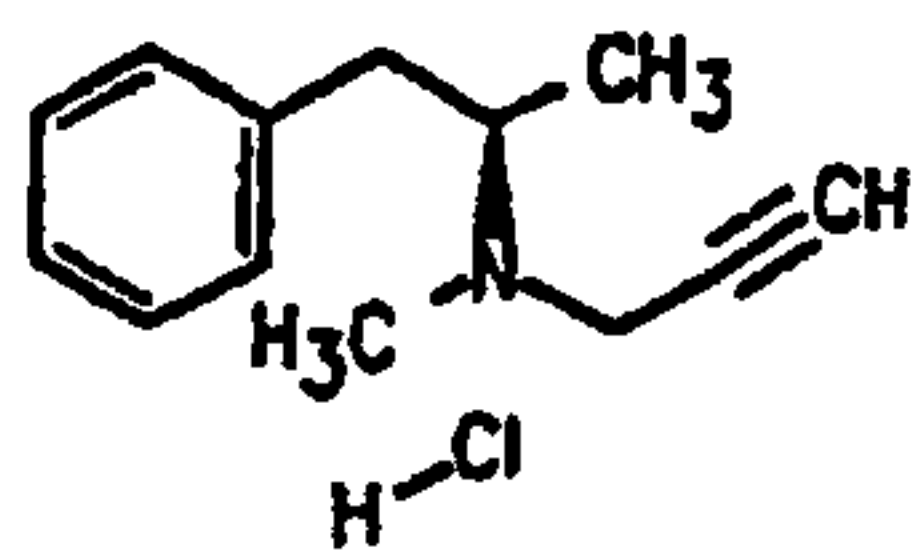
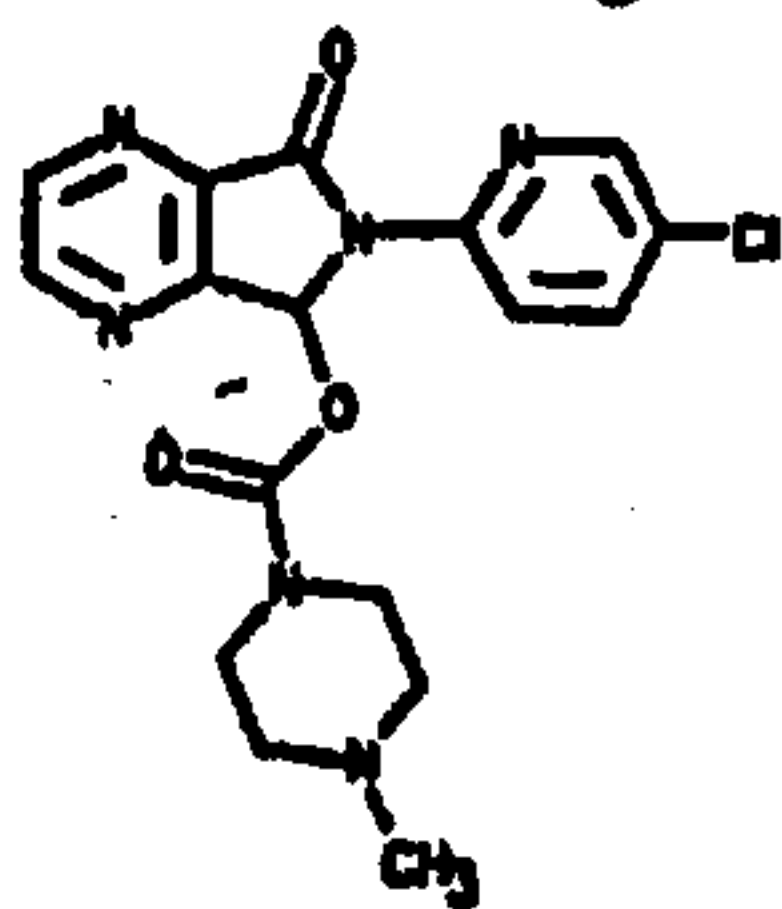
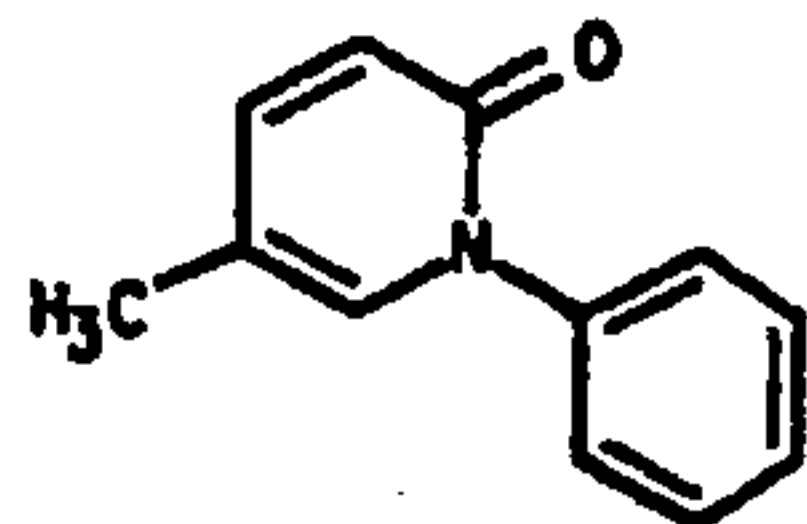
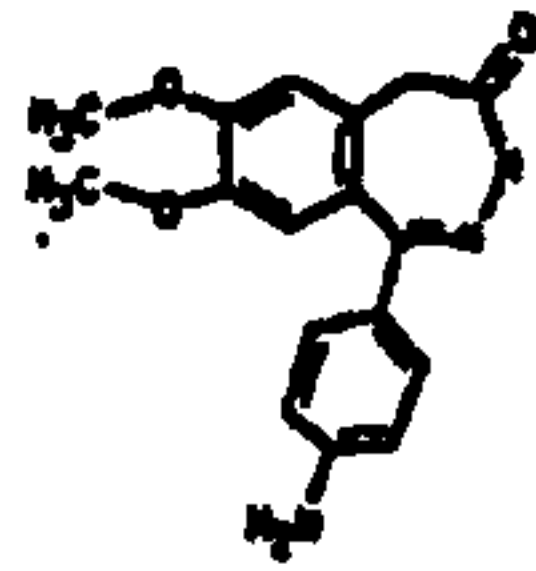
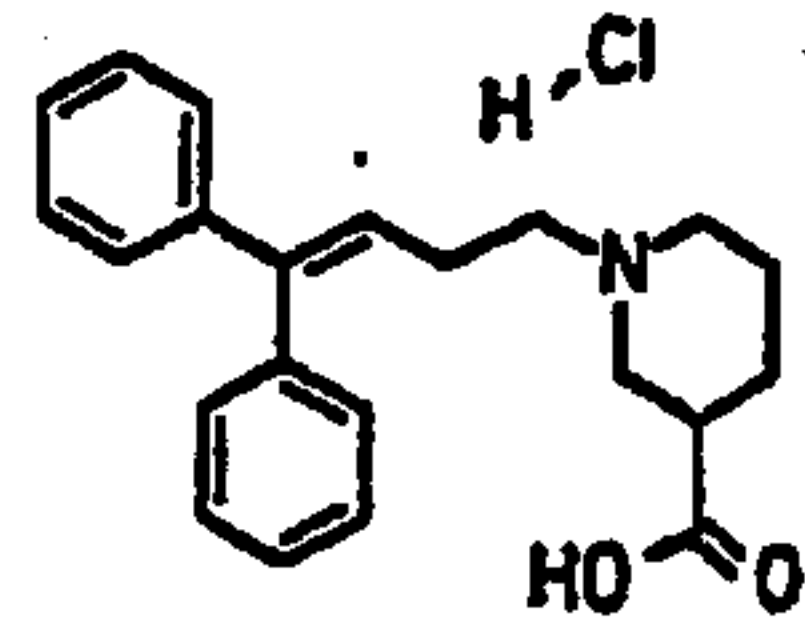


FIG. 17ooo

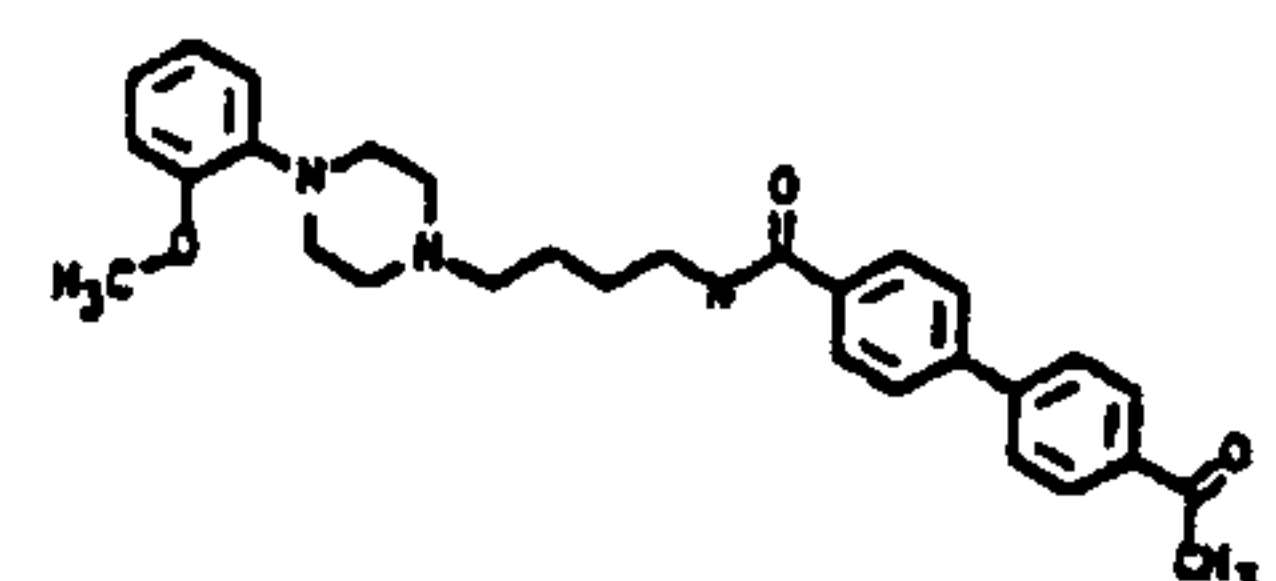
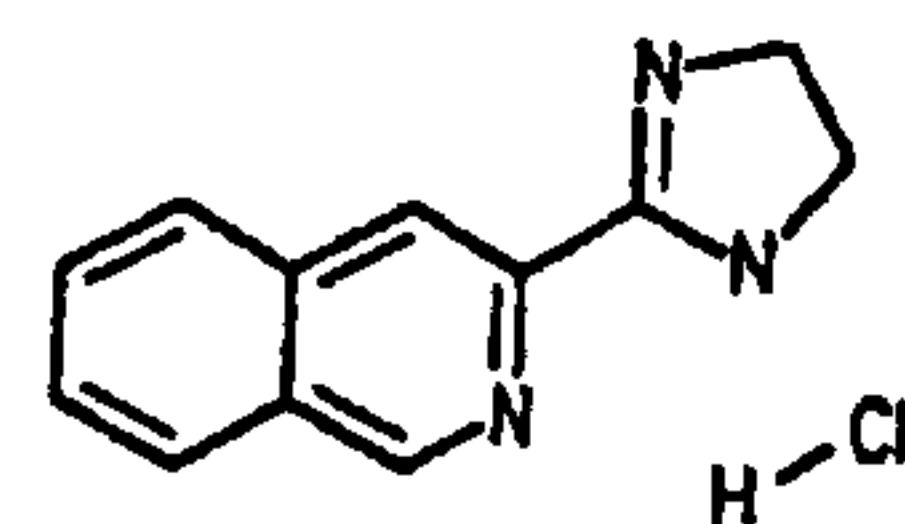
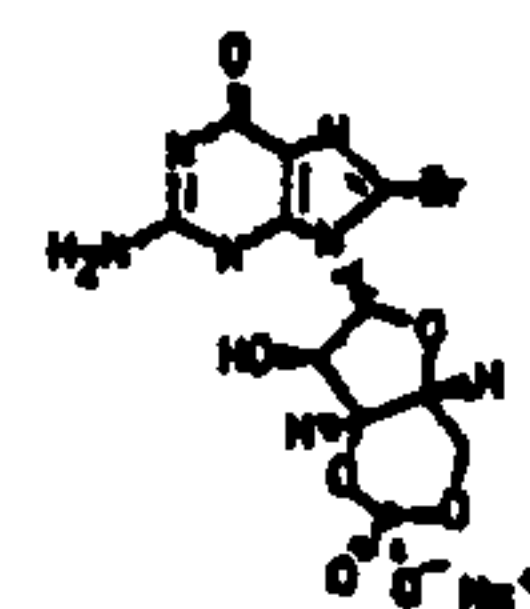
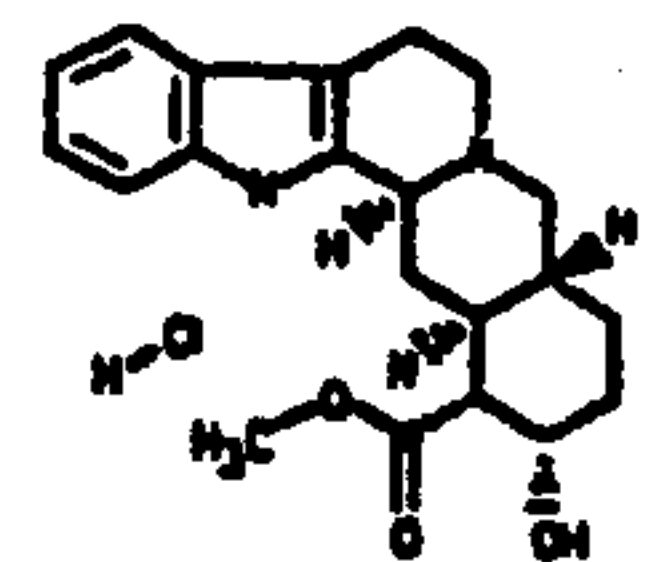
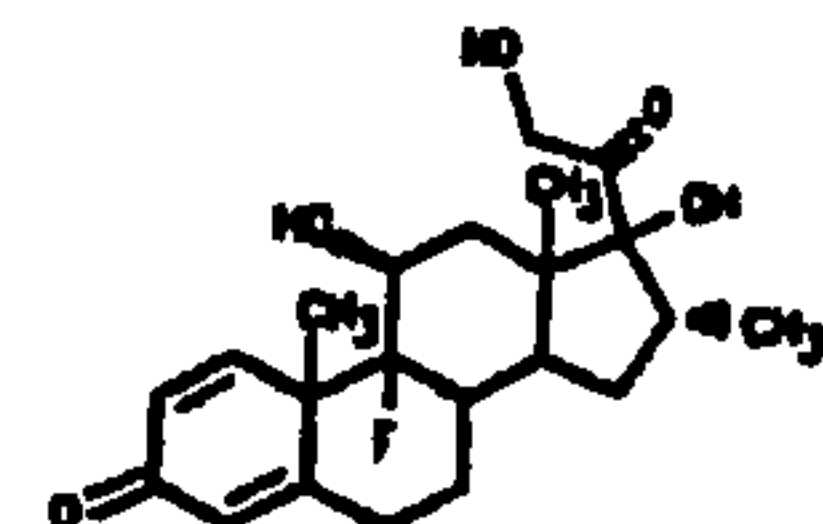
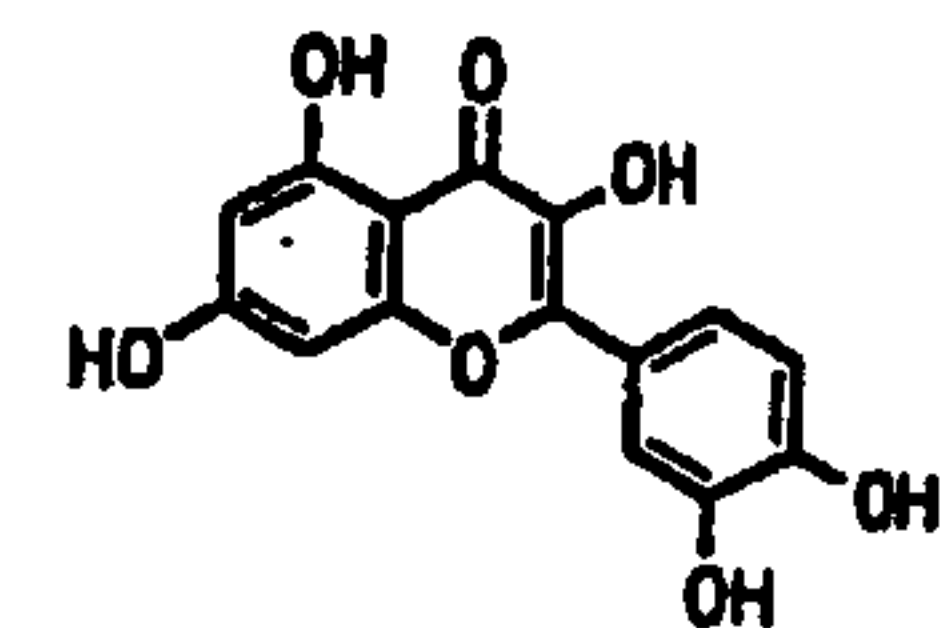
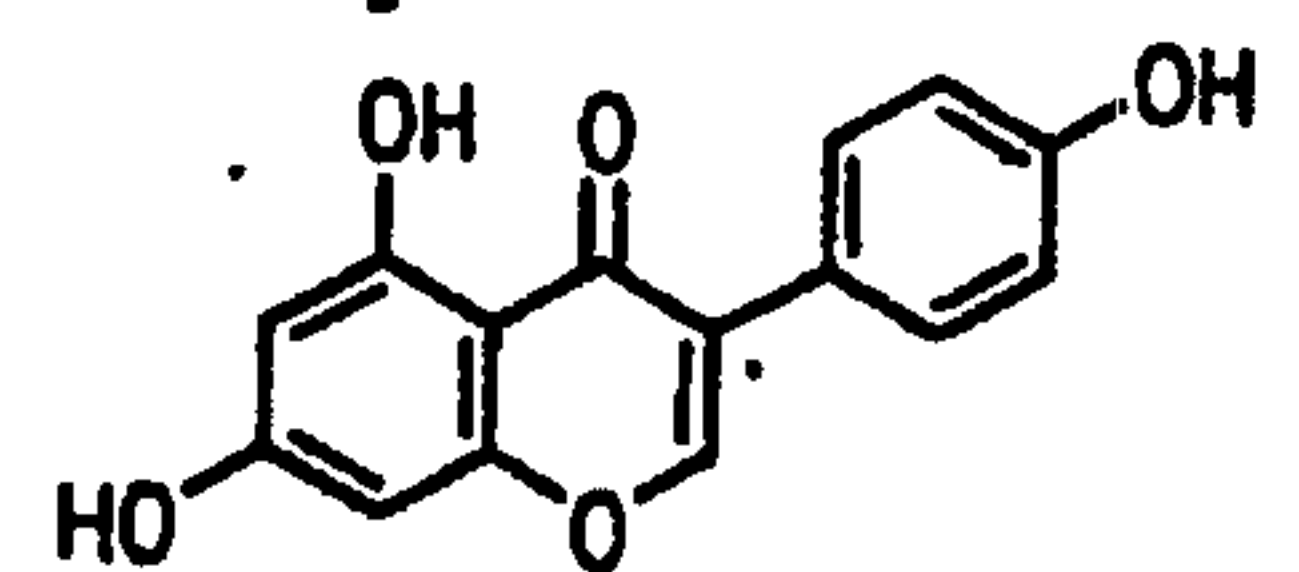
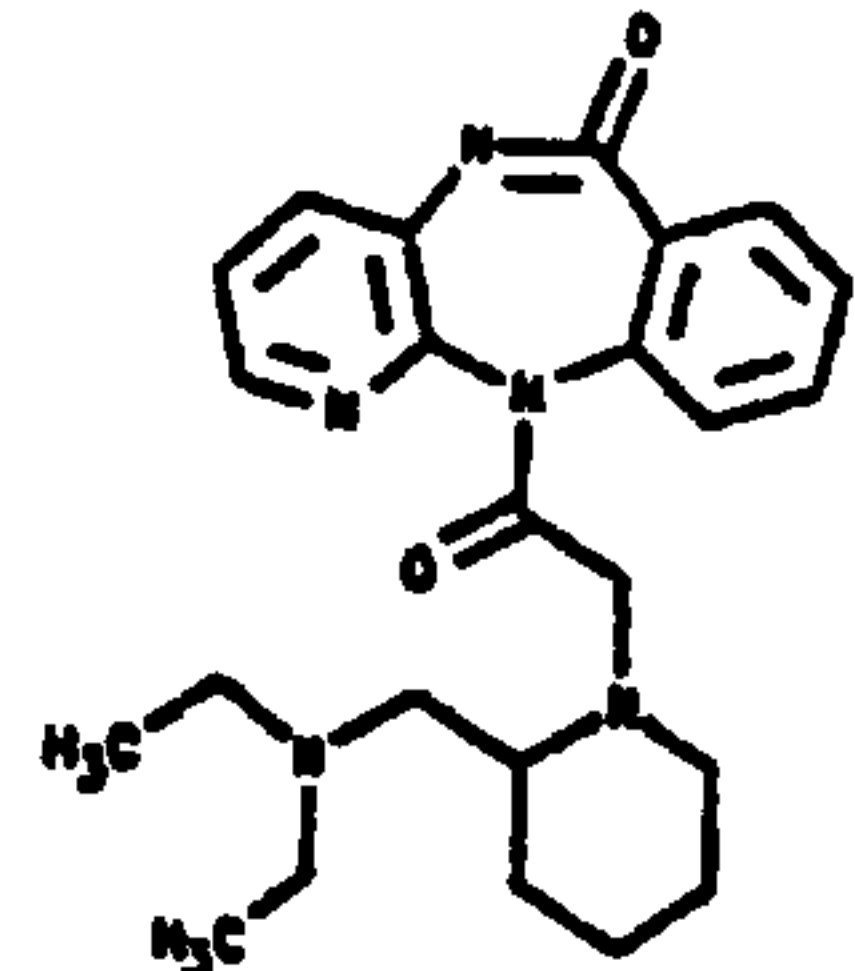
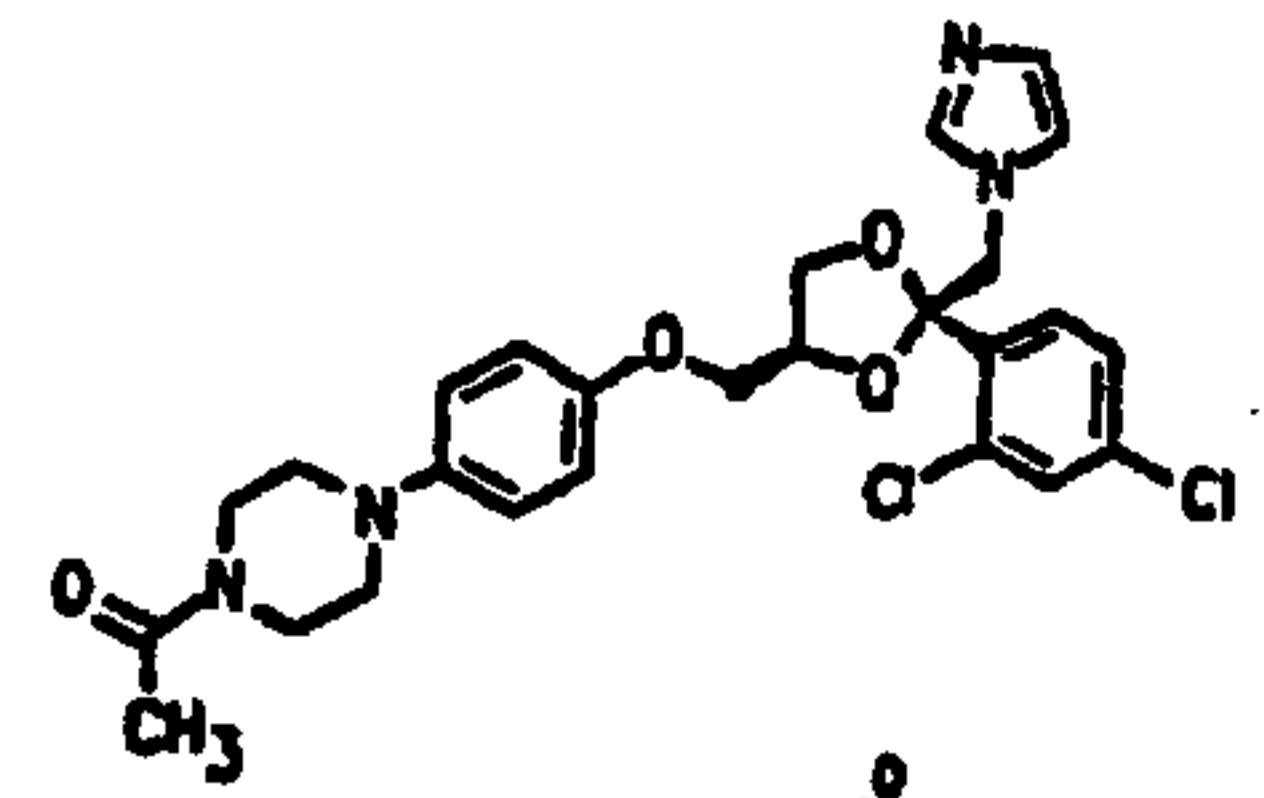
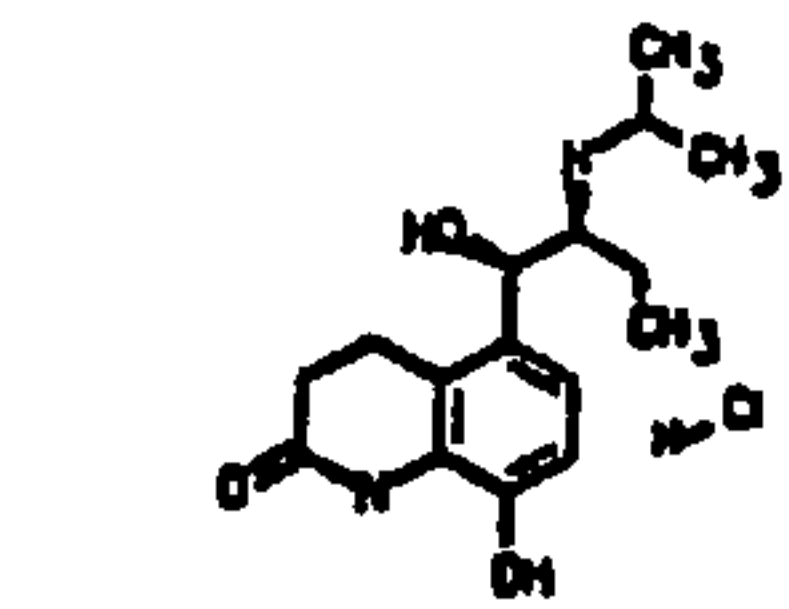


FIG. 17ppp

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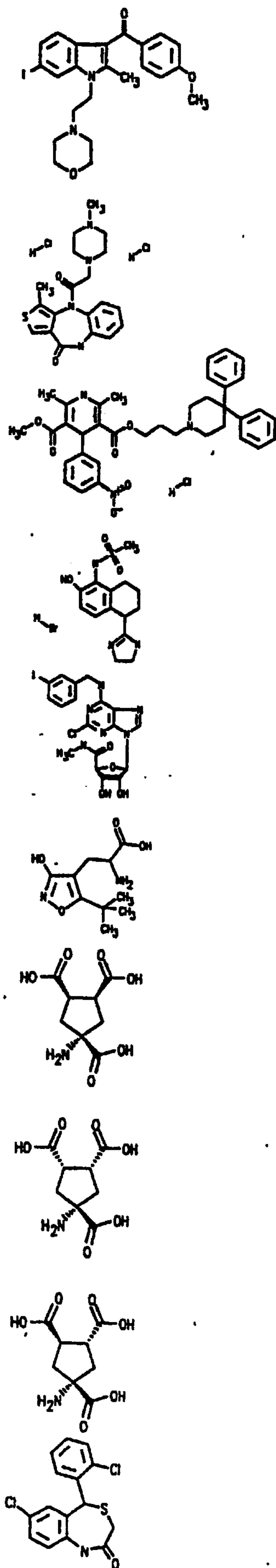


FIG. 17qqq

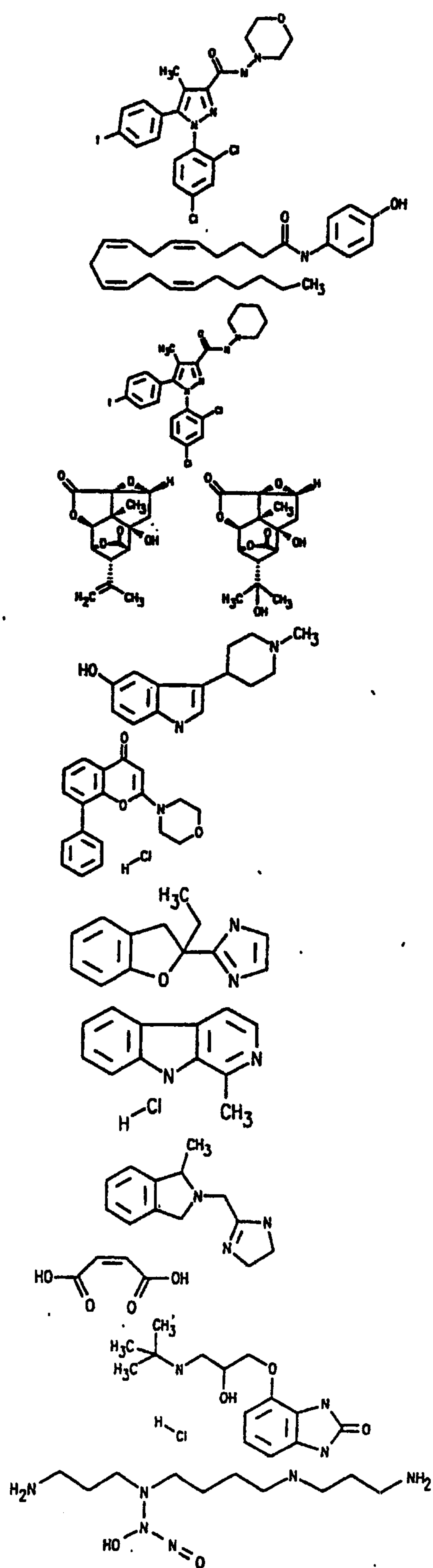


FIG. 17rrr



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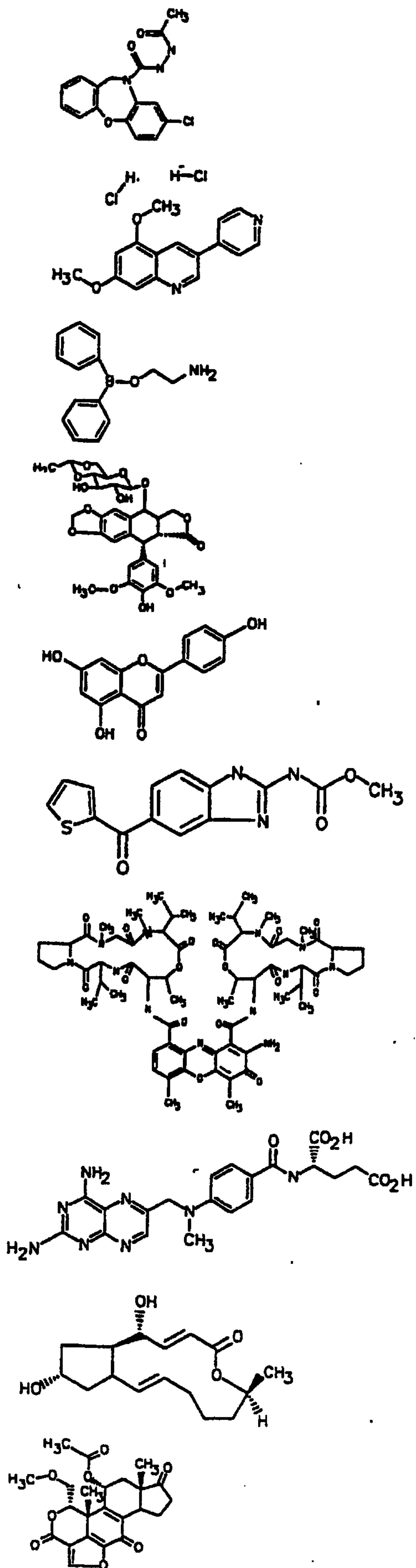


FIG. 17uuu

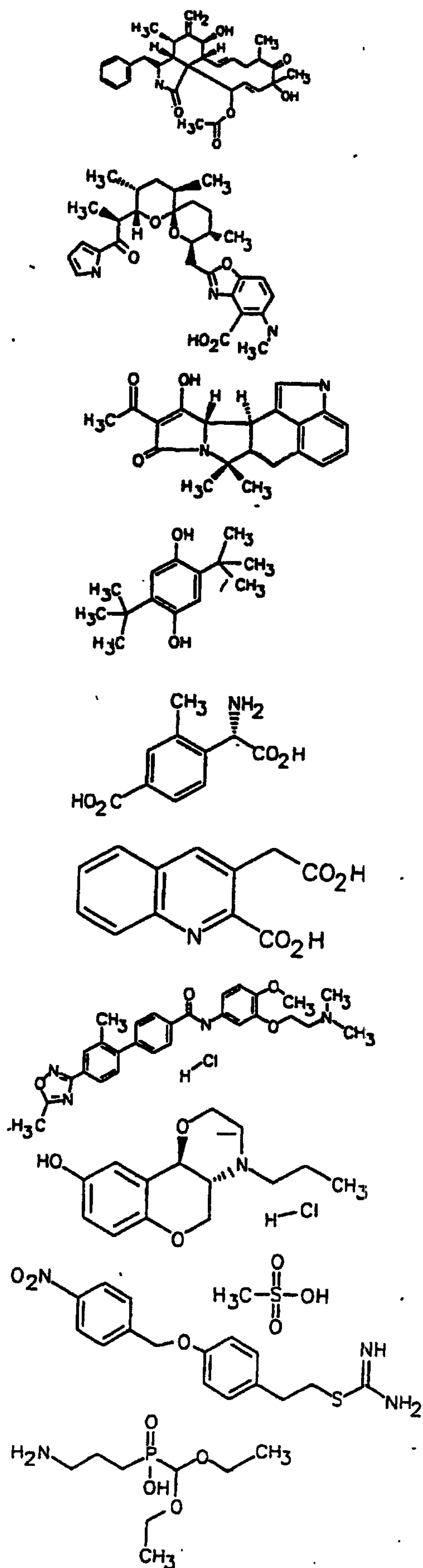


FIG. 17vvv

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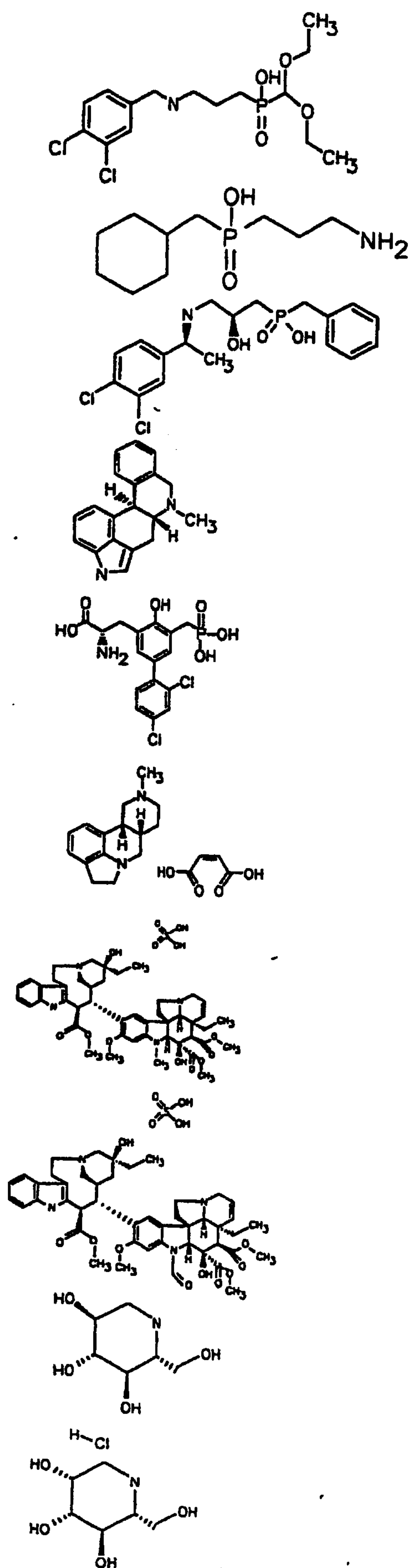


FIG. 17www

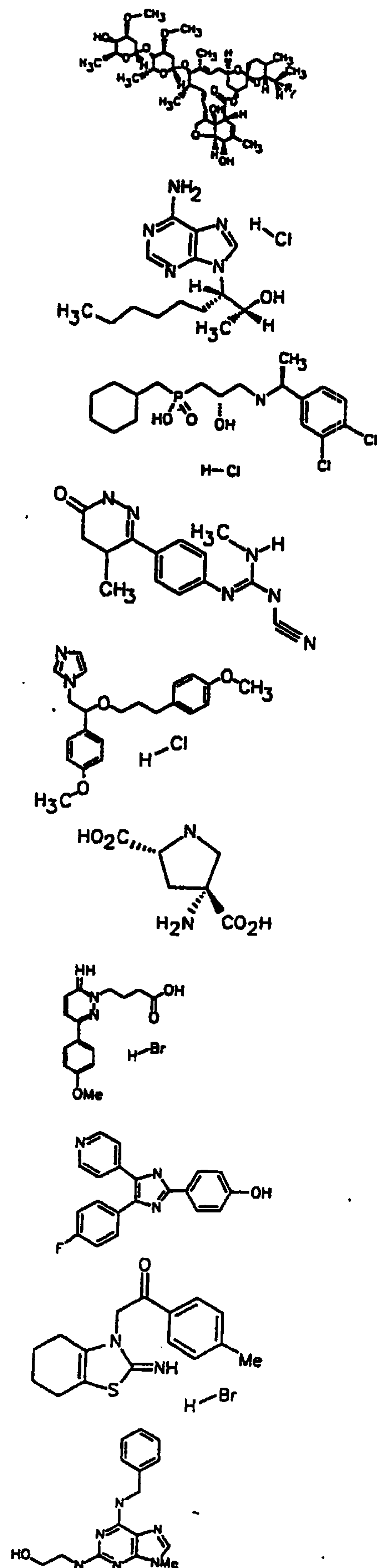


FIG. 17xxx

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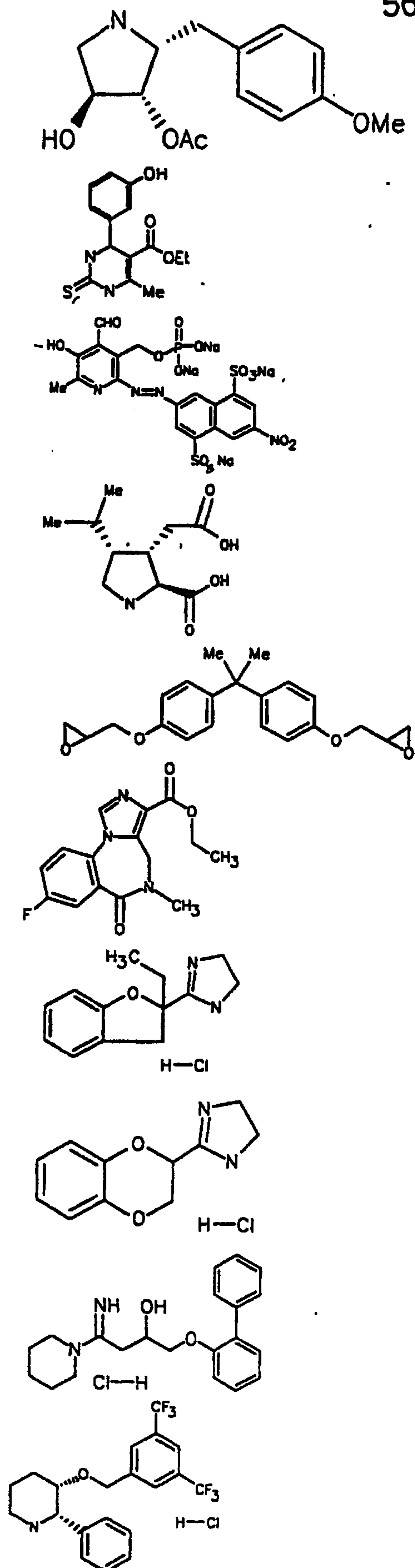


FIG. 17yyy

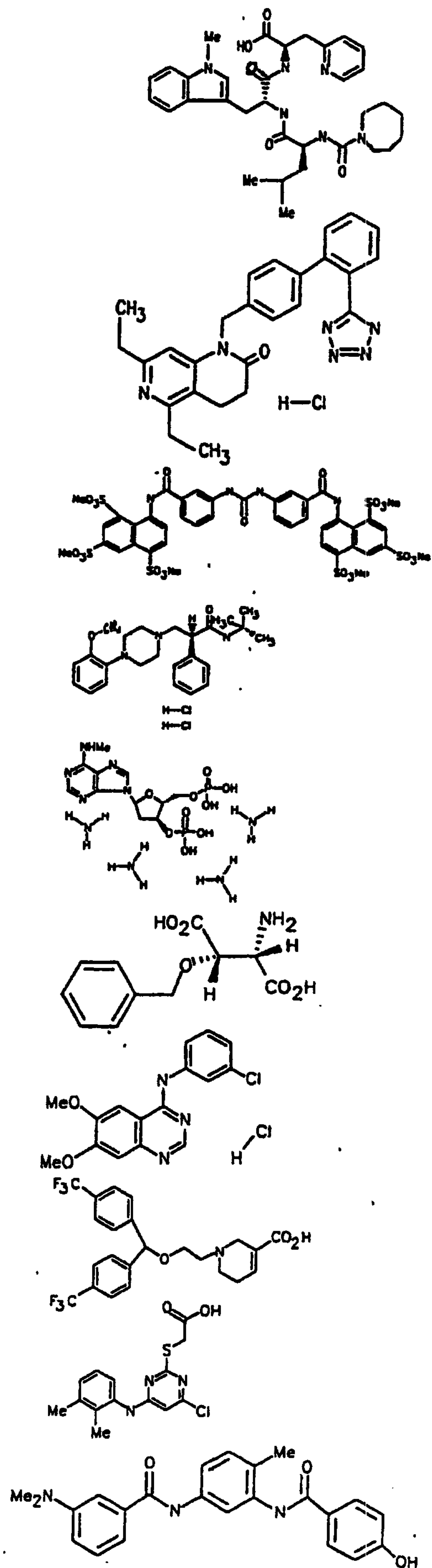
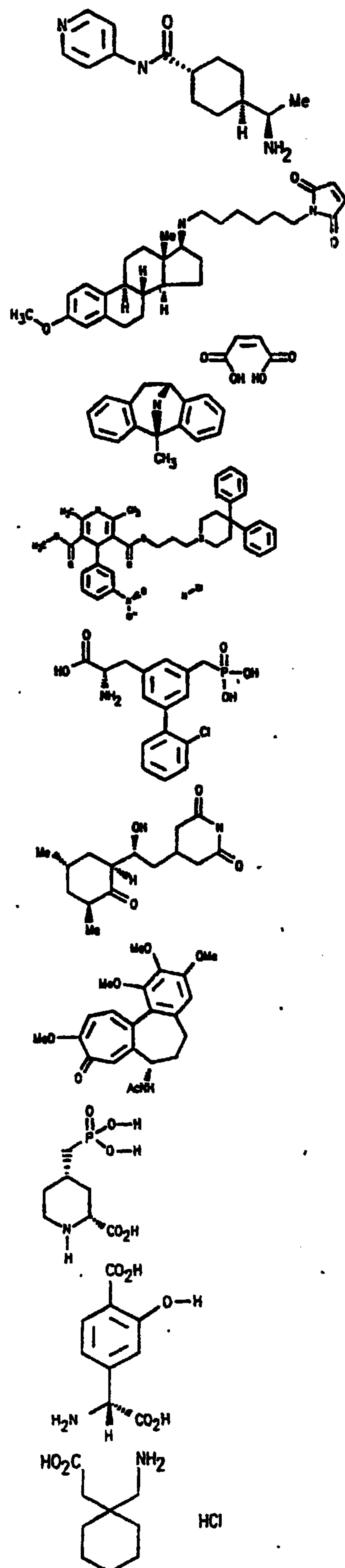
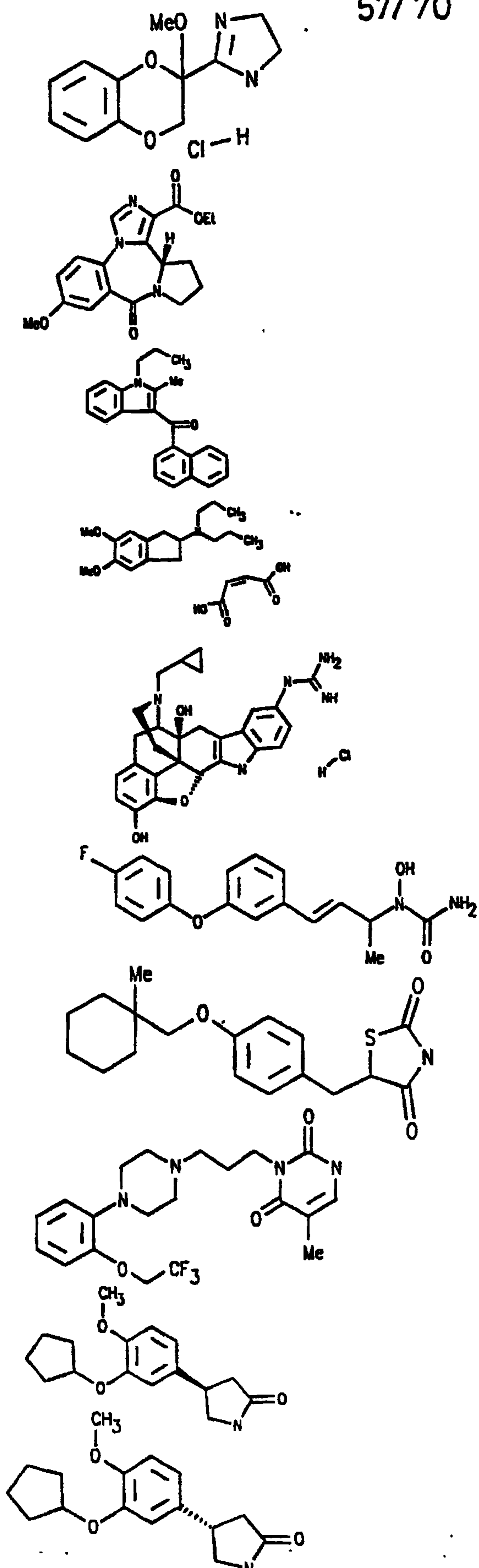


FIG. 17zzz

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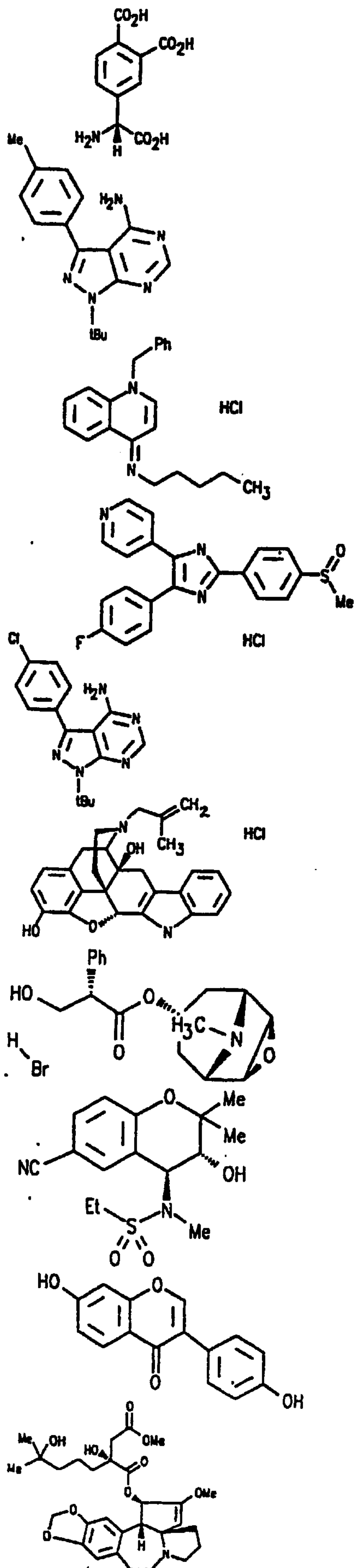


FIG. 17e

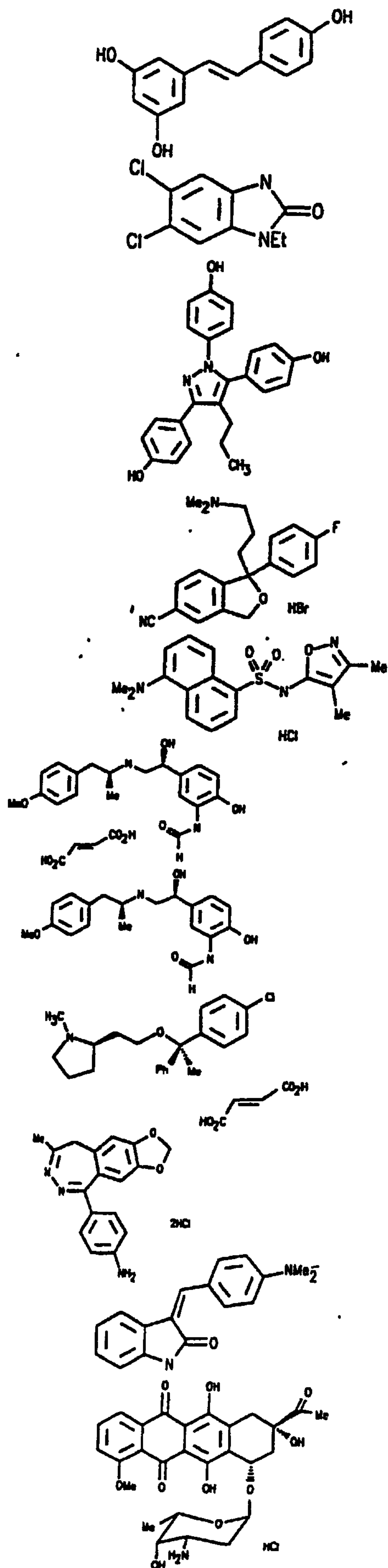


**FIG. 17ffff**

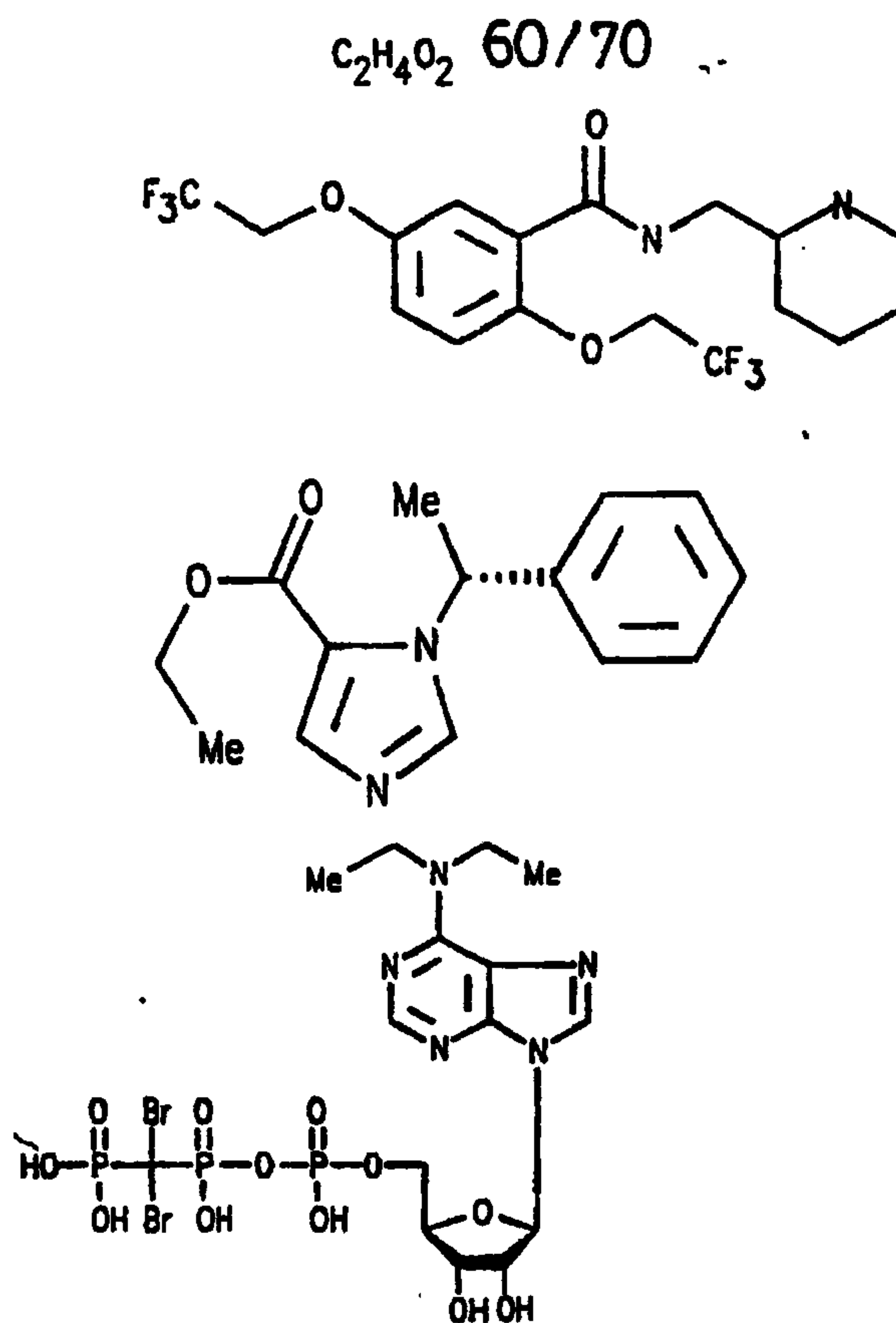


FIG. 17gggg

**PEPTIDES AND ANTIBODIES PROBES FOR CENTRAL NERVOUS  
SYSTEM DISEASES**

$\beta$ -Amyloid Precursor Protein  
 $\beta$ -Secretase Inhibitor III  
 $\gamma$ -Secretase Inhibitor XII  
 (+)-Ibuprofen  
 (S)-(+)-Ibuprofen  
 Anti- $\beta$ -Amyloid(1-43)  
 Anti-BACE1, C-Terminal(485-501)  
 Anti-Nicastrin, C-Terminal  
 Anti-Nicastrin, N-Terminal  
 Anti-Reelin

**PEPTIDES FOR ANGIOGENESIS**

MT1-MMp Hemopexin Domain, His-Tag<sup>®</sup>, Human, Recombinant  
 MT2-MMp Hemopexin Domain, His-Tag<sup>®</sup>, Human, Recombinant  
 VEGF Inhibitor

FIG. 17hhhh

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### Points for Regulation of Various Metabolic Control Mechanisms

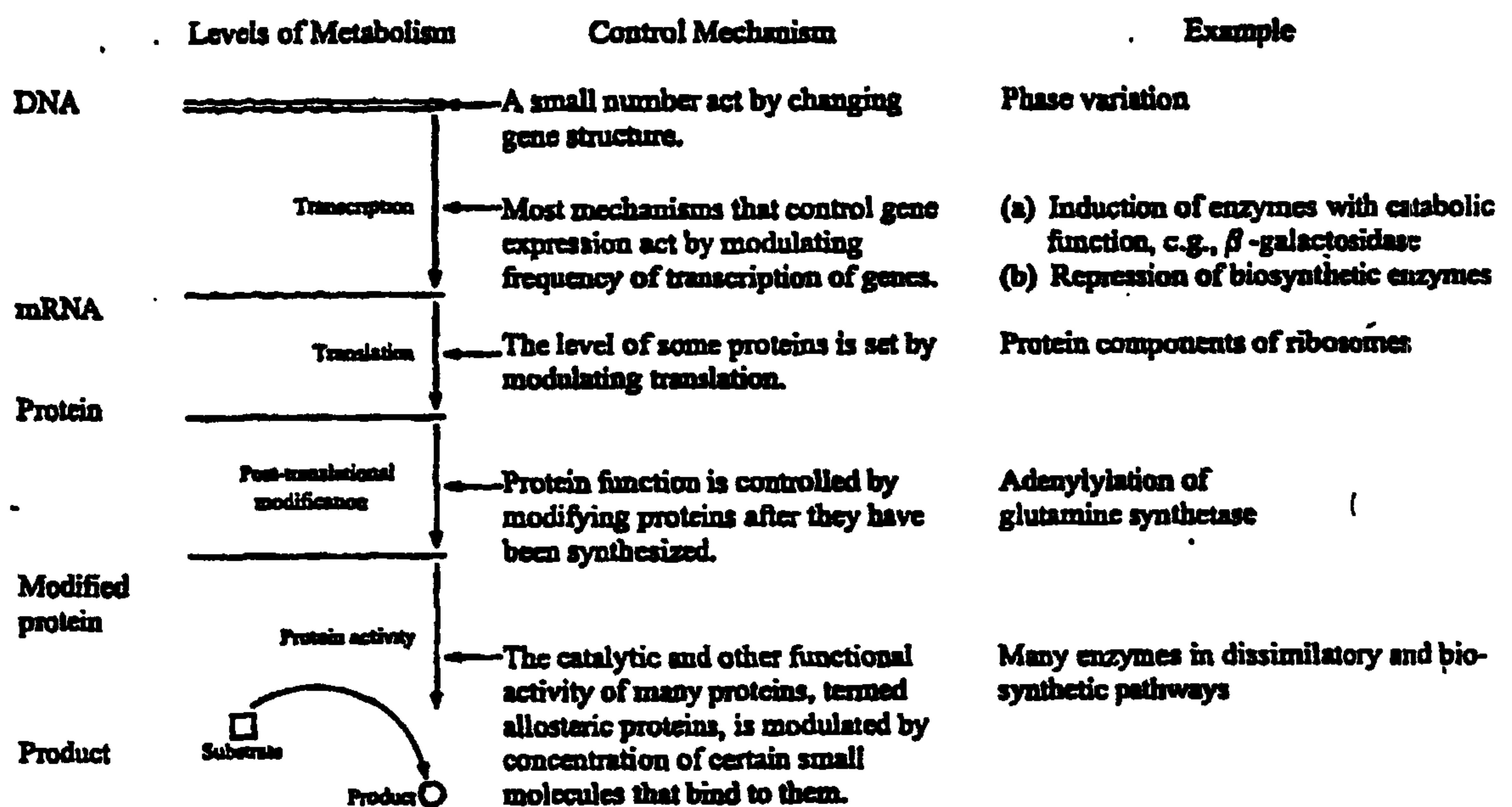


FIG. 18

# From Tissue to Cellular Target



Multiple separations from a single patient  
(Cytomation MoFlo High Speed Flow Cytometry)

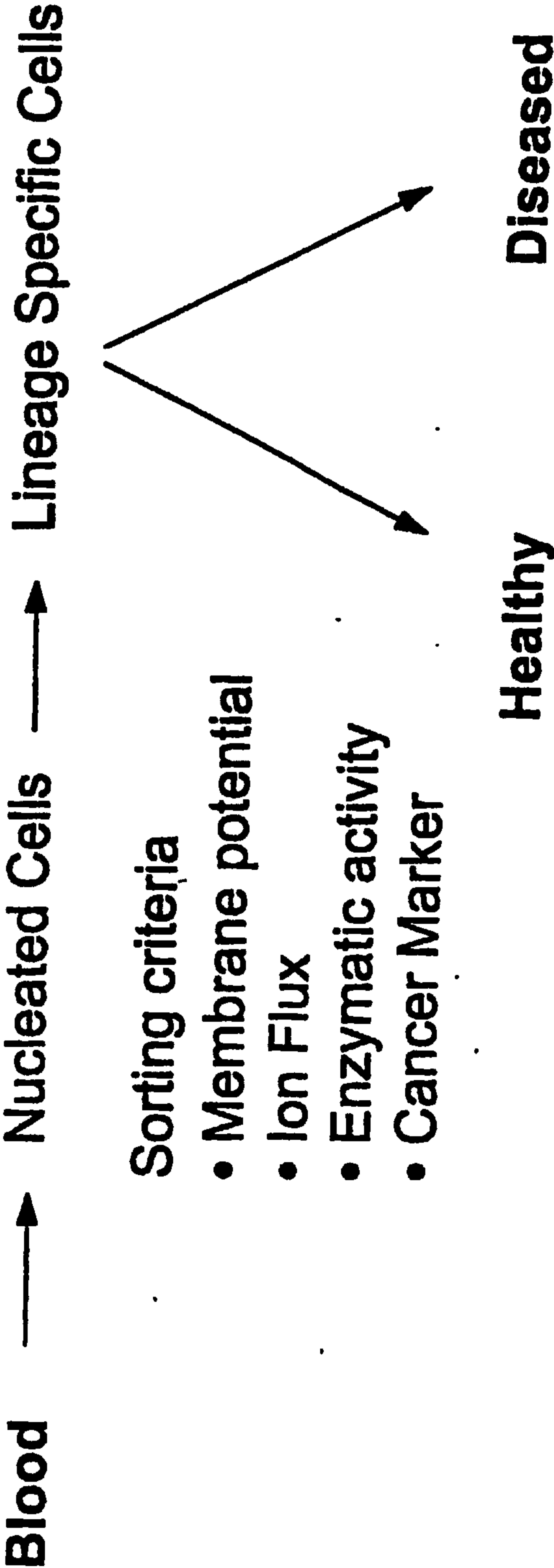
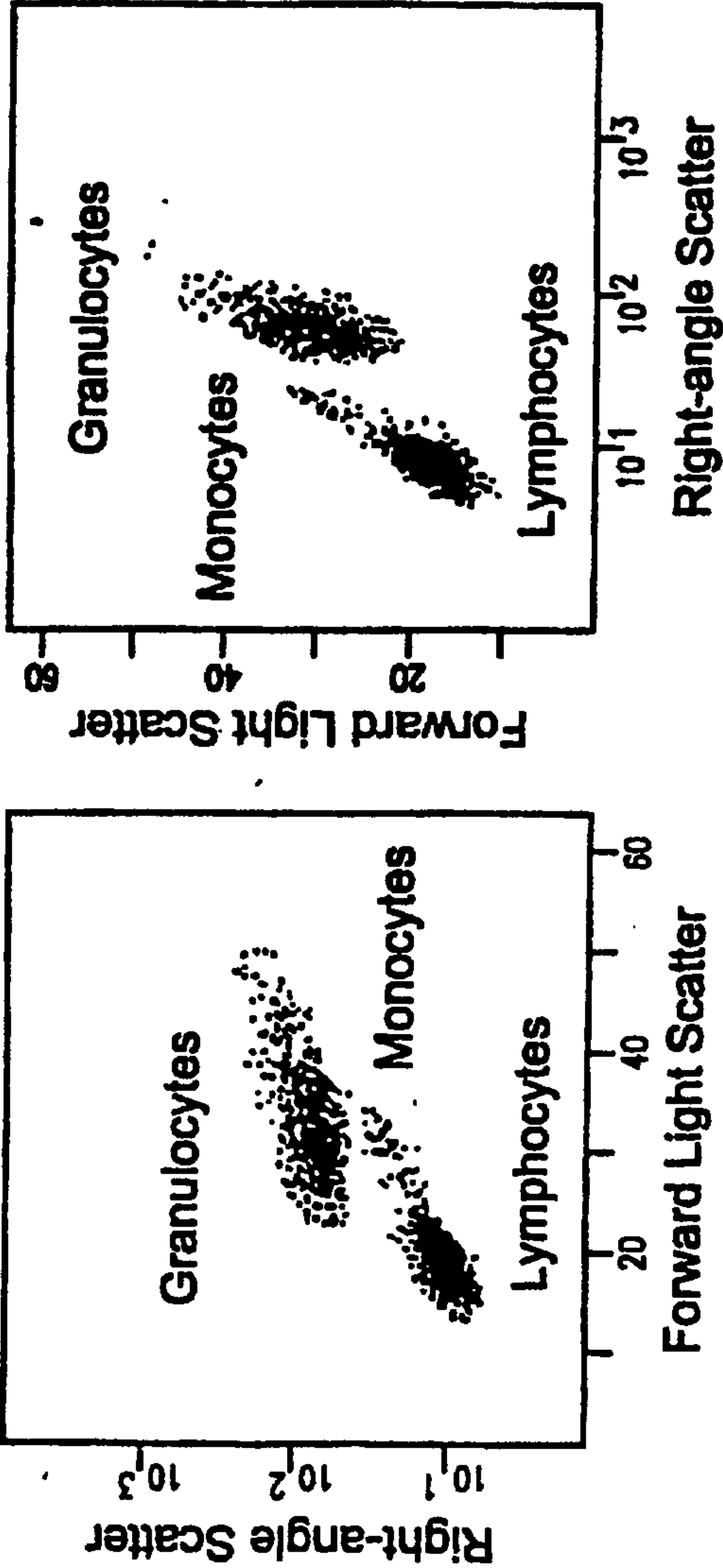
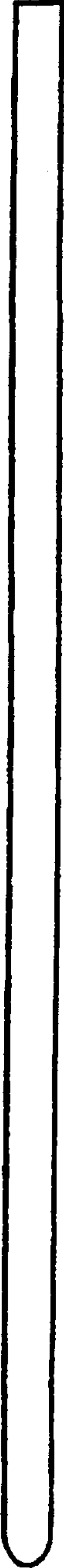


FIG. 19a

# Sorting Cell Types From Blood



Cell types from peripheral blood cause light scatter in reproducible manners that allow separation without labeling

FIG. 19b

# Cell Cycle Differences

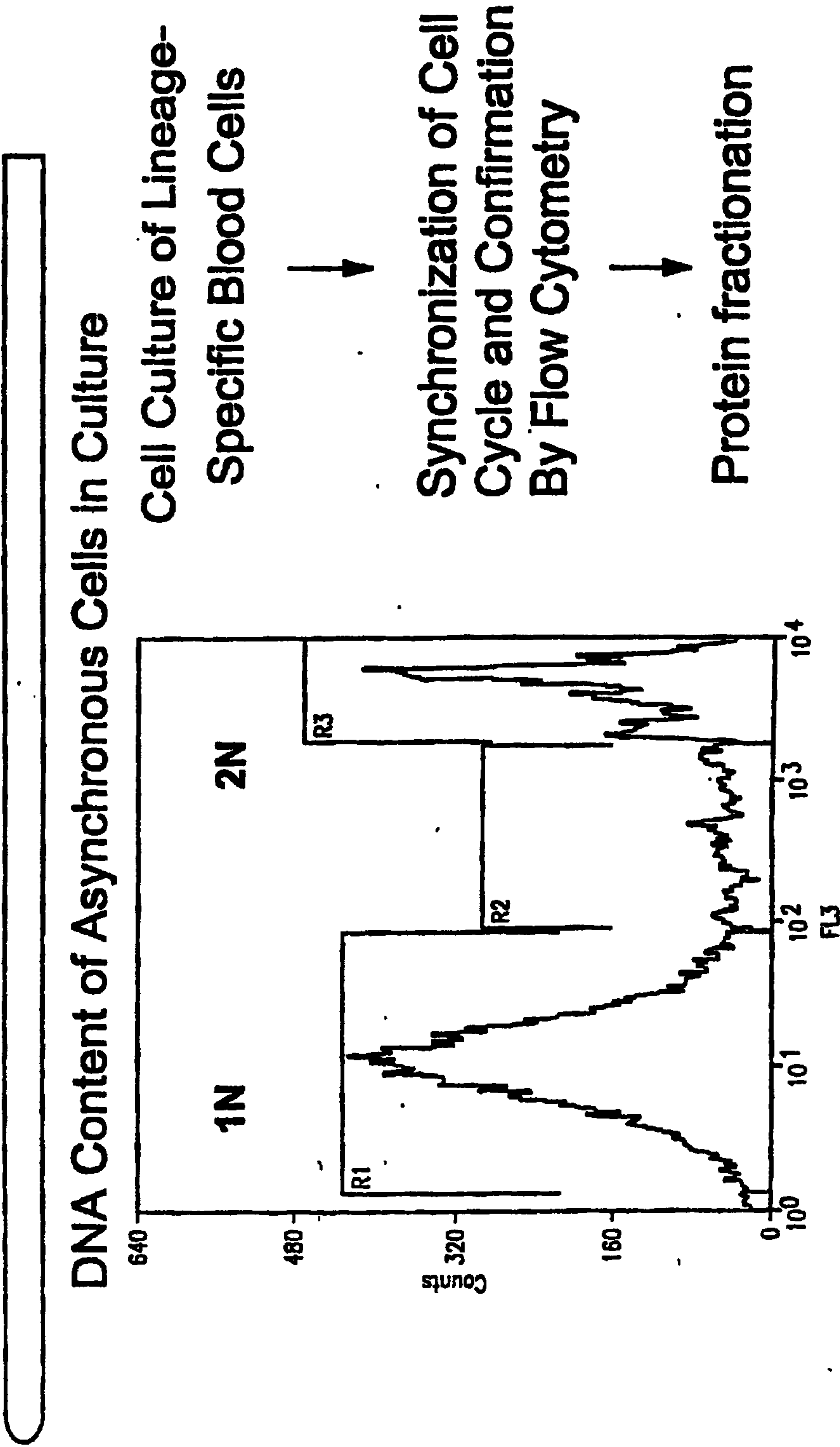
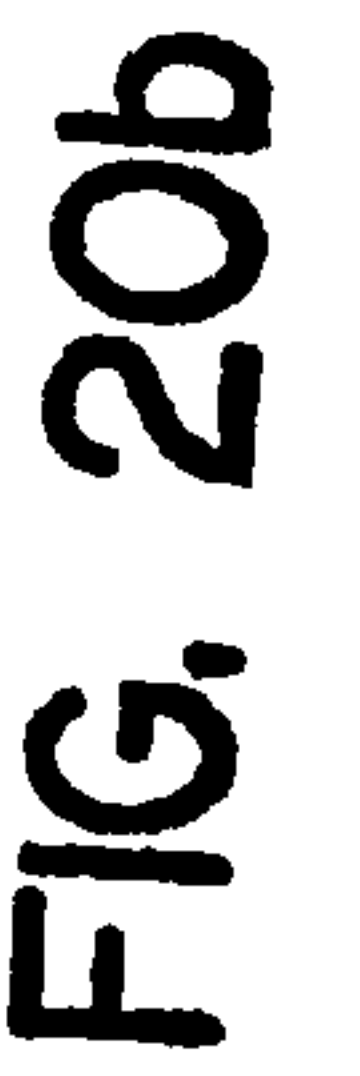


FIG. 19c





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## Cytochrome C

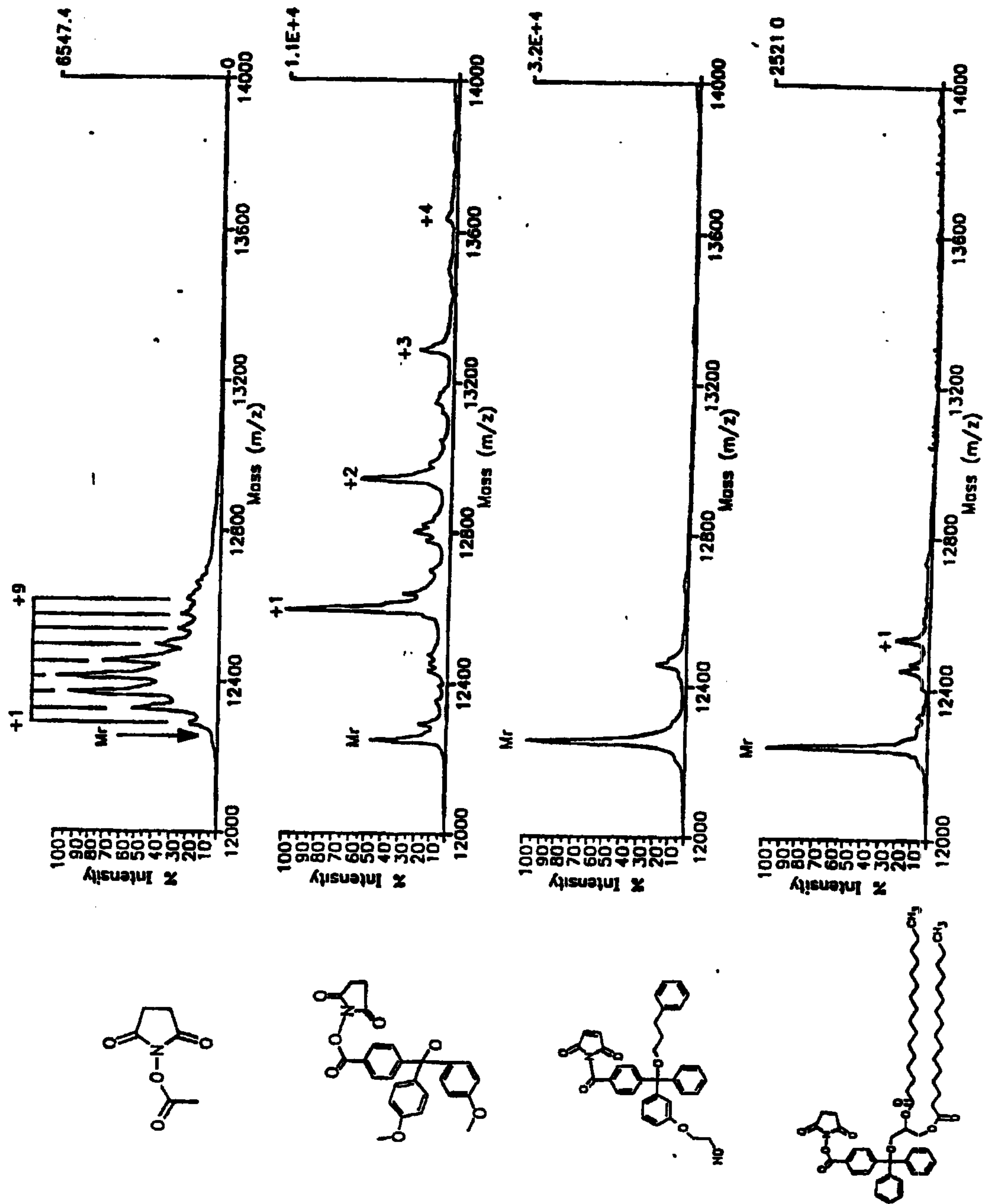
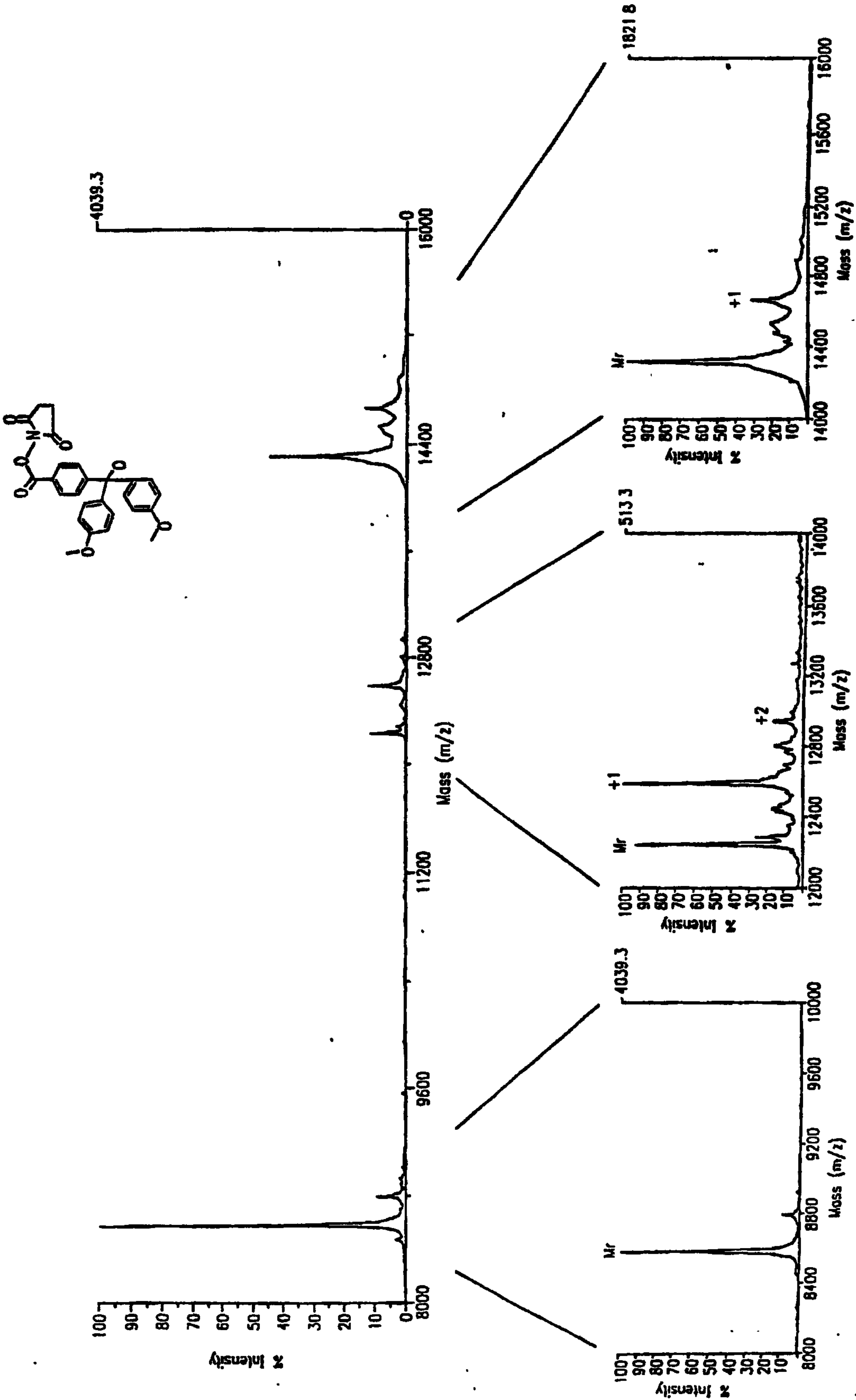


FIG. 20c

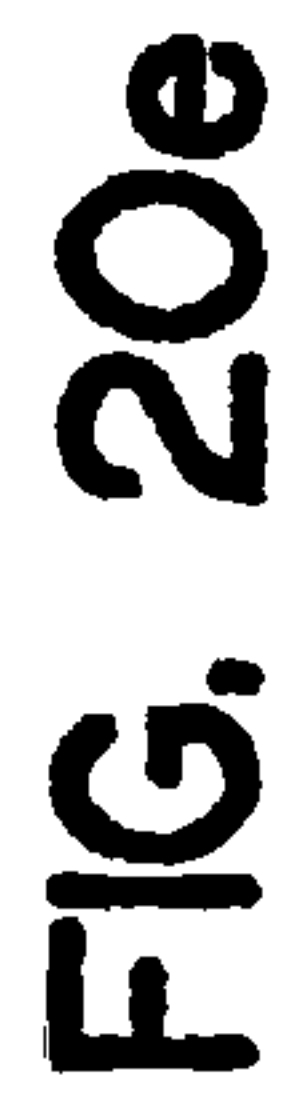


Ubiquitin

Cytochrome C

Lysozyme

FIG. 20d



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## Cytochrome C (18 Lys) Reaction with Non-Specific Compound

