PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
C07B 43/06, B01J 19/00	A1	WO 95/02566
		(43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/US94/08141

(22) International Filing Date: 15 July 1994 (15.07.94)

(30) Priority Data:

08/092,862	16 July 1993 (16.07.93)	US
08/180,863	13 January 1994 (13.01.94)	US

(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 21st floor, 300 Lakeside Drive, Oakland, CA 84612-3550 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: ARMSTRONG, Robert, W. [US/US]; 1907 Veteran Avenue, Los Angeles, CA 90025 (US).

(74) Agents: ADRIANO, Sarah, B. et al.; Merchant & Gould, Suite 400, 11150 Santa Monica Boulevard, Los Angeles, CA 90025 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SYNTHESIS OF COMBINATORIAL ARRAYS OF ORGANIC COMPOUNDS THROUGH THE USE OF MULTIPLE COMPONENT COMBINATORIAL ARRAY SYNTHESES

(57) Abstract

The present invention relates to an array of compounds having a common core structure wherein the compounds of the array comprise the products of a multiple component combinatorial array synthesis having at least three components. The present invention also relates to the method of synthesizing that array. A further embodiment of the present invention relates to the use of solid phase synthesis to synthesize the combinatorial array of compounds. The present invention also relates to a method of creating a combinatorial array of compounds with a common core structure by identifying the desired core structure, identifying an MCCA reaction capable of generating that core structure, followed by preparing an array of compounds using the identified MCCA reaction according to the aforementioned method. The present invention also relates to a method for conducting *in vitro* assays of biological material using the combinatorial arrays of the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

SYNTHESIS OF COMBINATORIAL ARRAYS OF ORGANIC COMPOUNDS THROUGH THE USE OF MULTIPLE COMPONENT COMBINATORIAL ARRAY SYNTHESES

Field of the Invention

The present invention relates to a method for generating arrays of compounds having a common core structure through a combinatorial array synthesis using a multiple component combinatorial array (MCCA) synthesis. The present invention also relates to arrays of organic compounds wherein each array is comprised of a group of structural analogues having a common core structure, the core structure being generated by a multiple component one pot synthesis.

Background of the Invention

Understanding the relationship between a molecule's structure and its biological function is essential to the development of new and improved therapeutic agents. For example, the contours of a receptor's binding site or an enzyme's active site can be effectively analyzed by testing the binding affinity of several structural analogues to these sites.

In nature, many biologically active molecules possess one of a relatively small group of common core structures. These common core structures include β-lactams, peptides, sugars, nucleosides, aromatics, pyridines, steroids, tetrazoles, pyrazines, terpines and alkaloids. Biological activity is often associated with the presence of one of these core structures. Structure-function studies using a group of compounds that share a common core structure but where the members of the group have differing substituents have been used to elucidate numerous biological mechanisms. Several of these studies and the mechanisms that were determined are cited in Goodman & Gilman's The Pharmacological Basis of Therapeutics, Macmillian (6th Edition, 1980).

In addition to being a valuable research tool for understanding biological mechanisms, structure-

function analysis using substituent and structural variants of a known biologically active molecule is an invaluable tool for developing new therapeutic agents. For example, improved penicillin based antibiotics were developed by mimicking penicillin's β-lactam core structure with a core structure that is not susceptible to degradation by β-lactamase. Many antiviral drugs, such as the A.I.D.S. drug 3-azidothymidine (AZT), are designed to mimic the core structure of nucleosides based on the notion that these nucleoside structural analogues will become incorporated into and interfere with the virus's genetic machinery.

The rate limiting step in the analysis of structure-function relationships is the synthesis of the necessary structural analogues. The traditional strategy for evaluating structure-function relationships involves an iterative process whereby successive groups of compounds, all having a common core structure, are synthesized and assayed. After each iteration, the assay results are evaluated in order to design the next group of compounds to be synthesized and assayed. This process is repeated until the substituents or structural factors influencing biological activity are determined and/or optimal therapeutic agents are identified.

The traditional approach to evaluating structure-function relationships is extremely time consuming and labor intensive, often requiring the synthesis of greater than one thousand compounds over the course of several years. The chemical syntheses of these compounds are often complex, generally requiring a multistep synthesis for each compound. As a result, a high degree of skill in the synthetic chemical arts is generally required to synthesize the compounds needed to perform these structure-function studies. This greatly limits the number of scientists that can perform this type of structure-function analysis research. Further, the labor demands for this type of research is very

intense, often requiring the full time efforts of several organic chemists per project. Because of these labor and skill demands, performance of this type of research and hence the development of new therapeutic agents is often limited to larger chemical firms. Researchers possessing a strong understanding of the biological system being studied are often prevented from participating in the development of new therapeutic agents because of their inability to access the compounds needed for performing these studies.

The labor demands associated with synthesizing structural analogues has also greatly limited the range of compounds for which structure-function analysis has been performed. The synthetic effort required to produce structural analogues has made it impractical to probe core structures not currently associated with known biologically active molecules for biological activity. However, it is likely that the number of core structures associated with biologically active compounds greatly exceeds the number of structures that have been identified. A more efficient method for synthesizing structural analogues would make it feasible to evaluate a far greater range of structures for biological activity.

In contrast to the effort required to synthesize compounds, the effort required to screen the synthesized compounds for biological activity is relatively low. A simple and efficient method for synthesizing compounds useful for probing for biological activity would significantly accelerate the rate of new drug development and enable a greater number of scientists to participate in the research.

One of the factors that makes the synthesis of compounds used in structure-function research studies so time consuming and research intensive is the fact that the chemical syntheses of these compounds are performed separately through stepwise linear transformations.

Since the time required to screen compounds is significantly smaller than the time required to synthesize the compounds, it would be advantageous from a time management standpoint to synthesize more compounds by a more rapid method, even if the total number of compounds that would have to be screened were increased.

The general approach for screening monoclonal antibodies to a specific antigen presents a useful model for how structure-function studies should be conducted. After antigen stimulation of a host, the monoclonal antibody producing B-cells of the host are immortalized and diluted over a large array of microtiter wells. Most of the monoclonal antibodies produced by the individual immortalized cells in each titer well have little utility. However, assays exist to identify the useful antigen binding antibodies in the array. Similarly, it is possible to rapidly assay large arrays of organic compounds for biological activity. Unfortunately, however, there are no known biological systems that can be stimulated to produce an array of organic chemicals from which a desired compound can be isolated. Thus, the need exists for a simple and efficient method for generating an array of organic compounds from which biologically active agents can be identified.

The methodology developed for the automated synthesis of biopolymers such as RNA, DNA, polypeptides and most recently oligosaccharides also presents a useful model for how structure-function studies could be conducted. The automated synthesis of these biopolymers has greatly accelerated the development of the biotechnology arts, primarily because RNA and DNA probes, as well as small polypeptides, are readily obtainable by all researchers. The benefits derived from automated biopolymer synthesis technology highlights the current need for a simple, efficient and

rapid method for generating numerous organic compounds to probe structure-function relations in route to the development of new therapeutic agents.

The automated synthesis of these biopolymers is achieved by performing a repetitive series of reactions on the biopolymer which is attached to a solid support. Unfortunately, biopolymer synthesis technology cannot be readily redirected to the production of structural analogues of organic compounds. Unlike biopolymer synthesis, the synthesis of structural analogues of organic compounds involves numerous different chemical reactions. Also, because of the wider variety of chemical reactions and reagents involved, a wider variety of solid phase supports and chemical linkers are needed.

SUMMARY OF THE INVENTION

The present invention relates to an array of compounds having a common core structure wherein the compounds of the array comprise the products of a multiple component combinatorial array synthesis having at least three reactive components. Each component of the multiple component combinatorial array synthesis comprises a group of reactants having a common functional group. The array synthesis is conducted under appropriate conditions such that the common functional group of each component in the reaction reacts with functional groups on the other components to form an array of compounds having a common core structure. Each component of the combinatorial array synthesis can itself comprise an array of compounds having a common core structure, the array of component reactants being synthesized by a multiple component combinatorial array synthesis. Each component can also comprise a group of reactants wherein each reactant comprising a mixture of compounds having a common functional group. In a further embodiment of the

present invention, the array of compounds is formed while bound to a solid support.

The present invention also relates to a method of making an array of compounds having a common core structure using a multiple component combinatorial array synthesis with at least three components. Each component comprises a group of reactants having a common functional group. The method comprises organizing a series of reaction vessels in an n dimensional array wherein each reaction vessel is identifiable by its coordinates in the n dimensional array. Each axis in the n dimensional array corresponds to a different component in the array synthesis. Each position on each axis corresponds to a different reactant of the corresponding component. The reactants of the n components are added to the n dimensional array of reaction vessels such that the same reactant is added to all of the reaction vessels in the array having a position on the array corresponding to that reactant. The components in each reaction vessel are then reacted under appropriate conditions to form the compounds of the array. Each component of the combinatorial array synthesis can itself comprise an array of compounds having a common core structure, the array of component reactants being synthesized by a multiple component combinatorial array synthesis. Each component can also comprise a group of reactants, each reactant comprising a mixture of compounds having a common functional group. A further embodiment of the present invention relates to solid phase multiple component array synthesis which comprises the further step of binding one of the components to a solid support.

The present invention also relates to a method of creating a combinatorial array of compounds with a common core structure by identifying the desired core structure, identifying a MCCA reaction capable of generating that core structure, followed by preparing an

array of compounds using the identified MCCA reaction according to the aforementioned method.

The present invention also relates to a method for conducting in vitro assays of biological material by adding biological material to an array of compounds, each member compound of the array having a common core structure and being bound to a solid support followed by measuring the effect each member compound of the array has on the biological material's biological activity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a-p provides a summary of some of the common core structures synthetically accessible by MCCA reactions.

Figure 2 depicts the synthesis of the core structures depicted in Figure 1, showing the correspondence between the substituents (R, R_1, R_2, R_3, R_4, etc.) in the product and in the reaction components. Figure 2a depicts a synthesis described in Joucia, et al., J. Tetrahedron Lett., (1985) 26:1221. Figure 2b depicts a synthesis described in Kelly, et al., J. Am. Chem. Soc., (1985) 107:3879. Figure 2c depicts a synthesis described in Banville, et al. Can. J. Chem., (1974) 50:80; Cameron, et al., J. Chem. Soc. Chem. Commun., (1976) 275; and Cameron, et al., J. Chem. Soc. Chem. Commun., (1977) 297. Figure 2d depicts a synthesis described in Kelly, et al., J. Amer. Chem. Soc. (1985) 107:4998. Figure 2e depicts a synthesis described in Posner, et al., Tetrahedron (1981) 37:3921. Figure 2f depicts a synthesis described in Davis, et al., J. Org. Chem., (1979) 44:3755. Figure 2g depicts a synthesis described in Bestmann, et al., Angew Chem. Int. Ed. Engl, (1985) 24:790. Figure 2h depicts a synthesis described in Weis, et al., Tetrahedron Lett., (1981) 22:1453. Figure 2i depicts a synthesis described in Ugi, et al., Justus Liegis Ann. Chem., (1967) 709:1.

Figure 2j depicts a synthesis described in Ugi, et al., Chem. Ber. (1961) 64:734. Figure 2k depicts a synthesis described in Sebti, et al., Synthesis (1983) 546. Figure 2l depicts a synthesis described in Isenring, et al., Synthesis (1981) 385 and Isnering, et al., Tetrahedron (1983) 39:2591. Figure 2m depicts a synthesis described in Kunz, et al., J. Amer. Chem. Soc. (1988) 110:651. Figure 2n depicts a synthesis described in Boehm, et al., J. Org. Chem. (1986) 51:2307. Figure 2o depicts a synthesis described in Murakami, et al., J. Org. Achem., (1993) 58:1458. Figure 2p depicts a synthesis of a c-linked disaccharide wherein the stereochemistry of the first ring is determined by the sugar used in the synthesis and the stereochemistry of the second ring is determined by the oxidation catalyst.

Figure 3 depicts the fact that MCCA array syntheses produce analogues on a geometric scale where the total number of analogues synthesized equals the product of the number of structural variants of each component of the MCCA array synthesis used.

Figure 4 depicts the results of a two dimensional MCCA array synthesis using the Passerini reaction wherein the aldehyde and acid reaction components are varied.

Figure 5 depicts the mechanism of action for carzinophilin/azinomycin.

Figure 6 depicts the α-acyloxy amine core structure of the antitumor antibiotic carzinophilin/azinomycin. By labelling the substituents off of the α-acyloxy amine core structure R_1, R_2 and R_3, a Passerini reaction scheme for the synthesis of carzinophilin/azinomycin and its derivatives is identified.

Figure 7 depicts the Ugi reaction which, upon removal of the protecting groups, provides nonhydrolizable peptide analogues that mimic a natural peptide's structure.

Figure 8 depicts the peptide backbone core structure of phosphotyrosine peptides and the synthesis of structural variants of phosphotyrosine by varying the first and second amino acids employed (aa1 and aa2) as well as the other components of the Ugi reaction.

Figure 9 depicts the synthesis of a series of tyrosine-based peptide analogues. Figure 9a depicts the synthesis of pseudosubstrate peptide inhibitors. Figure 9b depicts the synthesis of low molecular weight tyrosine-based peptide analogues where the tyrosine structural unit is intact. Figure 9c depicts the synthesis of low molecular weight tyrosine-based peptide analogues where the tyrosine structural unit is modified. Figure 9d depicts the synthesis of additional low molecular weight tyrosine-based peptide analogues.

Figure 10 depicts the synthesis of a support and linker system.

Figure 11 depicts the solid phase synthesis of peptidomimetics by means of the Ugi reaction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a simple and efficient method for synthesizing arrays of organic compounds having a common core structure. More specifically, the present invention relates to the use of multiple component combinatorial array (MCCA) syntheses to synthesize arrays of structurally related analogues having a common core structure.

Multiple component combinatorial array (MCCA) syntheses correspond to reactions where the reactants combine synchronously or asynchronously in one reaction vessel to form the product. Over the years, numerous MCCA reactions have been developed. In some cases, the reactions were developed in order to show an efficient synthesis of a known natural product. Cameron, et al., J. Chem. Soc. Chem. Comm., (1976) 275 (synthesis of m-deoxygenated benzoquinones in the application of the total synthesis if anthraquinone insect pigments); Schopf, Angew. Chem., (1937) 50:779-797 (double Mannich reaction in the total synthesis of tropane alkaloids); Posner, et al., Tetrahedron, (1981) 39:3921. In other cases, the reactions were developed as a general entry into methodology involving the formation of multiple bonds in a cascade process. In any event, these reactions represent a simple and efficient means for generating numerous structural variants sharing the core structure generated by the given MCCA reaction. A summary of some of the core structures that are made synthetically accessible by MCCA reactions is provided in Figure 1. The reaction sequence for generating these core structures is summarized in Figure 2.

Traditionally, structural analogues have been synthesized by multiple step linear syntheses. Linear syntheses involve the sequential reaction of several separate reactants in order to achieve the final product. Linear syntheses are generally not one pot reactions, requiring the isolation and purification of intermediate products. Unlike linear syntheses, MCCA reactions, because they are one pot syntheses, do not require the isolation and purification of intermediate reaction products. As a result, MCCA reactions are simpler and more efficient to perform than multiple step linear syntheses. Products of MCCA reactions are therefore more accessible to those lacking a high level of skill in the synthetic organic chemical arts.

Further, since no intermediate isolation and purification is required, MCCA reactions are more readily adaptable to automation.

MCCA reactions, when used in the form of an array synthesis, enable the synthesis of structural analogues on a geometric rather than linear scale. In an MCCA array synthesis, at least three reaction components are used. Each component comprises a group of reactants possessing a common functional group that participates in the MCCA reaction. The combinatorial array synthesis is conducted by reacting the different combinations of the various MCCA components. Combinatorial array syntheses are depicted in Figures 3, 7 and 9.

A MCCA array synthesis can be characterized by the number of reaction components involved and the number of variants of each reaction component employed. In a MCCA array synthesis where the MCCA reaction has n components, an n dimensional array of structural analogues can be produced. Each array of analogues is equivalent to a library of analogues. When one reactant is held constant (or when a component is comprised of a group of one reactant), the n dimensional array simplifies to an n-1 array and can be visualized as a sublibrary of a greater array where the reactant used for that component is held constant.

As depicted in Figure 3, an MCCA array synthesis is analogous to the multiplication of a series of one dimensional arrays. Each component of the array synthesis is represented by a different one dimensional array, the elements of the one dimensional array corresponding to the group of reactants used for a given component in the combinatorial array synthesis. Mathematically, an n dimensional array is generated when n one dimensional arrays are multiplied. In the case of an MCCA array synthesis, the reaction vessels are organized in an n dimensional array wherein each

reaction vessel is identifiable by its coordinates in an n dimensional array. Each axis of the array of reaction vessels corresponds to a different component in the MCCA reaction. Each position on each axis corresponds to a different reactant of the corresponding component. During the combinatorial array synthesis, the reactants of the n components are added to the n dimensional array of reaction vessels such that the same reactant is added to all of the reaction vessels in the array having a position on the array corresponding to that reactant. The reaction of the components in the array of reaction vessels is analogous to the multiplication of the n one dimensional arrays since the reaction of the various reactants in each reaction vessel in the n dimensional array results in the "product" of the reactants added to that reaction vessel.

MCCA array syntheses have the advantage over linear syntheses in that they produce analogues on a geometric scale rather than a linear scale. The ability to synthesize structural analogues on a geometric scale significantly decreases the time and effort required to synthesize these compounds.

As depicted in Table 1, the total number of analogues synthesized equals the product of the number of each reactant employed. For example, if 10 different variants of each component of a three component MCCA are employed, a 10x10x10 three dimensional array of analogues is produced which corresponds to an array of 1000 structural analogues.

TABLE 1

No. of Components	No. of Reactants Per Component	No. of Analogues
3	3	27
3	5	125
3	10	1000
4	5	625
5	5	3125

The combinatorial array synthesis described above provides a wide variety of structural analogues limited only be the reactive limits of the underlying MCCA reaction. For example, in a further embodiment of the present invention, the reactants of a component of a combinatorial array synthesis can themselves be the product of a combinatorial array synthesis. In yet a further embodiment of the present invention, individual reactants of a component of an MCCA array synthesis can comprise mixtures of different compounds having a common functional group. When the individual reactants used comprise mixtures of compounds, the analogues produced by the synthesis are a mixture of compounds. Hence, the term "analogue," as it is used in this application, can include more than one compound. Further, depending on the particular reaction, cis, trans, exo, endo and diasteriometric isomers can also be produced by the array synthesis.

It should be understood that the MCCA array syntheses of the present invention encompass all type of chemical reactions including, but not limited to solution, solid phase, photochemical, electrochemical, free radical and enzymatic reactions.

It should also be understood that MCCA array syntheses involving the condensation of four or more components can also be conducted according to the present invention. In the case of a four component array synthesis, it is simplest to view the reaction as a series of three dimensional (n-1) array syntheses where the only variable between the three dimensional

arrays is the structure of the fourth component used in each array. Higher order MCCA array syntheses are also possible.

The present invention also relates to the arrays (libraries) of structural analogues that can be synthesized by an MCCA array synthesis. Assaying compounds requires very little time relative to the time presently required to synthesize compounds by linear syntheses. The present invention enables the rapid production of large libraries of structurally related analogues all having a common core structure. Given the speed with which compounds can be analyzed, access to libraries of compounds sharing a common core structure would be an invaluable tool for analyzing the structure-function relationships governing a molecule's biological activity.

At present, a relatively small group of core structures appear to predominate in nature. Reference arrays of structural analogues of these common core structures would be valuable for making at least preliminary screenings regarding which core structures and which analogues having those core structure arrays possess some biological activity. Although these reference arrays may not always be specifically designed for the biological system being tested, the observation of some change in biological activity with regard to certain analogues within the arrays would provide valuable clues as to the governing structure-function relations. These clues could then be used to design more specifically tailored combinatorial arrays and/or specific target compounds.

Access to combinatorial arrays of structural analogues of core structures found in nature would also be invaluable for expeditiously performing preliminary structure-function analyses on newly isolated biomolecules that have not been fully characterized regarding their biological function. Further, these

combinatorial arrays would provide smaller research groups and, in particular, non-synthetic research groups, with access to compounds for doing structure-function analyses that were previously inaccessible.

The ability of the present invention to generate large quantities of structural analogues now makes it feasible, for the first time, to investigate the biological activity of arrays of compounds with core structures not currently associated with biologically active molecules. Previously, the synthetic effort required to produce structural analogues made it impractical to probe core structures not associated with known biologically active molecules for biological activity. The large arrays of structural analogues that can be generated by the present invention now make it feasible to assay a wider range of core structures and structural analogues of these core structures for biological activity.

A very small amount of material is needed to assay a compound for the presence of biological activity. Therefore, combinatorial arrays of structural analogues would not need to contain large amounts of any given compound. In addition, since a small quantity of compound is needed per assay, even combinatorial arrays containing small quantities of each compound would be capable of repeated use.

The organizational sense of the combinatorial arrays of the present invention greatly simplifies the mental aspect of structure-function analysis. In order to appreciate this utility, the organizational nature of the combinatorial arrays of the present invention needs to be explained. The combinatorial array of compounds generated by a MCCA synthesis is best thought of in terms of a geometric array having a dimension equal to the number of reaction components employed in the MCCA array synthesis. For example, a four component MCCA reaction would generate a four dimensional combinatorial

array. Each element of the array is identifiable by its coordinate in the array which corresponds to the precise components used to synthesize that analogue. Hence the product of the second variant of each component of a three dimensional array would have an array coordinate of (2,2,2).

The coordinate system of the combinatorial array assists the evaluation of results derived from assaying the elements of a particular combinatorial array for biological activity. Each n dimensional array can be envisioned as a series of (n-1) dimensional arrays or sublibraries wherein all of the members of each sublibrary share a common core structure formed by at least one common component. Thus, as depicted in Figure 3, a three component condensation reaction can be broken down into a series of two dimensional arrays where one reactant is held constant.

The formation or destruction of activity across a sublibrary reveals the biological significance of the structure contributed by the component common to all of the members of the sublibrary. Thus, by interpreting MCCA arrays as multidimensional arrays that can be divided into sublibraries, the present invention provides a systematic framework for evaluating structure-function relationships. Since each sublibrary, independent of its size, corresponds to a class of compounds where one structural subunit is held constant, a finding of biological activity across a particular sublibrary would indicate that the unvaried functional subunit was significant to the biological activity realized for that group of compounds.

The organizational sense created by the combinatorial arrays of the present invention provide an invaluable tool for evaluating the structure-function correlations of a very large number of compounds. The linear synthetic approach currently employed for the identification of new therapeutics is driven largely by

evaluating the binding constants of the molecules assayed. The rational as to why certain analogues exhibit stronger binding is not necessarily readily apparent from those studies. In contrast, the approach of the present invention provides an organizational framework whereby structure-function effects become readily apparent. Thus, in addition to providing a more rapid and efficient route to analogues for testing structure-function relationships, the present invention provides a more effective means for evaluating structure-function relationships in route to the identification of superior therapeutic agents.

The present invention also relates to the solid phase synthesis, storage and use of these MCCA arrays. Solid phase synthesis is valuable for simplifying purification. By binding one of the reaction components to a solid support, for example, a microtiter well, it is possible to isolate the compounds synthesized in high purity. One of the disadvantages of any multiple step reaction is low yields. A three step synthesis having a 70% yield for each step only produces a product in a 35% yield. Thus, when synthesizing complex molecules, simplification of the purification process is highly desirable as a means for optimizing the resulting yield. By synthesizing the compounds of the combinatorial array on a solid support where one of the reactants is attached to a detachable linker, purification and isolation of the product is greatly simplified. This is especially important when complex, lower yielding MCCA reactions are involved since all that is needed in the present invention is an adequate amount of each analogue to test for biological activity.

Solid phase synthesis of the combinatorial arrays of the present invention improves the storage stability of these arrays by enabling these compounds to be isolated in high purity and in a solid state. In addition, storing the compounds while bound to a solid

support prevents the spillage or mixing of the compounds in the combinatorial array.

Solid phase synthesis also facilitates the use of these combinatorial arrays for conducting in vitro binding studies. Entire two dimensional arrays of compounds bound to microtiter trays can be assayed at one time for their ability to bind to a protein or cell. This enables entire arrays of analogues to be very rapidly assayed for binding to different biomolecules and cells.

Solid phase synthesis of combinatorial arrays by MCCA reactions presents very few limitations on the different types of compounds that can be synthesized. In general, the only limitation presented by the use of solid phase synthesis is the requirement that there be a means for attaching one of the reaction components to the solid support. This will generally involve the use of a functional group such as an alcohol, mercaptan, amine, carbonyl, carboxylate or selenide. No other limitations to the structure of the compound to be synthesized are created. Further, as new linkers are designed, the structural limitations presented will be further reduced.

Any solid support media capable of covalently binding to a linker can be used in the present invention. Polymers such as polystyrene-divinyl benzene, polyethylene grafted polystyrene, polyacrylamide-kieselguhr composites and controlled pore glass have all been commercialized with functional groups suitable for derivitization with various linkers.

Linkers are used to couple a variety of monomers to the solid support for the solid phase polymeric synthesis of biomolecules such as DNA and peptides. The linkers of the present invention must be capable of binding to one of the components of the MCCA synthesis and must also be capable of later releasing the synthesized molecules by some specific, regulatable

mechanism. Such regulatable methods include but are not limited to thermal, photochemical, electrochemical, acid, base, oxidation and reduction reactions.

There are several commercially available linkers which provide a variety of functional group coupling and cleavage strategies. Examples of acid labile linkers include the 4-hydroxy methyl phenoxy aliphatic acids which allow the coupling of ethers acetals and esters to the linker. Acid labile linkers bearing an amine, such as the p-[2,4-dialkoxylaminobenzyl] phenoxy acetic acid, allow for coupling through functional groups such as amides, imines, carbonates or ureas. The extent of the alkoxyl substituents on the aromatic rings of these linkers influence the pH at which the organic compound is cleaved from the solid support. Trityl-based linkers can be cleaved under mildly acid conditions.

Base labile linkers include [4-(2-bromopropionyl)phenoxy] acetic acid linkers. The ester coupling of these linkers can be cleaved by nucleophiles such as hydroxide, alkoxides, and amines to give the corresponding acid, ester and amide derivatives of the organic compounds. In addition, the [4-(2-bromopropionyl)phenoxy] acetic acid linker as well as the ortho-nitro benzyl linkers enable the photochemical cleavage of an organic compound from solid support under neutral conditions.

It is preferred that the linker be capable of photochemically or thermally releasing the product since no decoupling reactants are required. A linker having a thermal release mechanism is the most preferred embodiment because thermal reactions provide greater control over the rate at which the compound is released. Further, thermally labile linkers are more stable than photochemically labile linkers.

The types of linkers useful in the present invention is not limited to the linkers described herein

since additional suitable linkers may exist in the art or be later developed. The use of linkers in the solid phase synthesis of an MCCA array is described in Examples 6 and 7.

The present invention also relates to the use of solid support bound analogues in in vitro binding studies. In this application of the present invention, whole arrays of compounds can be simultaneously, quickly and efficiently tested for their ability to bind to a biological molecule. This greatly accelerates the process of assaying large arrays of compounds for biological activity.

Arrays of compounds bound to a solid support can also be used to study the effect of structural orientation on a compound's biological activity. This is accomplished by assaying combinatorial arrays of the same group of compounds where each array differs in that a different portion of the structural analogues in each array are used to bind the compounds to the solid support. Hence, the present invention provides a method for testing the binding affinity of different faces of the same array of compounds. The linear synthesis approach currently employed does not provide a means for obtaining this data short of obtaining crystal structures of bound complexes.

One of the limitations of using solid phase synthesis is the requirement that one of the components be attachable to the solid support and also be able to participate in the MCCA reaction. In order for a component to serve both these functions, the component must have two functional groups, one that participates in the reaction and the other which binds to the linker. The requirement that a component have two functional groups is not a significant problem when one does not seek to vary the structure of the component being bound to the solid support. However, the range of structural variants of a component that can be both bound to a

linker and at the same time participate in the reaction is often limited.

It should be noted that it is not always necessary to isolate and purify the products of an array synthesis. Many MCCA reactions are high yielding and all components can be reacted in solution. Further, many bioassays are operable on crude product mixtures. Hence, in these instances, the primary advantage of solid phase synthesis, namely simplified purification, is obviated.

In addition, a method for isolating reaction products from solution chemistry reaction mixtures involves sequestering the unreacted components of the reaction. An example of the use of sequestering agents to isolate the products of an Ugi reaction is provided in Example 9. By using sequestering agents to sequester the unreacted components of the reaction, the products of the array synthesis can be isolated at sufficient purity to use in bioassays.

The present invention also relates to a method for the automated production of structural analogues. The automated synthesis of MCCA arrays reduces the level of skill required for the preparation of arrays of structural analogues and thus enables the performance of structure-function studies without the assistance of synthetic organic chemists. Thus, much like automated DNA, RNA and peptide synthesizers, the present invention enables researchers without a high level of skill in the synthetic chemical arts to generate combinatorial arrays of structural analogues. The automated synthesis of the combinatorial arrays of structural analogues of the present invention is made possible by the logical organization of the arrays synthesized. Automation of the present invention is facilitated by the fact that the present invention can be organized so that the same reaction component can be added to all of the reaction compartments in the same row, column or layer.

Automated synthesis is also facilitated by the fact that the same MCCA reaction conditions can be used for the synthesis of all of the compounds within the MCCA array.

Automated combinatorial synthesis of MCCA arrays can be performed in both the solution phase as well as in the solid phase. In many cases, the MCCA reactions are high yielding such that the major component of the reaction mixture is the product. In such cases, the product can often be isolated by evaporation of the solvent and other volatiles.

In the case of lower yielding MCCA reactions, the automated combinatorial synthesis of MCCA arrays is preferably conducted in the solid phase because the purification and isolation of the synthesized compounds is greatly simplified.

The method of generating combinatorial arrays of structural analogues using multiple component condensation reactions as well as the combinatorial arrays generated are illustrated in the following examples. As is shown by these examples, the present invention, through the use of MCCA reactions, provides a simple and efficient method for synthesizing large numbers of structural analogues for most of the known common core structures of biologically active molecules. Further objectives and advantages other than those set forth above will become apparent from the examples and accompanying drawings.

It should be noted that some reactions may not function within the disclosed scope of the invention. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art. e.g., by appropriate protection of interfering groups, by changing to alternative conventional reactants, or by routine modification of reaction conditions. Alternatively, other reactions

disclosed herein or otherwise conventional will be applicable to the preparation of the corresponding compounds of the invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials; all temperatures are set forth uncorrected in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

EXAMPLES

EXAMPLE 1 Determination of the MCCA Array To Prepare

The present invention takes advantage of the organic chemistry synthesis design theory known as the retrosynthetic approach. The retrosynthetic approach teaches one to dissect a target molecule into its smaller, simpler components by looking for bonds in the molecule that can be formed by known synthetic methods. The present invention takes an analogous approach, teaching one to evaluate the target molecule for the presence of one or more of the core structures known to be accessible by MCCA reactions.

In order to determine which MCCA reaction to employ to generate a series of structural analogues of the target molecule, it is first necessary to compare the target compound with the core structures generated by MCCA reactions. Figure 1 summarizes fourteen core structures that can be generated by known MCCA reactions. Once a MCCA core structure in the target molecule is identified, the scheme for synthesizing the target molecule is determined by labelling the substituents attached to the core structure as R, R_1, R_2, R_3, etc. as indicated. Then, as shown in Figure 2, the same substituents are attached to the corresponding reaction components. A combinatorial array of structural analogues of the target molecule can be prepared by varying the structure of the substituents of the various components of the MCCA reaction. This procedure is further described in Example 3 which describes the synthesis of carzinophilin/azinomycin structural analogues using a Passerini MCCA reaction.

EXAMPLE 2 Synthesis of α-Acyloxy Amine MCCA Arrays Using The Passerini Reaction

The synthesis of an array of structural analogues having a common α-acyloxy amine core structure can be

prepared by a combinatorial array synthesis using the Passerini reaction. As depicted in Figure 3, the Passerini reaction involves the reaction of an aldehyde having the general structure RCHO with an isocyanide having the general structure R_1NC and an acid having a general structure R_2COOH.

A two-dimensional MCCA array synthesis employing the Passerini reaction was conducted using eight aldehydes, eight carboxylic acids and one isocyanide. The two dimensional combinatorial array of analogues generated by this synthesis, along with the observed yields, is depicted in Figure 4. The experimental protocol for this combinatorial array synthesis as well as the physical data for ten of the analogues synthesized is provided below. All of the reactants used in the two dimensional array synthesis are commercially available.

Experimental Protocol:

A. Reactant Preparation:

An anhydrous CH_2Cl_2 (17 mL) solution of diethyl cyanomethylphosphonate (700 mg, 70 equiv.) was prepared in a 25 mL round bottom flask and allowed to stir for 1 hour at room temperature under an N_2 atmosphere. Anhydrous CH_2Cl_2 (2.2 mL) solutions of aldehydes: benzaldehyde, heptaldehyde, propanaldehyde, trans-2-butenal, p-methoxybenzaldehyde, butyraldehyde, 4-N,N-dimethylaminobenzaldehyde, and cinnamaldehyde were prepared in 5 mL round bottom flasks such that each aldehyde represented 20 molar equivalents based on isocyanide. An anhydrous CH_2Cl_2 (2.2 mL) solution of carboxylic acids: acetic acid, 2-phenylacetic acid, 2',2-diphenylacetic acid, acrylic acid, benzoic acid, 1-naphthoic acid, cinnamic acid, 3,3-dichloropropionic acid were prepared in 5 mL round bottom flasks such that

each acid represented 10 molar equivalents based on isocyanide.

B. Reaction Protocol:

A shell vial box (Fisher, 1 dr, 15 X 45 mm, 72 vials) containing 64 glass vials (8X8) was used as the reaction chamber for the combinatorial array synthesis. The specific aldehyde used in the combinatorial array synthesis was varied by rows. The specific acid used in the combinatorial array synthesis was varied by columns.

Row 1:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of cinnamaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 1 in one single process.

Row 2:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 4-N,N-dimethylbenzaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 2 in one single process.

Row 3:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of butyraldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 3 in one single process.

Row 4:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of p-methoxybenzaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 4 in one single process.

Row 5:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of trans-2-butenal in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 5 in one single process.

Row 6:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of propanaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 6 in one single process.

Row 7:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of heptaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 7 in one single process.

Row 8:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of benzaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 8 in one single process.

Column A:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of acetic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column A in one single process.

Column B:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 2-phenylacetic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column B in one single process.

Column C:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 2,2-diphenylacetic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column C in one single process.

Column D:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of acrylic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column D in one single process.

Column E:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of benzoic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column E in one single process.

Column F:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 1-naphthoic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column F in one single process.

Column G:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of cinnamic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column G in one single process.

Column H:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 3,3-dichloropropionic acid in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in column H in one single process.

The 17 mL CH_2Cl_2 solution of cyanomethylphosphonate was dispensed in 0.25 mL aliquots into 64 vials of the array using a gas tight syringe in a single process. Upon completion of reactant additions, each reaction vessel contained 2 molar equivalents of aldehyde, one molar equivalent of acid, and one molar equivalent of isocyanide in a total volume of 0.75 mL in CH_2Cl_2. The reaction vessels (vials) were all capped and no further precautions were taken to maintain an anhydrous atmosphere. The entire array was manually shaken for 30 seconds and then allowed to stand at room temperature for 17 hours. Selected samples were each transferred to round bottom flasks and evaporated to dryness under reduced pressure without further purification. Each crude mixture was analyzed by ^1H NMR to determine the product composition and yield based on unreacted starting isocyanide. Data is provided below

for a selection of ten analogues. Each analogue is identified by its row and column number.

Analogue 3C: N-[(1-diethylphosphono)methyl]-2-diphenylacetoylpentamide: This reaction mixture contained n-butanaldehyde (80 uL, 2 equiv.), diphenylacetic acid 100 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv) ^{1}H NMR ($CDCl_3$) δ = 7.3 (m, Ar-H), 5.2 (t, OCH), 4.1 (m, OCH_2CH_3), 3.7 (m, CH_2P), 3.5 (m, CH_2P), 1.7 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 80% yield.

Analogue 3F: N-[(1-diethylphosphono)methyl]-2-(1-napthoyl)pentamide: This reaction mixture contained n-butanaldehyde (80 uL, 2 equiv.), 1-napthoic acid 80 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR ($CDCl_3$) δ = 9.0 (d, Ar-H), 8.2 (d, Ar-H), 8.0 (d, Ar-H), 7.6 (m, Ar-H), 7.5 (m, Ar-H), 7.3 (Ar-H), 5.5 (t, OC6CH), 4.1 (m, OCH_2CH_3), 3.8 (m, CH_2P), 2.1 (m, CH_2), 1.6 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 1.0 (t, CH_3). 80% yield.

Analogue 6C: N-[(1-diethylphosphono)methyl]-2-diphenylacetoylbutamide: This reaction mixture contained n-propanaldehyde (70 uL, 2 equiv.), diphenylacetic acid (100 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR ($CDCl_3$) δ = 7.3 (m, Ar-H), 6.6 (bt, NH), 5.2 (t, OCOCH), 5.1 (s, $CHAr_2$), 4.1 (m, OCH_2), 3.8 (m, CH_2P), 1.85 (m, OCH_2CH_3), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 60% yield.

Analogue 6F: N-[(1-diethylphosphono)methyl]-2-(1-napthoyl)butamide: This reaction mixture contained n-propanaldehyde (70 uL, 2 equiv.), 1-napthoic acid (80 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR ($CDCl_3$) δ = 9.0 (d, Ar-H), 8.2 (d, Ar-

H), 7.8 (m, Ar-H), 7.5 (m, Ar-H), 7.5 (bt, NH), 5.5 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.85 (m, CH_2P), 2.1 (m, CH_2), 1.4 (m, OCH_2CH_3), 1.3 (m, CH_2), 1.1 (t, CH_3). 75% yield.

Analogue 7A: N-[(1-diethylphosphono)methyl]-2-acetoxyoctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), acetic acid (30 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 6.6 (bt, NH), 5.1 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.65 (m, CH_2P), 2.1 (5, CH_3), 1.7 (m, CH_2), 1.55 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 95% yield.

Analogue 7B: N-[(1-diethylphosphono)methyl]-2-phenylacetoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), phenylacetic acid (65 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 7.3 (m, Ar-H), 6.7 (bt, NH),- 5.1 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.7 (m, CH_2P), 3.6 (s, CH_2), 3.55 (m, CH_2P), 1.8 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 50% yield.

Analogue 7D: N-[(1-diethylphosphono)methyl]-2-acyloyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), acrylic acid (35 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDC1_3$) δ = 6.9 (bt, NH), 6.4 (dd, CH_2CH), 6.1 (dd, CH_2CH), 5.8 (dd, CH_2CH), 5.15 (t, OCOCH), 4.6 (m, OCH_2C11_3), 3.7 (m, CH_2P), 1.8 (OCH_2), 1.5 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 100% yield.

Analogue 7E: N-[(1-diethylphosphono)methyl]-2-benzoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), benzoic acid (60 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 8.0 (d, ArH), 7.5 (d, ArH),

7.4 (d, Ar-H), 7.1 (bt, NH), 5.4 (t, OCOCH), 4.1 (m, O-CH_2CH_3), 3.8 (m, CH_2P), 2.0 (m, CH_2), 1.6 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 100% yield.

Analogue 7G: N-[(1-diethylphosphono)methyl]-2-cinnamoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), cinnamic acid (70 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 7.7 (d, CH), 7.5 (m, Ar-H), 7.4 (m, Ar-H), 7.3 (m, Ar-H), 6.5 (d, CH), 5.3 (t, OC6CH), 4.1 (m, $6CH_2CH_3$), 3.8 (m, CH_2P), 1.9 (m, CH_2), 1.6 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 70% yield.

Analogue 7H: N-[(1-diethylphosphono)methyl]-2-dichloroacetoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), dichloroacetic acid (70 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 7.15 (bt, NH), 6.2 (s, $CHCl_2$), 5.3 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.7 (m, CH_2P), 1.9 (m, CH_2), 1.6 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 95% yield.

EXAMPLE 3 Application Of The MCCA Synthesis Strategy To Structural Analogues Of Carzinophilin/Azinomycin

Carzinophilin (CZ) and Azinomycin B are antitumor/antibiotic compounds which function as a DNA bis-alkylating agent. The mechanism of action for CZ and Azinomycin B is depicted in Figure 5. As also shown in Figure 5, Carzinophilin and Azinomycin B are structurally very similar. Given the structural complexity of these molecules, chemical syntheses are long and low yielding. Azinomycin B has also been produced by fermentation but in very low yields (2 mg/250 L).

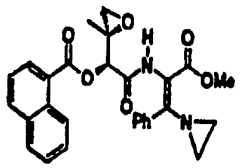
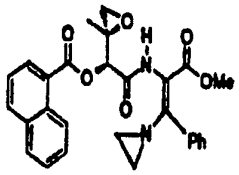
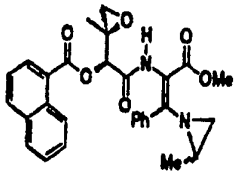
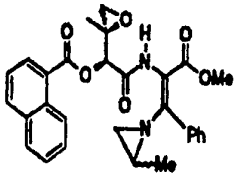
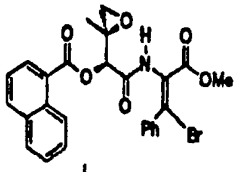
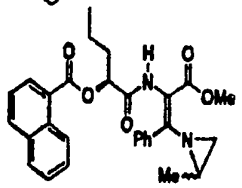
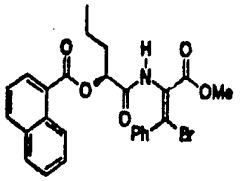
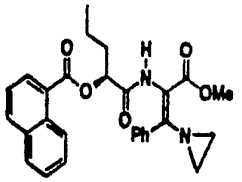
Given the difficulty associated with synthesizing these molecules, few derivatives of these molecules have been prepared. It therefore would be highly desirable to have an efficient means for producing a series of structural analogues of these molecules in order to probe the mechanism of action of these molecules.

As shown in Figure 6, both CZ and Azinomycin B have α-acyloxy amine core structures. By labelling the substituents off of the α-acyloxy amine core structure R_1, R_2 and R_3, a Passerini reaction scheme for the synthesis of Carzinophilin, Azinomycin B and their structural analogues is identified.

Once a Passerini reaction scheme for Carzinophilin and Azinomycin B has been identified, a Passerini multicomponent combinatorial array synthesis of Carzinophilin and Azinomycin B analogues can be designed by varying the R_1, R_2 and R_3 substituents of the reaction components. One possible three dimensional Passerini-type MCCA array synthesis of Carzinophilin and Azinomycin B structural analogues is provided in Figure 6.

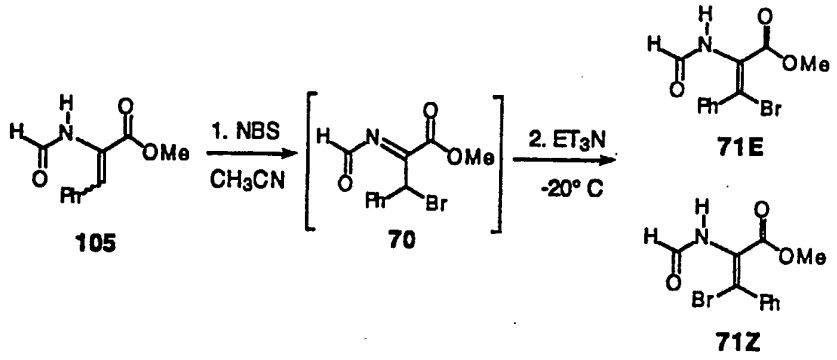
A two dimensional MCCA array synthesis of Carzinophilin and Azinomycin B structural analogues using three sets of diasteriometric isocyanates and two different aldehydes was performed. The combinatorial array synthesis and the yields obtained are provided in Table 2.

Table 2 Azinomycin Analogs Synthesized via Passerini Reactions

Cmpd	Isocyan	Aldehyde	Acid	Z/E	de[a]	% yield[b]	Major Product
109E	107E	(S)-2-methyl-glycidal	1-naphthoic acid	1/2.5	3.4:1	42%	
109Z	107Z	(S)-2-methyl-glycidal	1-naphthoic acid	3.9/1	3.4:1	53%	
110E	108E	(S)-2-methyl-glycidal	1-naphthoic acid	1/1.5	5:5:1:1	37%	
110Z	108Z	(S)-2-methyl-glycidal	1-naphthoic acid	3.3/1	3.4:3.4:1:1	51%	
111Z/E	106Z/E Z/E=1/1.4	(S)-2-methyl-glycidal	1-naphthoic acid	1.4/1	3:3:1:1	39%	
112Z/E	108Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1	1:1	68%	
113Z/E	106Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1.4	-----	69%	
114Z/E	107Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1.2	-----	57%	

[a]Major product. [b]Combined Z/E isolated yields.

The isocyanates used in the combinatorial array synthesis described in Table 2 were prepared according to the general reaction scheme provided below.



Methyl-isocyanoacetate and benzaldehyde were reacted to form the dehydroaminoacid precursor **105** according to the procedure described in Hoppe, D., et al., U. Liebigs Ann. Chem. (1972) 766:116-129. The mixture of geometric isomers were then brominated with N-bromosuccinamide (NBS) in CH_3CN to yield the α-bromo imine derivative **70** which tautermerized in Et_3N to afford the corresponding β-bromo-dehydroamino acids **71Z** and **71E** in 81% yield (Z/E = 1.4:1). The modified bromonation conditions (See Combs, A., et al., Tetrahedron Lett. (1992) 33:6419) gave substantially increased yields of the E-isomer as compared to the conditions reported by Matsumoto. Nunami, K., et al., Tetrahedron (1988) 44:5467 (60% yield, Z/E = 13:1).

The resulting vinyl bromide diasteriomers **71E/Z** were separated by silica gel chromatography and recrystallized to analytical purity. Their stereochemistries were assigned according to the

literature by ^1H NMR chemical shift comparison of the methyl proton resonances of the E- and Z- esters.

Isocyanides **106E** and **106Z** were formed from a β-bromo-dehydroamino acids **71E** and **71Z** with retention of the olefin configuration by dehydrating the N-formyl groups with $POCl_3$ and Et_3N in CH_2Cl_2 at 0°C for 30 minutes.

The corresponding vinyl aziridine compounds **107E** and **107Z** were formed from the isocyanates **106E** and **106Z** by adding ethyleneimine to the isocyanates in tetrahydrofuran and triethyl amine for 3-5 hours.

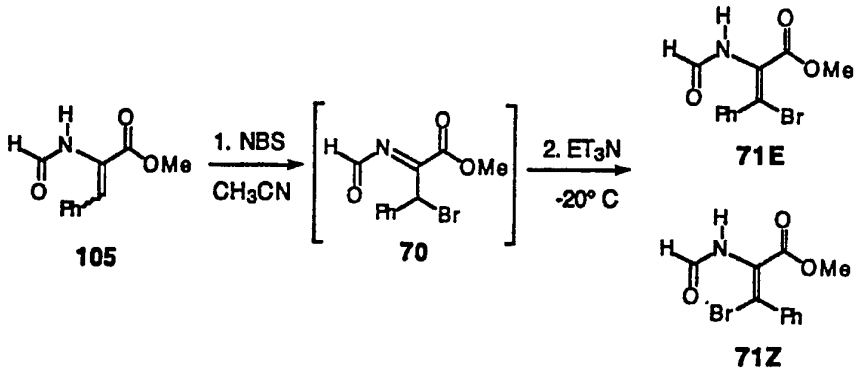
The reaction conditions and physical data for isocyanates and the structural analogues produced in the combinatorial synthesis described in Table 2 are provided below.

The above-described combinatorial synthesis of carzinophilin and azinomycin structural analogues exposes the power of the present invention to enable the synthesis of broad arrays of structural analogues. For example, in the present synthesis of carzinophilin and azinomycin structural analogues, it is possible to further vary the aziridine ring, the phenyl ring and the olefin portion of the isocyanate, the epoxide group of the aldehyde as well as the substituents on the aromatic ring of the acid. Thus, as can be seen from the present example, a very broad array of carzinophilin and azinomycin structural analogues may be produced according to the present invention.

It may be further noted that the above-described combinatorial array synthesis of carzinophilin and azinomycin structural analogues may be readily performed as a solid phase synthesis. For example, by using a carboxylic acid containing a hydroxy, thio or amino functional group, one could readily attach the carboxylic acid to a solid support. Then, by adding an aldehyde and an isocyanate under the conditions described in the present example, one could carry out

the above-described synthesis as a solid phase synthesis.

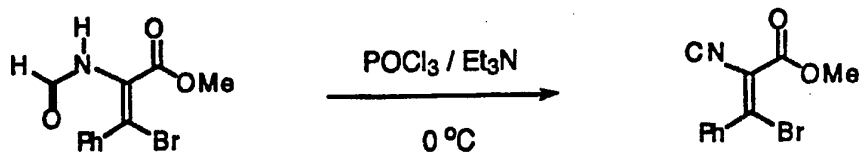
Experimental Data



NBS (5.4 g, 30.22 mmol) was added to a r.t CH_3CN (20 mL) solution of dehydoamino acid **105** (5.9 g, 28.78 mmol). The reaction was stirred at 25°C for 1 hr. and then heated to 45°C for 30 min. to give α-bromo imine intermediate **70**. The solution of **70** was then cooled to r.t. and Et_3N (4.0 mL, 31.7 mmol) added dropwise over a 5 min. period. This mixture was allowed to stir for 1 hr., after which the solvent was removed under reduced pressure. The crude oil was taken up in EtOAc and the insoluble succinimide removed by filtration. The solvent was rotoevaporated to a clear oil, which was further purified by flash silica gel chromatography to yield two fractions: pure **71Z** (2.0 g) and bromides **71Z** and **71E** (4.6 g, Z/E=1:1.2) for an 81% overall yield (Z/E=1:1.4) The Z- and E- isomers could be further separated by flash silica gel chromatography and then recrystallized from EtOAc/Hex to analytical purity. The E- and Z- isomer data was consistent with previous literature assignments: **71E** m.p. 110-112°C, lit.m.p. 109-110°C; **71Z** m.p. 138-140°C, lit.m.p. 136-138°C.

NBS (34 mg, 0.188 mmol) was added to a r.t. CD_3CN (0.5 mL) solution of dehydroamino acid **105** (35 mg, 0.171 mmol). After 3 hrs. the reaction gave the α-bromo imine **70** quantitatively by ^1H and ^{13}C NMR.

70: ^{1}H NMR (360 MHz, CD_3CN) δ 9.33 (s, 1H, CHO), 7.53 (m, 2H, o-ArH), 7.39 (m, 3H, m,p-ArH), 6.26 (s, 1H, CHBr), 3.81 (s, 3H, OCH_3); ^{13}C NMR (90 MHz, CD_3CN), δ 174.05 (CHO), 159.52 (C), 136.71 (C), 130.29 (CH), 130.25 (CH), 129.71 (CH), 54.86 (OCH_3), 48.86 (CHBr).

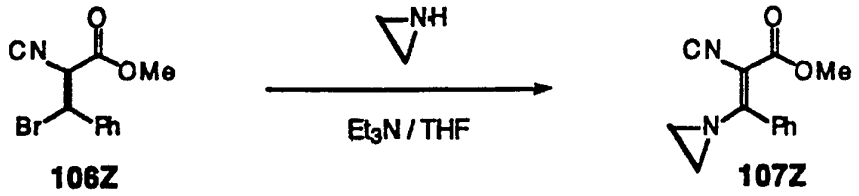


71E **106E**

$POCl_3$ (217 μL, 2.3 mmol) was added dropwise over a 15 min. period to a 0°C CH_2Cl_2 (40 ml) solution of bromide **71E** (600 mg, 2.12 mmol) and Et_3N (1.4 mL, 10.6 mmol). The solution was allowed to stir at 0°C for 30 min., after which, the reaction was quenched with 20 mL of aqueous Na_2CO_3 (450 mg, 4.24 mmol). This mixture was stirred for 15 min. and then the aqueous layer extracted 3X with Et_2O. The combined organic layers were dried over $MgSO_4$ and rotoevaporated to a brown oil. The crude oil was triturated with Et_2O (3X) and the ether fractions filter through a $MgSO_4$ plug. The ether was removed under reduced pressure to afford **106E** (590 mg, 105%) as a brown oil. The extracted material was sufficiently pure (>90%) for the next synthetic step without chromatography. (The crude could be further purified by flash silica gel chromatography, but with noticeable loss in material).

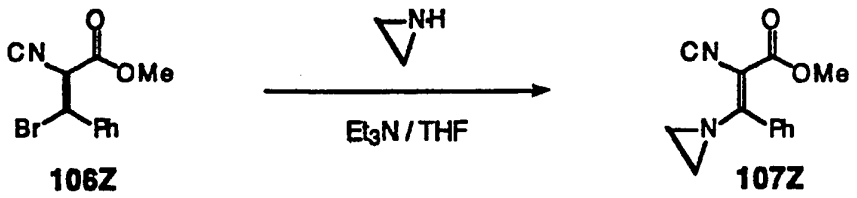
106E: IR (film) cm^{-1}: 2956, 2112 (CN), 1744, 1699, 1442, 1261, 1119, 893; ^{1}H NMR (360 $CHCl_3$) δ 7.46 (m, 2H, o-ArH), 7.39 (m, 3H, m,p-ArH), 3.86 (s, 3H, OCH_3); ^{13}C NMR (90 MHz, $CHCl_3$), δ 171.9 (CHO), 160.3 (C), 138.1 (C), 136.6 (C), 130.9 (CH), 128.5 (CH), 128.5

(CH), 128.5 (C), 53.4 (OCH_3). HRMS (CI) $[MH]^+$ calcd for $C_{11}H_8NO_2Br$ 265.9817, found 265.9817.



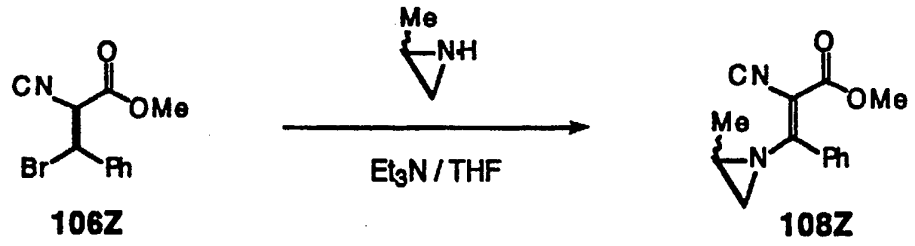
Ethyleneimine (125 μL, 2.43 mmol) was added to a r.t. THF (10 mL) solution of isocyanide bromide **106Z** (215 mg, 0.81 mmol) and Et_3N (110 μL, 0.90 mmol). The mixture was stirred for 3 hrs., after which the solvent and excess amines were removed under reduced pressure to afford a brown oil. Attempts to extract over aqueous solutions hydrolyzed the vinyl aziridine. The crude oil was therefore triturated with Et_2O and completely insoluble $EtNH^+Br^-$ removed by filtration through a $MgSO_4$ plug. The ether was rotoevaporated to a brown oil **107Z** (185 mg, 100%), which was sufficiently pure (>90%) for analytical data.

107Z: IR (film) cm^{-1}: 3350, 3000, 2952, 2116 (CN), 1560, 1290; ^1H NMR (360 MHz, $CHCl_3$) δ 7.41-7.26 (m, 3H, o,p-ArH), 7.16 (m, 2H, m-ArH), 3.59 (s, 3H, OCH_3), 2.34 (s, 4H, $N(CH_2)_2$); ^{13}C NMR (90 MHz, $CHCl_3$), δ 170.0 (C), 165.3 (C), 161.6 (C), 129.4 (CH), 128.0 (C), 127.9 (CH), 127.2 (CH), 51.9 (OCH_3), 29.9 ($N(CH_2)_2$); HRMS (CI) $[MH]^+$ calcd for $C_{13}H_{12}N_2O_2$ 229.0978, found 229.0977.



Ethyleneimine (145 μL, 2.89 mmol) was added to a r.t. THF (5 mL) solution of isocyanide bromide **106E** (225 mg, 0.962 mmol) and Et_3N (150 μL, 1.16 mmol). The mixture was stirred for 2 hrs. after which the solvent and excess amines were removed under pressure to afford a brown oil (attempts to extract over aqueous solutions hydrolyzed the vinyl aziridine). The crude oil was therefore triturated with Et_2O and the completely insoluble $EtNH^+Br^-$ removed by filtration through a $MgSO_4$ plug. The ether was rotoevaporated to afford a brown oil **107E** (255 mg, 103%), which was sufficiently pure (<90%) for analytical data.

107E: IR (film) cm^{-1}: 3325, 3001, 2952, 2117 (CN), 1717, 1554, 1445, 1390, 1257, 1148, 1124; ^1H NMR (360 MHz, $CHCl_3$) δ 7.40 (m, 5H, Ar**H**), 3.81 (s, 3H, OCH_3), 2.25 (s, 4H, $N(CH_2)_2$); ^{13}C NMR (90 MHz, $CHCl_3$), δ 168.1 (C), 166.6 (C), 161.5 (C), 135.3 (C), 129.8 (CH), 128.3 (CH), 128.2 (C), 127.3 (CH), 52.1 (OCH_3), 32.9 ($N(CH_2)_2$); HRMS (CI) $[MH]^+$ calcd for $C_{13}H_{12}N_2O_2$ 229.0977, found 229.0977.

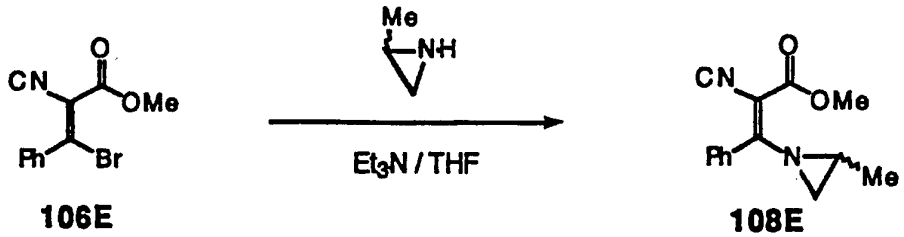


106Z **108Z**

Racemic 2-methylasiridine (210 μL, 2.91 mmol) was added to a r.t. THF (10 mL) solution of isocyanide bromide **106Z** (0.88 mmol) and Et_3N (125 μL, 0.97 mmol). The mixture was stirred for 2 hrs., after which the solvent and excess amines were removed under reduced pressure to afford a brown oil. The crude oil was extracted 3X with Et_2O over H_2O. The ether fractions were dried over $MgSO_4$ and rotoevaporated to afford a

brown oil **108Z** (198 mg, 93%), which was sufficiently pure (>90%) for analytical data.

108Z: IR (film) cm^{-1}: 3350, 2953, 2116 (CN), 1723, 1564, 1291; ^1H NMR (360 MHz, $CHCl_3$) δ 7.44-7.35 (m, 3H, o,p-ArH), 7.21 (m, 2H, m-ArH), 3.61 (s, 3H, OCH_3), 2.54 (ddq, J=3.8, 5.5, 6.1 Hz, 1H, $NCHCH_3$), 2.23 (d, 1=6.1 Hz, 1H, NCH_2), 2.18 (d, J=3.8 Hz, 1H, NCH_2), 1.36 (d, J=5.5 Hz, 3H, $NCHCH_3$); ^{13}C NMR (90 MHz, $CHCl_3$), δ 169.9 (C), 165.8 (C), 161.8 (C), 135.0 (C), 129.4 (CH), 128.3 (C), 128.2 (CH), 127.4 (CH), 52.0 (OCH_3), 38.2 ($NCHCH_3$), 36.4 (NCH_2), 18.3 ($NCHCH_3$); HRMS (CI) $[MH]^+$ calcd for $C_{14}H_{14}N_2O_2$ 243.1134, found 243.1129.

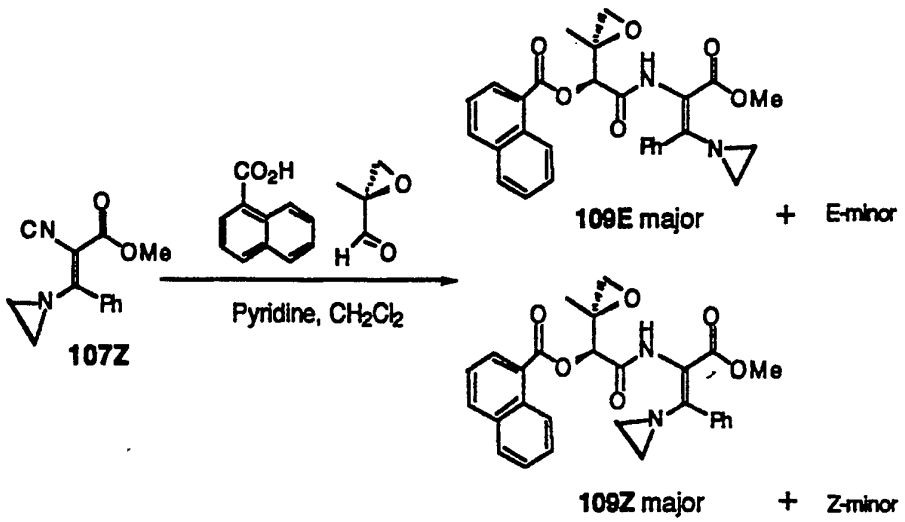


106E **108E**

Racemic 2-(methyl)-aziridine (250 μL, 3.0 mmol) was added to a r.t. THF (5 mL) solution of isocyanide bromide **106E** (263 mg, 0.992 mmol) and Et_3N (150 μL, 1.19 mmol). The mixture was stirred for 2 hrs., after which the solvent and excess amines were removed under reduced pressure to afford a brown oil. The crude oil was extracted 3X with Et_2O over H_2O to efficiently remove the $Et_3NH^+Br^-$. The combined ether fractions were dried over $MgSO_4$ and rotoevaporated to afford a brown oil **108E** (269 mg, 112%), which was sufficiently pure (>90%) for analytical data.

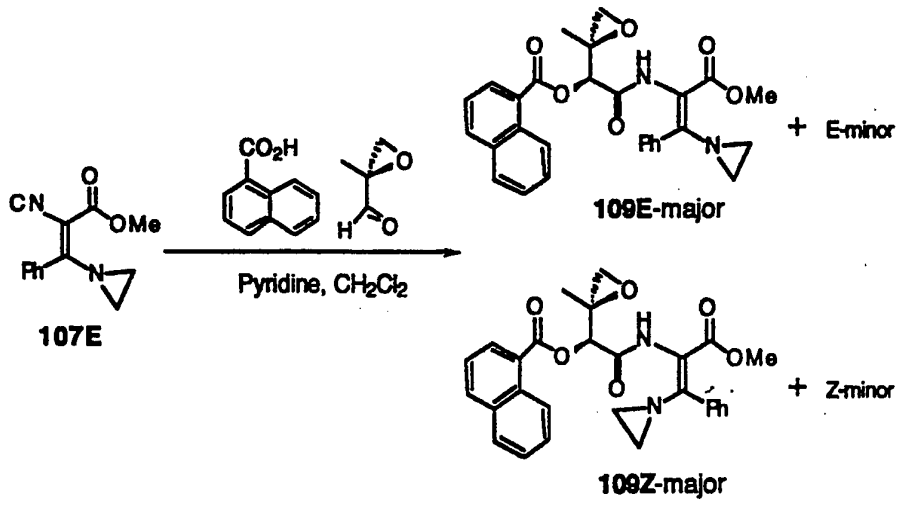
108E: IR (film) cm^{-1}: 3303, 2995, 2952, 2117 (CN), 1723, 1548, 1445, 1172, 1124; ^1H NMR (360 MHz, $CHCl_3$) δ 7.47-7.40 (m, 5H, ArH), 3.83 (s, 3H, OCH_3), 2.31 (ddq, J=1.5, 5.2, 6.1 Hz, 1H, $NCHCH_3$), 2.29 (d, J=1.5 Hz, 1H, NCH_2), 2.18 (d, J=5.2 Hz, 1H, NCH_2), 1.24 (d, J=6.1 Hz, 3H, $NCHCH_3$); ^{13}C NMR (90 MHz, $CHCl_3$), δ 167.8 (C),

166.7 (C), 161.7 (C), 135.8 (C), 129.8 (CH), 128.4 (C), 128.3 (C), 127.5 (CH), 52.1 (OCH_3), 40.2 ($NCHCH_3$) 39.9 (NCH2), 17.8 ($NCHCH_3$); HRMS (CI) $[MH]^+$ calcd for $C_{14}H_{14}N_2O_2$ 243.1134, found 243.1134.



Pyridine (72 μL, 0.89 mmol), (S)-2-(methyl)-glycidal (210 μL, 2.43 mmol), and 1-naphthoic acid (140 mg, 0.81 mmol) were added sequentially to a r.t. CH_2Cl_2 (5 mL) solution of isocyanide **107Z** (0.81 mmol). The mixture was stirred under N_2 for 20 hrs., after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) to afford four fractions: **109Z** (138 mg, de=2.9:1), **109Z** (28.5 mg, de=2:1), **109Z/109E** (13.0 mg, Z/E=2:1, de=1.4:1, de=1:2) and **109E** (30 mg, de=2.3:1) for a 53% overall yield (**109Z/109E**=5.1:1).

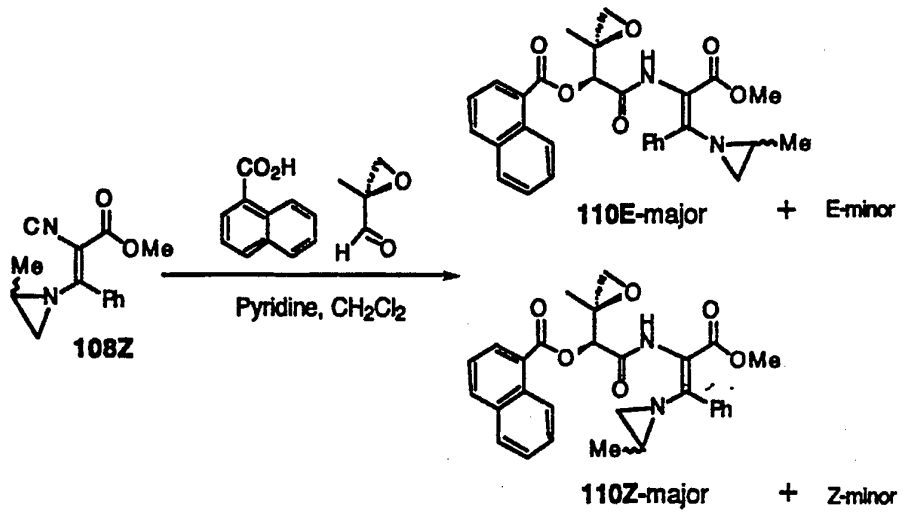
109Z (major diasteromer): IR (film) cm^{-1}: 3296, 3056, 2999, 2949, 1717, 1699, 1593, 1511, 1310, 1240, 1194, 1143, 1060; ^1H NMR (360 MHz, $CDCl_3$), δ 8.98 (d, 1=8.8 Hz, 1H, ArH), 8.38 (dd, J=1.2, 7.3 Hz, 1H, ArH), 8.07 (d, J=8.1 Hz, 1H, ArH), 7.91 (d, J=7.6 Hz, 1H, ArH), 7.67-7.51 (m, 3H, ArH), 7.55 (bs 1H, NH), 7.37-7.25 (m, 5H, ArH), 5.39 (s, 1H, OCH), 3.49 (s, 3H, OCH_3), 3.14 (d, J=4.5 Hz, 1H, OCH_2), 2.86 (d, J=4.6 Hz, 1H, OCH_2), 2.09 (bs, 4H, $N(CH_2)_2$), 1.66 (s, 3H, $OCCH_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.87, 165.24, 164.07, 164.02, 136.73, 133.80, 133.56, 131.24, 130.42, 128.63, 128.41, 128.23, 127.87, 127.01, 126.24, 125.70, 125.55, 124.30, 108.27, 75.53, 55.78, 52.66, 51.52, 32.32, 17.49; HRMS (FAB) $[MH]^+$ calcd for $C_{28}H_{26}N_2O_6$ 487.1869, found 487.1869.



Pyridine (55 μL, 0.68 mmol), (S)-2-(methyl)-glycidal (155 μL, 1.78 mmol), and 1-naphthoic acid (101 mg, 0.59 mmol) were added sequentially to a r.t. CH_2Cl_2 (3.0 mL) solution of isocyanide **107E** (135 mg, 0.59 mmol). The mixture was stirred under N_2 for 27 hrs., after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1

hex/EtOAc) affording two fractions: **109E** (84 mg, de=3.4:1) and **109Z** (30.5 mg, de=1.5:1), for a 42% overall yield (**109E/109Z**=2.8:1).

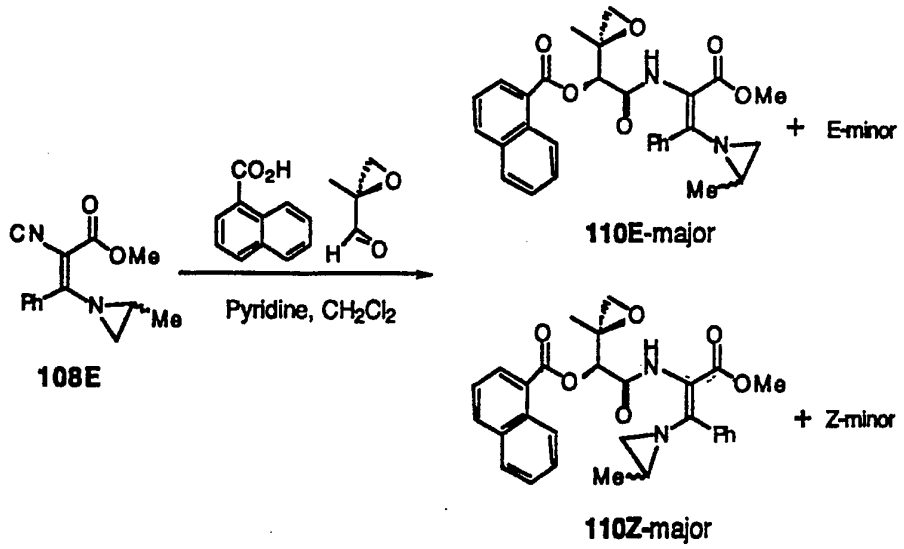
109E (major diasteromer): IR (film) cm^{-1}: 3311, 3058, 3001, 2948, 1722, 1511, 1279, 1244, 1194, 1138; ^1H NMR (360 MHz, $CDCl_3$), δ 8.85 (d, J=8.6 Hz, 1H, ArH), 8.10 (dd, J=1.1, 7.2 Hz, 1H, ArH), 8.02 (d, J=8.2 Hz, 1H, ArH), 7.86 (d, J=8.0 Hz, 1H, ArH), 7.62-7.43 (m, 3H, ArH), 7.28-7.15 (m, 5H, ArH), 6.99 (bs, 1H, NH), 5.12 (s, 1H, OCH), 3.76 (s, 3H, OCH_3), 2.82 (d, J=4.5 Hz, 1H, OCH_2), 2.63 (d, J=4.6 Hz, 1H, OCH_2), 2.21 (bs, 4H, $N(CH_2)_2$), 1.4 (s, 3H, $OCCH_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.87, 165.24, 164.07, 164.02, 136.73, 133.80, 133.56, 131.24, 130.42, 128.63, 128.41, 128.23, 127.87, 127.01, 126.24, 125.70, 125.55, 124.30, 108.27, 75.53, 55.78, 52.66, 51.52, 32.32, 17.49; HRMS (FAB) $[MH]^+$ calcd for $C_{28}H_{26}N_2O_6$ 487.1869, found 487.1869.



Pyridine (36 μL, 0.45 mmol), (S)-2-(methyl)-glycidal (107 μL, 1.24 mmol), and 1-naphthoic acid (72 mg, 0.42 mmol) were added sequentially to a r.t. CH_2Cl_2 (5 mL) solution of isocyanide **108Z** (100 mg, 0.41 mmol). The mixture was stirred under N_2 for 24 hrs., after which the solvent was removed under reduced pressure. The

crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording four fractions: 110Z (69 mg, de=3.4:3.4:1:1), **110Z** (11 mg, de= 1.5:1.5:1:1), **110Z** and **110E** (14 mg, **110Z**, de=1.5:1.5:1:1 and **110E**, de=1:1) and **110E** (22 mg, de=1.5:1.5:1:1), for a 56% overall yield (**110Z/110E**=3.2:1).

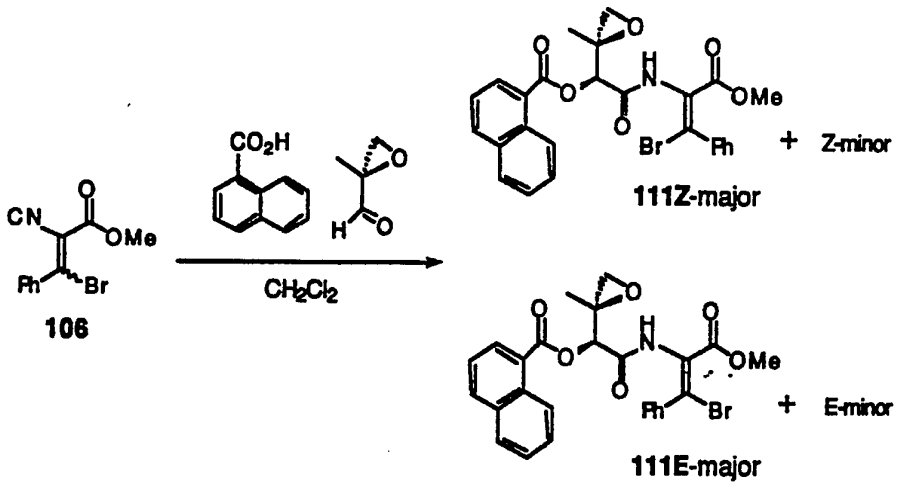
110Z (major diasteromers): IR (film) cm^{-1}: 3290, 3064, 2994, 1715, 1511, 1328, 1237, 1138; 1H NMR (360 MHz, $CDCl_3$), δ 8.97 (d, J=8.7 Hz, 1H, Ar**H**), 8.93 (d, J=9.0 Hz, 1H, Ar**H**), 8.38 (dd, J=1.1, 7.3 Hz, 1H, Ar**H**), 8.35 (dd, J=1.1, 7.4 Hz, 1H, Ar**H**), 8.08 (d, J=8.3 Hz, 211, Ar**H**), 7.91 (d, J=7.9 Hz, 2H, Ar**H**), 7.66-7.52 (m, 6H, Ar**H**), 7.41 (bs, 1H, N**H**), 7.40 (bs, 1H, N**H**), 7.37-7.26 (m, 10H, Ar**H**), 5.36 (s, 1H, OC**H**), 5.35 (s, 1H, OC**H**), 3.50 (s, 3H, CCH_3), 3.49 (s, 3H, OCH_3), 3.14 (d, J= 4.5 Hz, 1H, OCH_2), 3.12 (d, 4.6 Hz, 1H, OCH_2), 2.88 (d, J=1.3 Hz, 1H, OCH_2), 2.86 (d, J=4.0 Hz, 1H, OCH_2), 2.25 (m, 1H, $NCHCH_3$), 2.15 (m, 3H, $NCHCH_3$, NCH_2), 2.00 (d, J=6.1 Hz, 1H, NCH_2), 1.89 (d, J=3.7 Hz, 1H, NCH_2), 1.67 (s, 3H, $OCCH_3$), 1.66 (s, 3H, $OCCH_3$), 1.21 (d, J=6.1 Hz, 3H, $NHCH_3$), 1.09 (d, J=4.9 Hz, 3H, $NHCH_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.26, 165.99, 165.87, 165.81, 164.28, 164.28, 158.57, 158.57, 136.29, 136.21, 134.10, 134.02, 133.79, 133.79, 131.42, 131.37, 130.82, 130.71, 128.58, 128.58, 128.41, 128.41, 128.31, 128.31, 128.08, 128.08, 127.64, 127.64, 126.40, 126.40, 125.98, 125.86, 125.65, 125.65, 124.50, 124.50, 111.06, 111.06, 76.23, 76.14, 56.29, 56.29, 53.61, 53.35, 51.44, 51.44, 37.54, 37.39, 36.40, 36.15, 18.32, 17.97, 17.48, 17.47; HRMS (DCI) $[MH]^+$ calcd for $C_{29}H_{28}N_2O_6$ 501.2026, found 501.2026.



Puridine (40 μL, 0.50 mmol), (S)-2-(methyl)-glycidal (120 μL, 1.37 mmol), and 1-naphthoic acid (80 mg, 0.45 mmol) were added sequentially to a r.t. CH_2Cl_2 (3 mL) solution of isocyanide **108E** (110 mg, 0.45 mmol). The mixture was stirred under N_2 for 24 hrs., after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording four fractions, **110Z** (20.5 mg, de=3:3:1:1), **110Z** and **110E** (23.8 mg, **110Z** de=2:2:1:1 and **110E**, de=1.5:1.5:1:1), **110E** (9.5 mg, de= 1:1), and **110E** (29 mg, de=2:2:1:1), for a 37% overall yield (**110E/110Z**=1.5:1).

110E (major diasteromers): IR (film) cm^{-1}: 3322, 3055, 2995, 2946, 1711, 1505, 1275, 1239, 1136; ^1H NMR (360 MHz, $CDCl_3$), δ 8.86 (d, J=8.6 Hz, 1H, Ar**H**), 8.82 (d, J=8.6 Hz, 1H, Ar**H**), 8.14 (dd, J=1.2, 7.2 Hz, 1H, Ar**H**), 8.05-8.01 (m, 3H, Ar**H**), 7.87 (m, 3H, Ar**H**), 7.65-7.40 (m, 5H, Ar**H**), 7.3-7.2 (m, 10H, Ar**H**), 6.94 (bs, 1H, N**H**), 6.93 (bs, 1H, N**H**), 5.19 (s, 1H, OC**H**), 5.05 (s, 1H, OC**H**), 3.76 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 2.83 (d, J=5.0 Hz, 1H, OCH_2), 2.82 (d, J=5.7 Hz, 1H, OCH_2), 2.65 (d J=4.6 Hz, 1H, OCH_2), 2.63 (d, J=4.6 Hz, 1H, OCH_2), 2.30 (m, 1H, $NCHCH_3$), 2.25-2.10 (m, 3H, $NCHCH_3$, NCH_2), 2.15 (d, J=3.5 Hz, 1H, NCH_2), 2.14 (d, J=1.4 Hz, 1H,

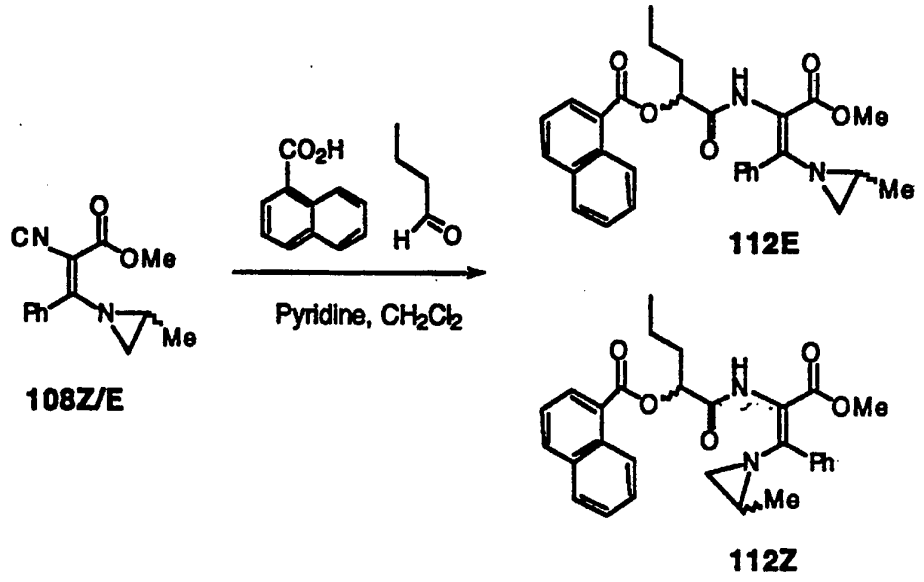
NCH_2), 1.43 (s, 3H, $OCCH_3$), 1.40 (s, 3H, $OCCH_3$), 1.22 (d, J=5.6 Hz, 3H, $NHCH_3$), 1.21 (d, J=5.5 Hz, 3H, $NHCH_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 167.07, 166.90, 165.43, 165.25, 164.17, 164.17, 164.14, 163.84, 137.10, 137.04, 133.88, 133.85, 133.69, 133.69, 131.35, 131.35, 130.54, 130.45, 128.66, 128.59, 128.49, 128.49, 128.24, 128.24, 127.97, 127.95, 127.20, 127.11, 126.34, 126.33, 125.91, 125.76, 125.67, 125.64, 124.41, 124.37, 107.72, 107.67, 75.72, 75.50, 55.92, 55.86, 52.95, 52.55, 51.59, 51.59, 39.91, 39.47, 39.32, 39.04, 18.03, 17.99, 17.82, 17.43; HRMS (DCI) $[MH]^+$ calcd for $C_{29}H_{28}N_2O_6$ 501.2025, found 501.2026.



(S)-2-(methyl)-glycidal (39 μL, 0.45 mmol) and 1-naphthoic acid (57.0 mg, 0.33 mmol) were added sequentially to a r.t. CH_2Cl_2 (2 mL) solution of isocyanide **106** (80.0 mg, 0.302 mmol, Z/E=1.4:1) and stirred under N_2 for 24 hours. Additional (S)-2-(methyl)-glycidal (20 μL, 0.23 mmol) was added and the mixture stirred for 24 hours after which the solvent was removed under reduced pressure. The crude oil was purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hexane to 1:1 hex/EtOAc). One fraction was isolated containing an

inseparable mixture of all four stereoisomers **111** (61.0 mg, Z/E 1.4:1, de=3:3:1:1) for a 39% overall yield.

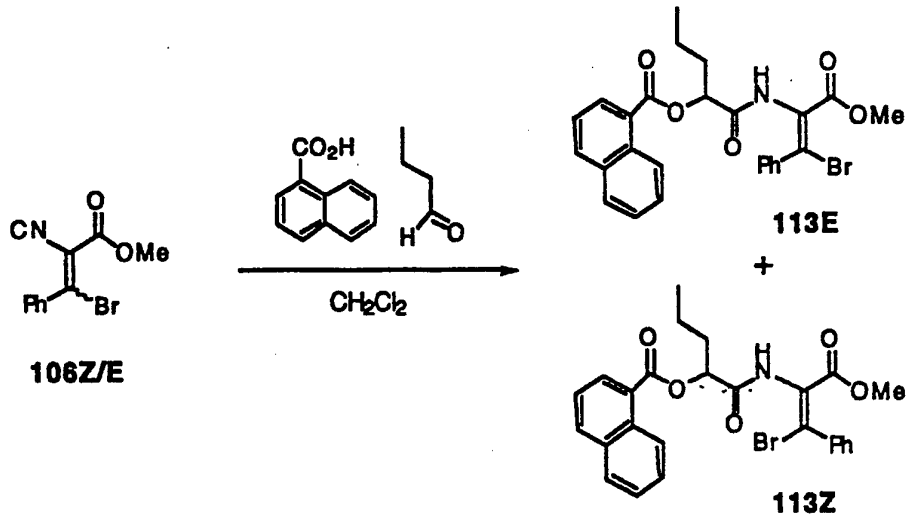
Major **111Z** and **111E**: IR (film) cm^{-1}: 3334, 2950, 1732, 1714, 1480, 1237, 1193, 1130, 783; ^1H NMR (360 MHz, $CDCl_3$), δ 9.00-8.85 (m, 2H, Ar**H**), 8.38 (m, 1H, Ar**H**), 8.37-8.05 (m, 3H, Ar**H**), 7.91 (s, 1H, **NH**), 7.89 (s, 1H, **NH**), 7.7-7.3 (m, 8H, Ar**H**), 7.2-7.0 (m, 2H, Ar**H**), 5.40 (s, 1H, O**CH**, Z-isomer), 5.24 (s, 1H, O**CH**, E-isomer), 3.92 (s, 3H, CO_2CH_3, E-isomer), 3.54 (s, 3H, CO_2CH_3, Z-isomer), 3.13 (d, J=4.4 Hz, 1H, CCH_2, Z-isomer), 2.92 (d, J=4.3 Hz, 1H, CCH_2, E-isomer), 2.87 (d, J=4.4 Hz, 1H, OCH_2, Z-isomer), 2.71 (d, J=4.4 Hz, 1H, OCH_2, E-isomer), 1.63 (s, 3H, CCH_3, Z-isomer), 1.63 (s, 3H, CCH_3, E-isomer); ^{13}C NMR (90 MHz, $CDCl_3$) δ 165.30, 164.95, 164.17, 163.83, 162.82, 136.89, 135.99, 134.72, 134.39,133.76, 131.46, 130.94, 130.72, 129.60, 129.52, 128.81, 128.61, 128.48, 128.40, 128.26, 127.79, 126.89, 126.59, 126.49, 126.45, 125.62, 125.49, 125.24, 124.45, 124.37, 120.13, 75.41, 75.21, 56.05, 55.76, 53.50, 52.76, 52.58, 17.86, 17.56, 17.37; HRMS (FAB) $[MH]^+$ calcd for $C_{26}H_{22}NO_6Br$ 524.0709, found 524.0709.



Pyridine (35 μL, 0.445 mmol), butyraldehyde (330 μL, 3.71 mmol), and 1-naphthoic acid (64 mg, 0.371 mmol) were added sequentially to a r.t. CH_2Cl_2 (3 mL) solution of isocyanide **108** (1:1.4 Z/E, 99.0 mg, 0.371 mmol). The mixture was stirred under N_2 for 22 hours, after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording two fractions: **112Z** (61 mg, de=1:1, 34%) and **112E** (61 mg, de=1:1, 34%), for a 68% overall yield.

112Z: 1R (film) cm^{-1}: 3433.8, 3059, 2962, 1705, 1667, 1512, 1312, 1196, 1138; ^1H NMR (360 MHz, $CDCl_3$), δ 9.01 (d, J=8.7 Hz, 1H, Ar**H**), 8.97 (d, J=8.5 Hz, 1H, Ar**H**), 8.34 (dt, J=1.2, 7.5 Hz, 2H, Ar**H**), 8.09 (d, J=8.2 Hz, 2H, Ar**H**), 7.92 (d, J=8.1 Hz, 2H, Ar**H**), 7.69-7.61 (m, 2H, Ar**H**), 7.60-7.53 (m, 4H, Ar**H**), 7.47 (bs, 1H, **NH**), 7.42 (bs, 1H, **NH**), 7.40-7.25 (m, 10H, Ar**H**), 5.76 (t, J=6.0 Hz, 1H, OC**H**CH_2), 5.72 (t, J=6.2 Hz, 1H, OC**H**CH_2), 3.48 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 2.20-2.11 (m, 7N, NCH_2, NC**H**CH_2, OCH_2CH_2), 2.03 (d, J=6.0 Hz, 1H, NCH_2), 1.95 (d, J=3.7 Hz, 1H, NCH_2), 1.85 (d, J=3.7 Hz, 1H, NCH_2), 1.71-1.60 (m, 4N, $CHCH_2H_2$), 1.09 (d, J=5.4 Hz, 6H, NHCH_3), 1.05 (t, J=7.8 Hz, 6H, CH_2CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.36, 169.28 (CO_2CH_3), 165.99, 165.99 (C), 164.36, 164.36 (C), 158.08, 157.51 (C), 136.06, 135.00 (C), 134.19, 134.09 (CH), 133.82, 133.81 (C), 131.43, 131.37 (C), 130.40, 130.34 (CH), 128.64, 128.64 (CH), 128.39, 128.33 (CH), 128.15, 8.10 (CH), 127.59, 127.59 (CH), 126.44, 126.44 (CH), 125.99, 125.81 (C), 125.49 (CH), 124.41 (CH), 111.50, 111.46 (C), 74.37, 74.10 (CH), 51.44, 51.44 (OCH_3), 36.89, 36.74 (CH_2N), 36.59, 36.59 (CHN), 34.11, 34.04 (CH_2), 18.35, 18.23 (CH_2), 18.03, 17.92 ($CHCH_3$, 13.82, 13.82 (CH_3CH_2); HRMS (DCI) $[MH]^+$ calcd for $C_{29}H_{30}N_2O_5$ 487.2233, found 487.2233.

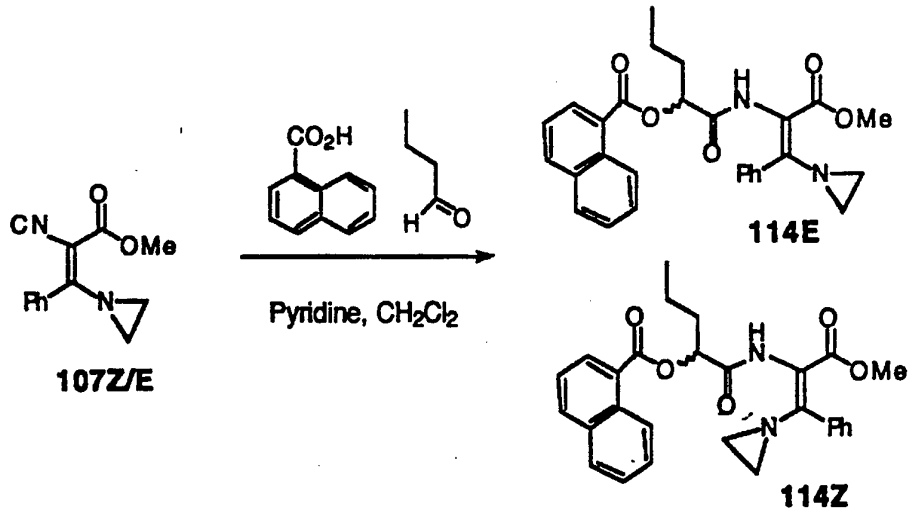
112E: 1R (film) cm^{-1}: 3420, 3065, 2962, 1703, 1510, 1290, 1137; ^1H NMR (360 MHz, $CDCl_3$), δ 8.79 (d, J=8.0 Hz, 1H ArH), 8.70 (d, J=8.7 Hz, 1H, ArH), 8.04-8.01 (m, 2H, ArH), 7.93-7.87 (m, 4H, ArH), 7.65-7.50 (m, 4H, ArH), 7.48 (m, 2H, ArH), 7.30-7.10 (m, 10H, ArH), 6.90 (bs, 2H, NH), 5.42 (t, J=6.1 Hz, 1H, $OCHCH_2$), 5.41 (t, J=6.6 Hz, 1H, $OCHCH_2$), 3.74 (s, 6H, OCH_3), 2.32 (m, 1H, $NCHCH_3$), 2.26 (m, 1H, $NHCH_3$), 2.22 (d, J=7.4 Hz, 2H, NCH_2), 2.16 (d, J=1.8 Hz, 2H, NCH_2), 1.87 (m, 4H, $CH_2CH_2CH_3$), 1.40 (m, 4H, $CH_2CH_2CH_3$), 1.24 (d, J=5.7 Hz, 3H, $NCHCH_3$), 1.21 (d, J=5.7 Hz, 3H, $NCHCH_3$), 0.92 (t, J=7.4 Hz, 6H, CH_2CH_3); ^{13}C NMR (90 MHz:, $CDCl_3$) δ 170.07, 169.96, 165.56, 165.54, 164.25, 164.25, 163.27, 162.99, 137.02, 137.02, 133.82, 133.76, 133.72, 133.69, 131.31, 131.24, 130.04, 129.92, 128.56, 128.51, 128.16, 128.14, 127.95, 127.93, 127.12, 127.09, 126.35, 126.35, 126.02, 125.97, 125.59, 125.55, 124.31, 124.29, 108.08, 108.02, 74.21, 74.16, 51.61, 51.61, 39.72, 39.72, 39.32, 39.29, 39.04, 39.04, 33.67, 33.67, 18.06, 18.02, 13.74, 13.74; HRMS (DCI) $[MH]^+$ calcd for $C_{29}H_{30}N_2O_5$ 487.2233, found 487.2233.



Butyraldehyde (3.65 μL, 4.04 mmol) and 1-naphthoic acid (80.0 mg, 0.45 mmol) were added sequentially to a r.t. CH_2Cl_2 (3 mL) solution of isocyanide **106** (98.0 mg, 0.405 mmol, E/Z=1.4:1) and stirred under N_2 for 23 hours after which the solvent was removed under reduced pressure. The crude oil was purified by flash silica gel (deactivated with 5% Et_3H) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc). One fraction was isolated as a white powder containing an inseparable mixture of adducts **113** (140 mg, 69%, E/Z = 1.4:1).

113Z and **113E**: 1R (film) cm^{-1}: 3400, 3298, 3056, 2961, 2874, 1732, 1480, 1321, 1236, 1194, 1132, 783; ^1H NMR (360 MHz, $CDCl_3$), δ 113Z 9.01 (d, J=8.6 Hz, 1H, Ar**H**, Z-isomer), 8.36 (dd, J=1.2, 7.3 Hz, 1H, Ar**H**, Z-isomer), 8.10 (d, J=8.3 Hz, 1H, Ar**H**, Z-isomer), 7.91 (d, J=8.0 Hz, 2H, Ar**H**, Z-isomer), 7.7-7.5 (m, 3H, Ar**H**, Z-isomer), 7.4-7.2 (m, 3H, Ar**H**, Z-isomer), 7.32 (bs, 1H, **NH**, Z-isomer), 7.05-6.85 (m, 3H, Ar**H**), 5.69 (t, J=5.9 Hz, 1H, OCH_2CH_2, Z-isomer), 3.56 (s, 3H, CO_2CH_3, Z-isomer), 2.18-2.08 (m, 2H, OCH_2CH_2, Z-isomer), 2.01-1.92 (m, 2H, OCH_2CH_2, E-isomer), 1.68-1.56 (m, 2H, CH_2CH_3, Z-isomer), 1.03 (t, J=7.3 Hz, 3H, CH_2CH_3, Z-isomer), **113E** 8.87 (d, J=8.6 Hz, 1H, Ar**H**, E-isomer), 8.12 (d, J=8.3 Hz, 1H, Ar**H**, E-isomer), 8.05 (d, J=8.2 Hz, 1H, Ar**H**, E-isomer), 7.76 (dd, J=1.2, 7.2 Hz, 1H, Ar**H**, E-isomer), 7.7-7.5 (m, 3H, Ar**H**, E-isomer), 7.4-7.2 (m, 3H, Ar**H**, E-isomer), 7.32 (bs, 1H, **NH**, E-isomer), 5.50 (t, J=6.0 Hz, 1H, OCH_2CH_2, E-isomer), 3.94 (s, 3H, CO_2CH_3, E-isomer), 1.52-1.40 (m, 2H, CH_2CH_3, E-isomer), 0.954 (t, J=7.4 Hz, 3H, CH_2CH_3, E-isomer); ^{13}C NMR (90 MHz, $CDCl_3$) δ 168.10 (C), 167.18 (C), 165.58 (C), 165.06 (C), 163.92 (C), 162.89 (C), 136.71 (C), 135.77 (C), 134.43 (CH), 134.27 (CH), 133.85 (C), 133.75 (C), 131.46 (C), 131.41 (CH), 130.50 (CH), 130.15 (CH), 129.46 (CH), 129.42 (CH), 128.78 (CH), 128.74 (CH), 128.65 (CH), 128.59 (CH), 128.40 (CH), 128.40 (CH), 128.26 (CH), 128.23 (CH),

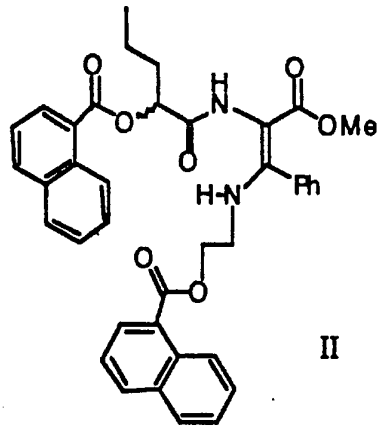
127.86 (C), 127.23 (C), 126.49 (CH), 126.49 (CH), 125.57 (CH), 125.44 (CH), 125.38 (C), 124.86 (C) 124.38 (CH), 124.27 (CH), 118.98 (C), 114.48 (C), 73.78 (CH), 73.41 (CH), 52.72 (CH3), 52.57 (CH_3), 33.78 (CH_2), 33.60 (CH_2), 18.06 (CH_2), 17.98 (CH_2), 13.76 (CH_3), 13.67 (CH_3); HRMS (FAB) $[MH]^+$ calcd for $C_{26}H_{24}O_5NBr$ 510.0916, found 510.0916.



Pyridine (35 μL, 0.441 mmol), butyraldehyde (330 μL, 3.67 mmol), and 1-naphthoic acid (63 mg, 0.367 mmol) were added sequentially to a r.t CH_2CL_2 (3 mL) solution of isocyanide **107** (90.0 mg, 0.367 mmol, Z/E=1:1.4). The mixture was stirred under N_2 for 12 hours after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording 2 fractions: **114Z** (54.5 mg, 31%) and **114E** (44.5 mg, 26%), for a 57% overall yield (Z/E=1.2:1).

114Z: IR (film) cm^{-1}: 3281, 3002, 2964, 1723, 1697, 1414, 1198, 1138; ^1H NMR (360 MHz, $CDCl_3$), δ 9.01 (d, J=8.3 Hz, 1H, Ar**H**), 8.34 (dd, J=1.2, 7.2 Hz, 1H Ar**H**), 8.10 (d, J=8.1 Hz, 1H, Ar**H**), 7.92 (d, J=7.7 Hz, 1H Ar**H**), 7.66 (dt, J=1.4, 5.8 Hz, 1H Ar**H**), 7.60-7.54 (m, 2H, Ar**H**), 7.43 (bs, 1H, **NH**), 7.35-7.33 (m, 3H, Ar**H**), 7.28-7.29 (m, 2H, Ar**H**), 5.69 (t, J=6.12 Hz, 1H, OC**H**CH_2), 3.47 (s, 3H, O$C\mathbf{H}_3$), 2.19-2.13 (m, 2H, OCHC$\mathbf{H}_2$), 2.04-1.97 (AB, 4H, $(C\mathbf{H}_2)_2N$), 1.7-1.6 (m, 2H, $C\mathbf{H}_2CH_3$), 1.05 (t, J=7.3 Hz, 3H, $CH_2C\mathbf{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.24 (C), 166.11 (C), 164.38 (C), 157.49, 136.06, 134.29, 133.91, 131.51, 130.56, 128.72, 129.49, 128.34 (CH), 128.25, 127.75 (CH), 126.50 (C), 125.78 (C), 125.55 (C), 124.50 (C), 112.01 (C), 74.46 ($OCHCH_2$), 51.56 (OCH_3), 34.06 ($CHCH_2$), 29.64 ($N(CH_2)_2$), 18.42 (CH_2CH_3), 13.85 (CH_2CH_3); HRMS (DCI) $[MH]^+$ calcd for $C_{28}H_{28}N_2O_5$ 473.2077, found 473.2070.

114E: IR (film) cm^{-1}: 3291, 2960, 2874, 1713, 1683, 1505, 1279, 1246, 1195, 1135, 784; ^1H NMR (360 MHz, $CDCl_3$), δ 8.75 (d, J=8.4 Hz, 1H, ArH), 8.03 (d, J=8.1 Hz, 1H, Ar**H**), 7.92 (dd, J=1.2, 7.4 Hz, 1H, Ar**H**), 7.89 (dd, J=1.4, 7.8 Hz, 1H, Ar**H**), 7.59 (AB, J=1.6, 6.9 Hz, 1H, Ar**H**), 7.55 (AB, J=1.3, 6.9 Hz, 1H, Ar**H**), 7.44 (dd, J=7.3, 8.2, 1H, Ar**H**), 7.27-7.18 (m, 4H, Ar**H**), 7.17-7.10 (m, 1H, Ar**H**), 6.92 (bs, 1H, **NH**), 5.41 (t, J=6.2 Hz, 1H, OC**H**CH_2), 3.75 (s, 3H, O$C\mathbf{H}_3$), 2.24-2.21 (m, 4H, $(C\mathbf{H}_2)_2N$), 1.90 (m, 2H, OCHC$\mathbf{H}_2$), 1.42-1.35 (m, 2H, $C\mathbf{H}_2CH_3$), 0.917 (t, J=7.3 Hz, 3H, $CH_2C\mathbf{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.00, 165.56, 164.24, 163.28, 136.80, 133.84, 133.74, 131.31, 130.03, 128.68, 128.53, 128.26, 127.99, 127.06, 126.39, 125.98, 125.60, 124.32, 108.71, 74.19, 51.67, 33.67, 32.33 ($(CH_2)_2N$), 18.06, 13.75; HRMS (DCI) $[MH]^+$ calcd for $C_{28}H_{28}N_2O_5$ 473.2077, found 473.2076.



II

The nucleophilic addition of 1-naphthoic acid to the vinyl aziridine isocyanides is a common side reaction observed when excess 1-naphthoic acid or no pyridine was added to these Passerini reactions.

II : 1R (film) cm^{-1}: 3281, 3057, 2959, 1717, 1663, 1591, 1515, 1280, 1241, 1138; ^1H NMR (360 MHz, $CDCl_3$), δ 9.31 (t, J=5.8 Hz, 1H, $NHCH_2$), 8.85 (d, J=9.0 Hz, 1H, Ar**H**), 8.77 (d, J=8.3 Hz, 1H, Ar**H**), 8.25 (d, J=8.4 Hz, 1H, Ar**H**), 8.04 (t, J=8.0 Hz, 2H, Ar**H**), 7.98 (d, J=7.3 Hz, 1H, Ar**H**), 7.89 (t, J=9.1 Hz, 2H, Ar**H**), 7.63-7.46 (m, 6H, Ar**H**), 7.34-7.22 (m, 5H, Ar**H**), 6.62 (bs, 1H, **NH**CO), 5.29 (t, J=6.2 Hz, 1H, **CH**O), 4.33 (t, J=5.4 Hz, 2H, CH_2O), 3.66 (s, 3H, OCH_3), 3.34 (q, J=5.8 Hz, 2H, CH_2NH), 1.7-1.6 (m, 2H, CH_2CH), 1.3-1.2 (m, 2H, CH_2CH_3), 0.84 (t, J=7.2 Hz, 3H, CH_3CH_2); LRMS (CI^+) $[MH]^+$ calcd for $C_{39}H_{36}N_2O_7$ 645.25, found 645.0.

EXAMPLE 4 Structural Activity Profile of the *In vitro* Cytotoxicities Of The Carzinophilin and Azinomycin Analogs Of Example 3

A structural activity relationship (SAR) of the *in vitro* cytotoxicities of the Carzinophilin and Azinomycin analogs of Example 3 is provided in Table 3 below.

Table 3 ***In Vitro*** **Cytotoxicities of Azinomycin Analogs in HCT116 Human Colon Carcinoma Cell Lines**

	IC_{50} (μM)		
Cmpd No.	HCT116	HCT116/VM46	HCT116/VP35
109E	4.39	5.56	5.27
109Z	5.4	1.6	2.6
110E	12.4	13.2	11.0
110Z	6.76	7.7	6.4
111E/111Z	>30	>30	>30
112E	>30	>30	>30
112Z	25.3	27.2	25.5
113E/113Z	>30	>30	>30
114E	>30	>30	>30
114Z	28.6	38.4	27.3
azinomycin B	0.838	—	—

[1]Cytotoxicity assessed by XTT assay after 72 hours continuous drug exposure.

Each analog was incubated for 72 hours with the human carcinoma cell line HCT116 and two drug resistant sublines HCT116/VM46 and HCT116/VP35. HCT116/VM46 was selected for resistance to VM-26 and expresses the multidrug resistant (MDR) phenotype. HCT116/VP35 was selected for resistance to VP-16 and is resistant to topoisomerase II active drugs. The results of the

Analogs **109E**, **109Z**, **110E** and **110Z** which all contain vinyl-aziridine, epoxide and naphthoate moieties displayed IC_{50}'s well within an order of magnitude of the natural product, Azinomycin B. Interestingly, the racemic 2-(methyl)-aziridine analogs **110E** and **110Z** behaved nearly identically in the *in vitro* assays to the correspondingly unsubstituted aziridine analogs **109E** and **109Z**.

Analogs lacking the vinyl-aziridine moiety gave no measurable cytotoxicity (IC_{50}<30μM) in all cases studied. Substitution of the epoxide fragment for the n-butyl group in analogs **114E**, **114Z**, **115E** and **115Z** displayed significantly less activity when compared to the most active compounds which contain the epoxide moiety.

In addition, these compounds did not show decreased potency when tested with Multiple Drug Resistance (MDR) and topoisomerase II resistant cell lines. This suggests that these compounds are not substrates for the P-glycoprotein efflux pump nor do they interact with topoisomerase II.

EXAMPLE 5 Synthesis of Peptidomimetic Polymer MCCA Array Using The Ugi Reaction

A combinatorial array of peptidomimetic polymers having a non-hydrolyzable ethylenediamine core structure can be prepared using the Ugi reaction. As depicted in Figure 7, the Ugi reaction is a four component condensation involving a first amino acid, a

second amino acid having a Boc-protected alpha amino group, methylisocyanide and acetic acid. The Ugi reaction is conducted at room temperature in aqueous solvents. As is illustrated in Figure 7, once the protecting groups have been removed, the Ugi reaction provides nonhydrolizable peptide analogues that mimic the structure of natural peptides.

EXAMPLE 6 Synthesis Of A Phosphotyrosine Peptide Structural Analog MCCA Array Using The Ugi Reaction.

One application for an Ugi MCCA array synthesis is the preparation of phosphotyrosine peptide structural analogues for use as structure-based competitive inhibitors. As depicted in Figure 8, phosphotyrosine peptides have a peptide backbone core structure. Structural variants of phosphotyrosine can be synthesized by varying the first and second amino acids employed (aa1 and aa2) as well as the other components of the Ugi reaction.

Several different combinatorial arrays of phosphotyrosine peptide analogues can be produced using the Ugi reaction. For example, as depicted in Figure 9a, pseudosubstrate peptide inhibitors can be prepared. The amino acid side chains of the tyrosine peptide analogues are determined by which R_1 and R_2 groups are used for the isocyanide and the acid. Branched chain tyrosine-based peptide analogues, not otherwise accessible using standard solid phase synthesis, can be prepared when R_3 on the amine is an amino acid or derivative thereof.

As depicted in Figure 9b, low molecular weight analogues of tyrosine that possess an intact tyrosine structural unit can be prepared wherein the R groups of the isocyanides, acids and amines employed are alkyl, aryl or acyl groups.

As depicted in Figure 9c, low molecular weight derivatives of tyrosine where the tyrosine structural unit is also modified can be prepared by using different aldehydes.

As depicted in Figure 9d, low molecular weight tyrosine derivatives can be prepared where the acid and amine are the same molecule. Further variations of these tyrosine-based peptide analogues can also be made according to the present invention by using different reaction components.

EXAMPLE 7 Synthesis of Solid Support And Linker System For Use In Solid Phase Synthesis Of MCCA Array.

A proposed synthesis of a novel support and linker system is depicted in Figure 10. The linker can be synthesized by condensing ethylene oxide with the lithium enolate of succinic anhydride. The resulting alcohol can then be converted to its mesylate and displaced with sodium azide to provide the azide functionality. This anhydride is then heated in the presence of the polymer in dimethylformamide to afford a free carboxylate linker.

EXAMPLE 8 Synthesis of Passerini-type MCCA Array Using Solid Phase Synthesis

A solid phase MCCA array synthesis utilizing the Passerini reaction can be conducted using the linker described in Example 7. The carboxylic acid linker-resin system formed in Example 7 can be treated with dicyclohexylcarbodiimide and hydroxybenzotriazole in the presence of the methyl ester of alanine. The progress of the reaction can be monitored by nuclear magnetic resonance based on the disappearance of free alanine. Filtration of reactants and subsequent washing with fresh DMF should provide a purified resin-linker-amino

acid system. Hydrolysis of the ester of alanine under base conditions should then provide the free carboxylic acid. Again, washing the resin with water provides a resin-linker-alanine carboxylic acid free of impurities or reactant contamination. Addition of cinnamaldehyde and 2,2-diethylphosphonomethylisocyanide to the resin reaction chamber stirring in an ethyl acetate solution should result in the three component Passerini condensation reaction. These reactants can be removed after the reaction by washing. Addition of triphenyl phosphine to a DMF solution of resin followed by washing with solvent should provide an amine side-chain on the linker. Heating this material should then cause an intramolecular reaction wherein the linker forms a lactam and releases the amine into solution.

EXAMPLE 9 Synthesis Of Peptidomimetics Using Solid Phase Synthesis

The use of the Ugi reaction as a means for synthesizing peptide mimics is described in Example 5 and 6 and as depicted in Figure 11. By coupling the first amino acid (aa_1) to a solid phase support as described in Example 8, the solid state synthesis of these peptide mimics can be performed. Reaction of the linked amino acid with an isocyanide, an aldehyde and an amine should yield a solid phase linked peptidomimetic analog. Removal of the tBoc blocking group under Ugi reaction conditions set forth in Figure 8 should afford a product containing two amino acid components. This process can be repeated over several cycles to provide 9^n products where n is the number of cycles. Thus, this application of the Ugi reaction provides numerous analogues despite the use of a very small number of different components.

EXAMPLE 10 Isolation Of Products From Ugi-type MCCA Array Synthesis By Sequestering The Unreacted Components

The reaction products of an Ugi-type reaction can be efficiently recovered from the reaction media by sequestering the unreacted components thereby yielding a substantially pure product.

In an Ugi-type reaction, the unreacted components consist of an aldehyde, an amine, an acid and an isocyanide. A mixed bed resin containing strongly acidic and basic resins can be used to remove the amine and acid respectively. In order to isolate the product of an Ugi reaction, the solvent is first removed by evaporation at reduced pressure. Distilled water is then added to the reaction mixture. To the resulting aqueous solution is added a mixed bed resin containing Biorad AG 501-X8/Bio-Rex MSZ 501, a strongly basic anion exchange and strongly acidic cation exchange resin. Once the mixed bed resin has been added, the solution is stirred for 10 min. The mixture is then filtered and concentrated to yield a substantially pure Ugi-type reaction product.

The use of exchange resins to sequester unreacted acids or amines can be extended to other unreacted components having different functional groups, such as esters, where the unreacted component is selectively converted to either an acid or amine after the reaction, thereby enabling its removal by the resin.

Metal sequestering agents can also be used where organometallic solution chemistry is employed in the MCCA reaction. For example, the addition of an EDTA molecule tethered to a polymer can be used to sequester palladium salts from the reaction mixture. Strongly acidic cation exchange resins can be used to remove boronic acids.

Example 11 Synthesis And Cleavage Of Passerini Reaction Products From Photocleavable Carbamate Linker

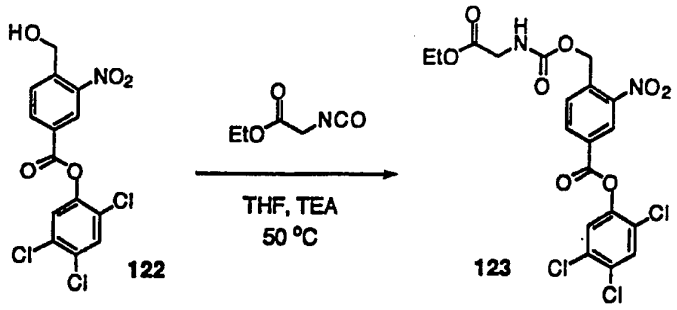
Synthesis of photocleavable carbamate linkers is depicted below. The photocleavable carbamate linkers **120** and **121** were constructed by reacting isocyanates **123** and **124** with the hydroxyl group from the linker alcohol described in Williams, P., et al., Tetrahedron (1991) 47:9867-9880.

The carbamate linkers **120** and **121** were then coupled to methylbenzhydrylamine (MBHA) Gly-resin **125** using HOBt in DMF to yield the polymer supported photocleavable carbamates **126** and **127**, respectively. Hydrolysis of the ester of carbamate **126** with LiOH, THF/H_2O yielded the corresponding polymer supported acid **128** in less than one hour. Surprisingly, the benzoate ester of carbamate **127** was resistant to hydrolysis under a variety of reaction conditions (LiOH, THF/H_2O, K_2CO_3, MeOH, NaOH, THF/H_2O and NaOH, MeOH).

Using the polymer supported acid **128**, a solid phase Passerini reaction was conducted. A CH_2Cl_2 solution containing methyl isocyanoacetate and butyraldehyde were added to the polymer supported acid **128** to yield the resin bound Passerini adduct **129**.

Initially, carbamate cleavage was attempted in methanol which yielded only the α-acyloxy hydrolysis Passerini product **131**. In order to avoid hydrolysis and capture the free amine product, photolysis at 350nm in a rayonet for 24 hours was conducted in CH_3CN in the presence of acetic anhydride (10 equiv.). Excess solvent acylating agent were then removed under vacuum to yield **134** as the only product. No other products were detectable from the crude product by ^1H NMR and TLC.

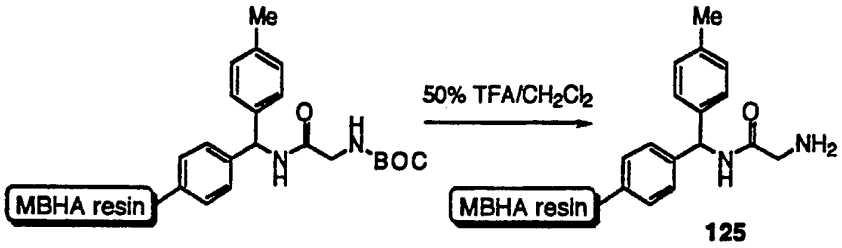
Experimental Data



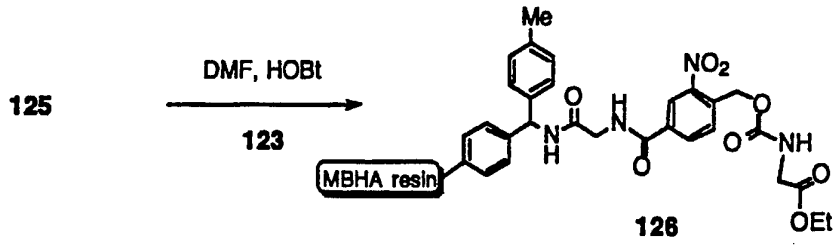
Ethyl-isocyanatoacetate (155 μL, 1.37 mmol) was added to a THF (5 mL) solution of linker **122** (450 mg, 1.14 mmol). Triethylamine was then added and the mixture allowed to stir at 25°C overnight. TLC showed that the reaction was slow, so the reaction was heated to 50°C. TLC showed that the reaction was complete in 2 days. Upon cooling the flask to -20°C, a precipitate formed and was subsequently filtered off. ^{1}H NMR in CD_3CN showed that this compound was the isocyanate decomposition product, glycine ethyl ester. The filtrate solvent was then removed under reduced pressure and the crude oil was extracted 3X with CH_2Cl_2 over KH_2PO_4 buffer. The combined organic layers were dried over Na_2SO_4 and evaporation of the solvent under reduced pressure gave a yellow solid. Purification by flash silica gell chromotography (gradient eluted 100% hex to 1:1 hex/EtOAc) afforded carbamate **123** (373 mg, 64%) as a white solid (mp 131-133°C).

123: IR (film) cm^{-1}: 3323, 3097, 2985, 1750, 1699, 1538, 1461, 1351, 1248, 1206, 1084, 1060; ^{1}H NMR (360 MHz, $CDCl_3$), δ 8.88 (d, *J*=1.7 Hz, 1H, Ar**H**), 8.42 (dd, *J*=1.7, 8.2 Hz, 1H, Ar**H**), 7.82 (d, *J*=8.2 Hz, 1H, Ar**H**), 7.60 (s, 1H, Ar**H**), 7.44 (s, 1H, Ar**H**), 5.62 (s, 2H, OCH_2Ar), 5.61 (bt, *J*=5.3 Hz, 1H, $HNCH_2$), 4.22 (q, *J*=7.2 Hz, 2H, OCH_2CH_3), 3.99 (d, *J*=5.6 Hz, 2H, $HNCH_2$), 1.28 (t, *J*=7.3 Hz, 3H, CH_2CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.75,

161.44, 155.43, 147.07, 145.38, 139.34, 134.88, 131.63, 131.12, 131.08, 128.87, 128.65, 126.74, 126.00, 124.14, 63.28, 61.61, 42.75, 14.05; HRMS (CI^+ $[MH]^+$ calcd for $C_{19}N_{15}N_2O_8Cl_3$ 504.9972, found 504.9972.



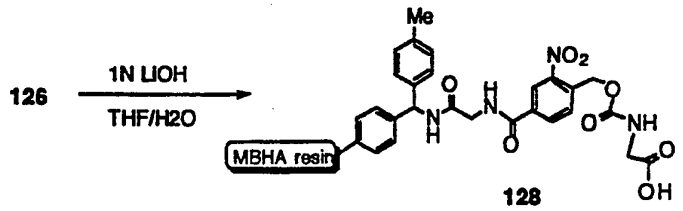
BOC-Gly-resin was deprotected with 33% TFA in CH_2Cl_2 for 30 min. and then washed exhaustively with CH_2Cl_2. The resin was then free baeed with 5% Hunig's base in CH_2Cl_2 and the solution filtered after 2 minutes. The resin was then washed exhaustively with sequential applications of CH_2Cl_2/MeOH/CH_2Cl_2 until the resin appeared colorless. The resin was dried *in vacuo* overnight. The entire procedure was repeated again with 50% TFA in methylene chloride (20 mL) affording Gly-MBHA-resin **125** (1.39 g) as colorless beads. The qualitative ninhydrin test (Kaiser Test) was positive (dark blue).



Gly-carbamate **123** (1.2 g, 2.37 mmol) in 2 mL DMF was added to a 5 mL DMF suspension of MBHA-resin **125**

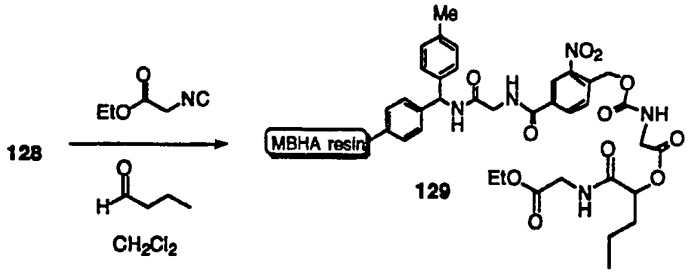
(1.34 g, 1.12 equiv./gram, obtained from Novabioehem.) HOBt (470 mg, 3.4 mmol) was then added and the mixture allowed to stir at 25°C for 36 hours. The resin was washed exhaustively in sequential applications of CH_2Cl_2/MeOH/CH_2Cl_2. After two iterations of this process, resin **126** (1.83 g) was afforded as pale yellow beads. The qualitative ninhydrin test (Kaiser Test) was negative.

Capping of residual free amines on the dried resin **126** (1.7 g, ≈1.7 mmol) was performed by suspending the resin in a pyridine (10 mL) solution of Ac_2O (1.7 mL, 16.9 mmol) and a catalytic amount of DMAP (25 mg, 0.2 mmol). The solution was stirred at 25°C for 1 hr. and then filtered and washed exhaustively with sequential applications of CH_2Cl_2/MeOH/CH_2Cl_2. The resin was dried *in vacuo* overnight affording resin **126** (1.7 g) as pale yellow beads. The qualitative ninhydrin test (Kaiser Test) was negative.

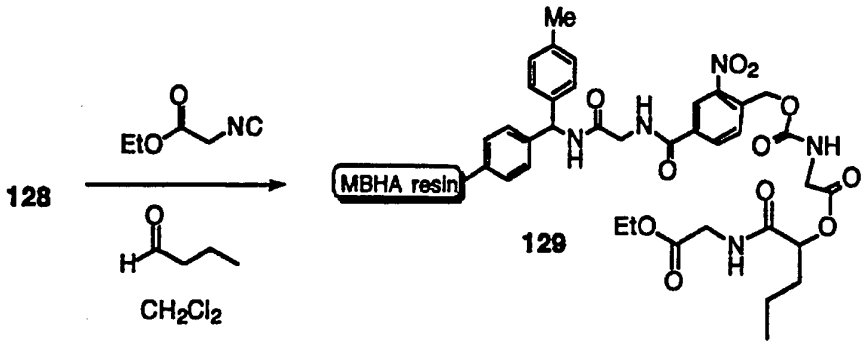


(Ethyl)-glycinate-MBHA-Gly-resin **126** (1.6 g, ≈ 1.5 mmol) was stirred in 45 mL THF with 1N LiOH (15 mL, 15.0 mmol) for 1 hr. The mixture was then acidified with 1N KH_2SO_4 (≈17 mL) to pH=2-3 and the resin was filtered. Exhaustive sequential washings with H_2O/THF/CH_2Cl_2 and subsequent drying *in vacuo* overnight afforded (glycinic acid)-Gly-MBHA resin **128** (1.46 g) as pale yellow beads. Photolysis of this resin in CH_3N and AC_2O (≈10 equiv.) did <u>not</u> give the unhydrolyzed N-acetyl-(ethyl)-glycinate

product by TLC (R_f=0.3, 100% EtOAc, yellow anisaldehyde stain).



Methyl isocyanoacetate (23 μL, 0.23 mmol) and butyraldehyde (23 μL, 0.23 mmol) were added to a CH_2Cl_2 (200 μL) suspension of (glycinic acid)-Gly-MBHA resin **128** (25 mg, ≈0.025 mmol). The mixture was allowed to stand at 25°C for 48 hours. The resin was then filtered, washed exhaustively with sequential applications of CH_2Cl_2/MeOH/CH_2Cl_2 and dried *in vacuo* overnight to afford (Mica-But-Gly)-Gly-MBHA resin **129** (≈27 mg) as yellow beads.



General procedure for the photolytic cleavage of *o*-nitrobenzyl carbamates and *in situ* acylation of Passerini adducts:

Acetic anhydride (10 equiv.) was added to a CH_3CN suspension of (Mica-But-Gly)-Gly-MBHA resin **128** (25

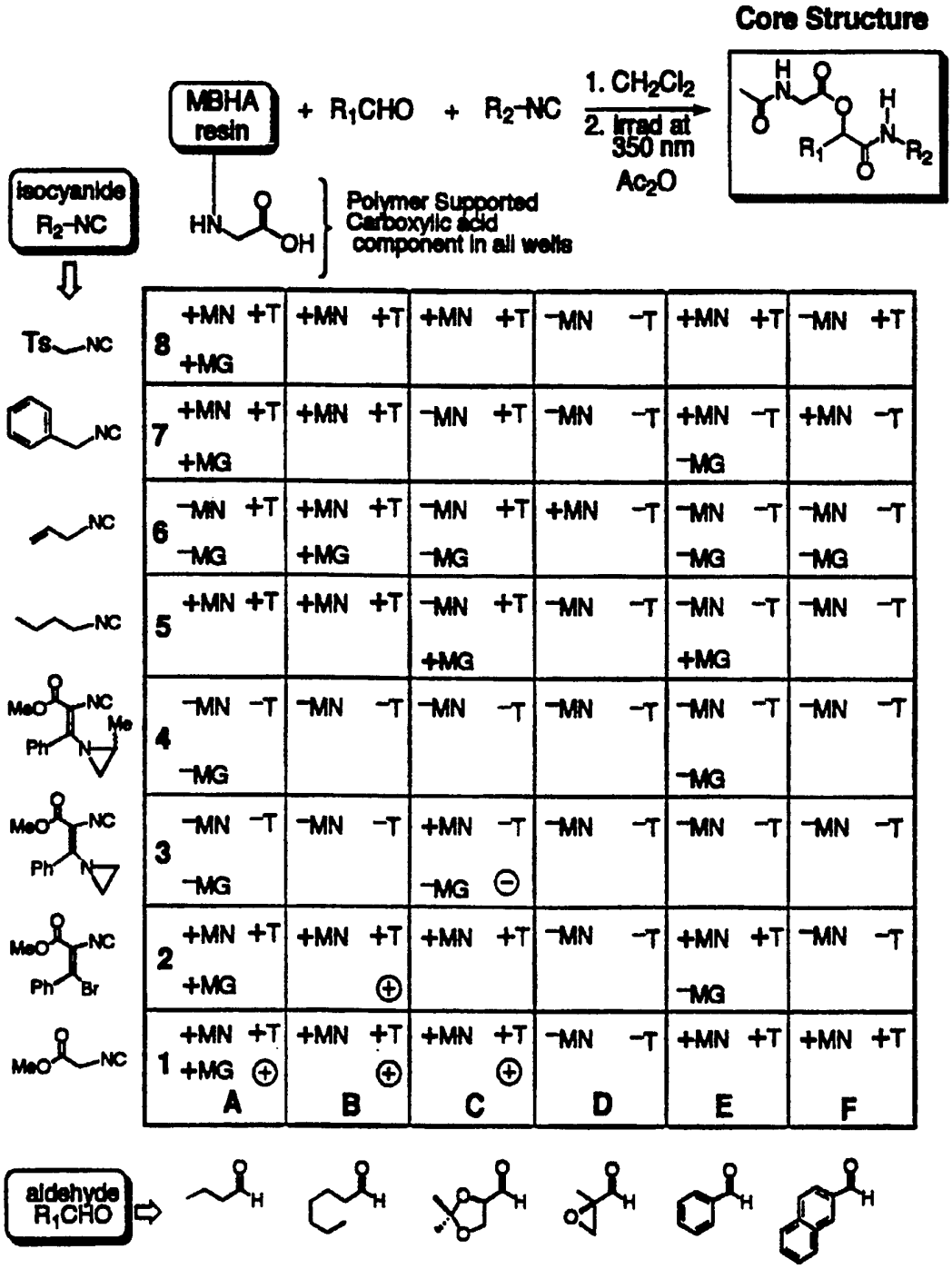
mg, ≈0.025 mmol) and irradiated at 350 nm in a Rayonet for 12-48 hours at 30°C. The solution was transferred into a round bottom flask by pipet with multiple washings (CH_3CN) of the resin. The solvent, excess acetic anhydride, and acetic acid was then efficiently removed in vacuo to afford Mica-But-(N-acetyl-Gly) adduct **129** (≈2-4 mg by crude ^1H NMR) as a colorless oil. ^1H NMR and TLC demonstrated this was the only compound present.

129: ^1H NMR (360 MHz, $CDCl_3$), δ 6.93 (bt, *J*=5 Hz, 1H, **NH**), 6.11 (bt, *J*=5 Hz, 1H, **NH**), 5.28 (t, *J*=6.0 Hz, 1H, O**CH**), 4.13 (dd, *J*=5.7, 18.2 Hz, 1H, $HNCH_2CO_2CH_3$), 4.08 (5, *J*=5.5 Hz, 2H, $HNCH_2CO_2CH_3$), 3.97 (dd, *J*=5.3, 18.1 Hz, 1H, $HNCH_2CO_2CH_3$), 3.76 (s, 3H, CO_2CH_3), 2.07 (s, 3H, $HNCOCH_3$), 1.89 (m, 2H, $OCHCH_2$), 1.41 (m, 2H, $OCHCH_2CH_2$), 0.93 (t, *J*=7.4 Hz, 3H, CH_2CH_3); LRMS (FAB) $[MH]^+$ calcd for $C_{12}H_{20}N_2O_6$ 289.14, found 289.5.

Example 12 Solid Phase Passerini MCCA Array Synthesis Using The Photocleavable Carbamate Linker Of Example 11

A solid phase MCCA array synthesis using the photocleavable carbamate linker of Example 11 was conducted and is depicted in Table 4. In this synthesis, 8 isocyanides, 6 aldehydes, 1 carboxylic acid and 1 acylating agent were employed. It should be understood that a variety of different carboxylic acids and acylating agents could have been used.

TLC and LRMS Data for Polymer Supported Passerini MCCAS



	A	B	C	D	E	F
8	+MN +T +MG	+MN +T	+MN +T	−MN −T	+MN +T	−MN +T
7	+MN +T +MG	+MN +T	−MN +T	−MN −T	+MN −T −MG	+MN −T
6	−MN +T −MG	+MN +T +MG	−MN +T −MG	+MN −T	−MN −T −MG	−MN −T −MG
5	+MN +T	+MN +T	−MN +T +MG	−MN −T	−MN −T +MG	−MN −T
4	−MN −T −MG	−MN −T	−MN −T	−MN −T	−MN −T −MG	−MN −T
3	−MN −T −MG	−MN −T	+MN −T −MG ⊖	−MN −T	−MN −T	−MN −T
2	+MN +T +MG	+MN +T ⊕	+MN +T	−MN −T	+MN +T −MG	−MN −T
1	+MN +T +MG ⊕	+MN +T ⊕	+MN +T ⊕	−MN −T	+MN +T	+MN +T

+T = TLC showed expected spot(s); −T = TLC did not show expected spot(s); +MN= LR Mass Spectra (NBA matrix) showed expected product; −MN= LR Mass Spectra (NBA matrix) did not showed expected product; +MG= LR Mass Spectra (Glycerol matrix) showed expected product; − MG= LR Mass Spectra (Glycerol matrix) did not showed expected product; ⊕=^{1}H NMR showed expected product; ⊖ =^{1}H NMR did not showed expected product.

All reactions were performed in Fisher shell vials arranged in two 4x6 microtiter trays set side by side to form the requisite 8x6 array. 25mg of resin was added to each vial and swelled with CH_2Cl_2. The aldehydes and carboxylic acids were then delivered by gas tight syringe to each of their respective rows and columns and allowed to react at room temperature for 2-4 days. Filtration of the individual reactions and exhaustive washings followed by photolysis at 350 nm in CH_3CN (10 equiv. Ac_2O) for 24h gave 48 reaction wells to be analyzed for product composition. The results of the array synthesis is depicted in Table 4. Low resolution mass spectroscopy combined with thin layer chromatography of each of the reaction wells showed that 50% of the desired products were formed and isolated. ^1H NMR of the reaction wells that gave positive TLC and mass spectroscopy data revealed that the desired products were the only solid phase products produced in these wells. This example thus demonstrates that large arrays of compounds can be rapidly and efficiently prepared in high purity using solid phase synthesis according to the present invention.

While the invention of this patent application is disclosed by reference to specific MCCA reactions, it is understood that the present invention can be applied all other multicomponent syntheses capable of being conducted in a single reaction vessel without the isolation of an intermediate product. Further, the present invention is intended to be applicable to all future developed MCCA reactions.

It is also understood that the embodiment of the present invention encompassing solid phase synthesis is intended to be practiced with the linkers and solid supports disclosed herein as well as with other linkers and solid supports presently known or developed in the future.

Accordingly, the invention may be embodied in other specific forms without departing from its spirit or essential characteristics. It is to be understood that this disclosure is intended in an illustrative rather than limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

What is claimed is:

1. An array of compounds having a common core structure wherein the compounds of the array comprise the products of a multiple component combinatorial array synthesis having at least three components wherein the components of the multiple component combinatorial array synthesis comprise

a first component, the first component comprising a first group of reactants having a same first functional group;

a second component, the second component comprising a second group of reactants having a same second functional group;

a third component, the third component comprising a third group of reactants, the third group of reactants having a same third functional group;

wherein the array synthesis is conducted under appropriate conditions such that the functional groups of the first, second and third components react with each other to form the array of compounds having a common core structure.

2. The combinatorial array of compounds of claim 1 wherein the first group of reactants comprises more than one reactant and the second group of reactants comprises more than one reactant.

3. The combinatorial array of compounds of claim 1 wherein the reactants of the first component comprise the compounds of another combinatorial array of compounds.

4. The combinatorial array of compounds of claim 1 wherein the reactants of at least one component comprise mixtures of more than one compound having the same functional group.

5. The combinatorial array of compounds of claim 1 wherein each compound is formed while bound to a solid support.

6. The combinatorial array of compounds of claim 5 wherein the first group of reactants comprises more than one reactant and the second group of reactants comprises more than one reactant.

7. A method of making a combinatorial array of compounds having a common core structure using an n component combinatorial array synthesis, wherein n corresponds to the number of reaction components, n being at least 3, wherein each component comprises a group of reactants having the same functional group;

the method comprising:

a) organizing a series of reaction vessels in an n dimensional array wherein each reaction vessel is identifiable by its coordinates in the n dimensional array, each axis in the n dimensional array corresponding to a different component, each position on the axis corresponding to a different reactant;

b) adding the reactants of the n components to the n dimensional array of reaction vessels such that the same reactant is added to all of the reaction vessels in the array having a position on the array corresponding to that reactant;

c) reacting the contents of each reaction vessel under appropriate conditions to form the compounds of the array.

8. The method of claim 7 comprising the further step of binding one of the components to a solid support.

9. The method of claim 7 wherein the reactants of at least one component comprise mixtures of more than one compound having the same functional group.

10. The method of claim 7 wherein the reactants of the first component comprise the compounds of another combinatorial array of compounds.

11. The method of claim 7 wherein at least two of the components comprise a group of more than one reactant.

12. The method of claim 11 comprising the further step of binding one of the components to a solid support within each reaction vessel of the array.

13. A method of creating a combinatorial array of compounds with a common core structure comprising:

a. identifying the desired core structure;

b. identifying a MCCA reaction capable of generating the core structure;

c. preparing an array of compounds using the identified MCCA reaction according to the method of claim 7.

14. A method of creating a combinatorial array of compounds with a common core structure comprising:

a. identifying the desired core structure;

b. identifying a MCCA reaction capable of generating the core structure;

c. preparing a combinatorial array of compounds using the identified MCCA reaction according to the method of claim 8.

15. A method of creating a combinatorial array of compounds with a common core structure comprising:

a. identifying the desired core structure;

b. identifying a MCCA reaction capable of generating the core structure;

c. preparing a combinatorial array of compounds using the identified MCCA reaction according to the method of claim 9.

16. A method for conducting in vitro binding studies on a biological material comprising:

a) adding the biological material to an array of compounds, each compound in the array having a common core structure and being bound to a solid support; and

b) measuring the binding of each compound in the array to the biological material.

17. A linker bound to a polymer useful for binding compounds during solid phase synthesis, the linker having the chemical structure

[POLYMER] - $HNCOCH_2CH_2CH(CH_2CH_2N_3)CO_2H$

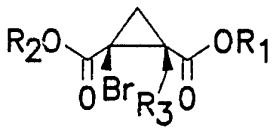


FIG. 1A

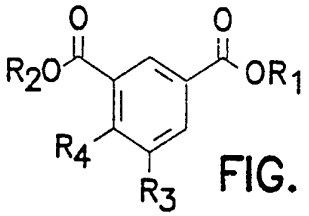


FIG. 1B

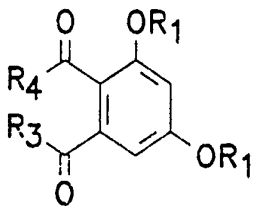


FIG. 1C

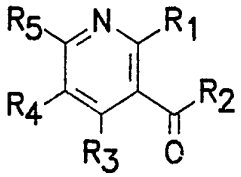


FIG. 1D

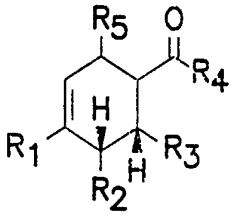


FIG. 1E

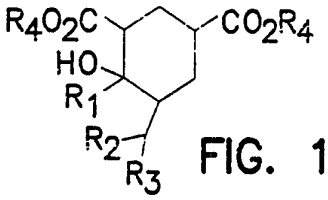


FIG. 1F

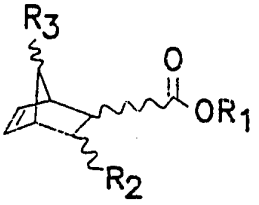


FIG. 1G

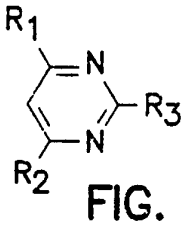


FIG. 1H

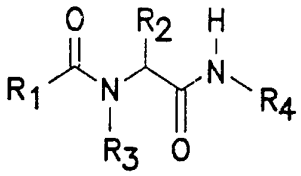


FIG. 1I

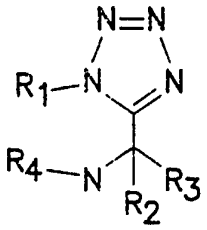


FIG. 1J

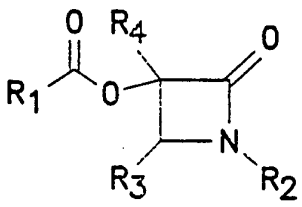


FIG. 1K

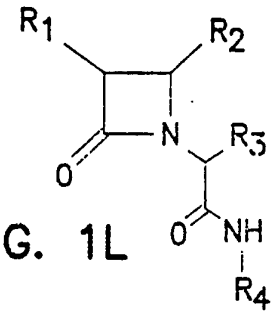


FIG. 1L

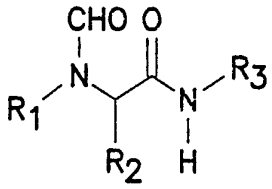


FIG. 1M

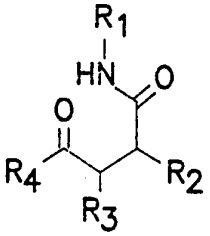


FIG. 1N

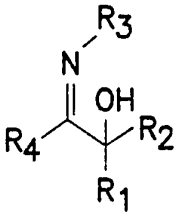


FIG. 1O

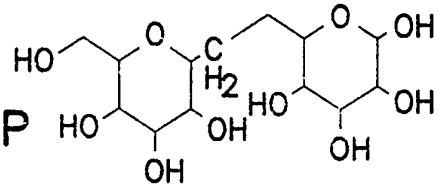


FIG. 1P

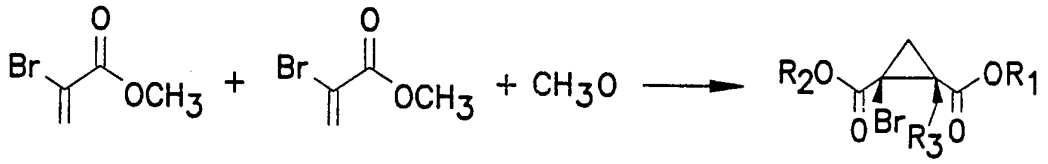


FIG. 2A

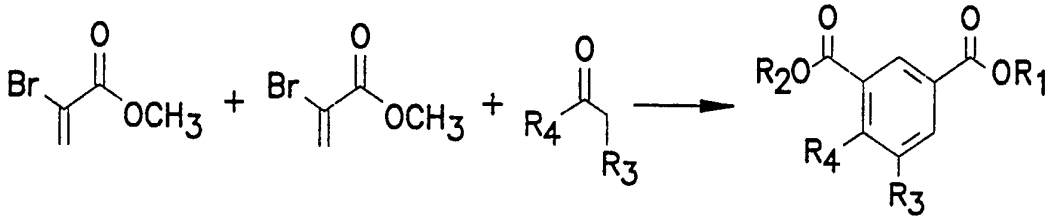


FIG. 2B

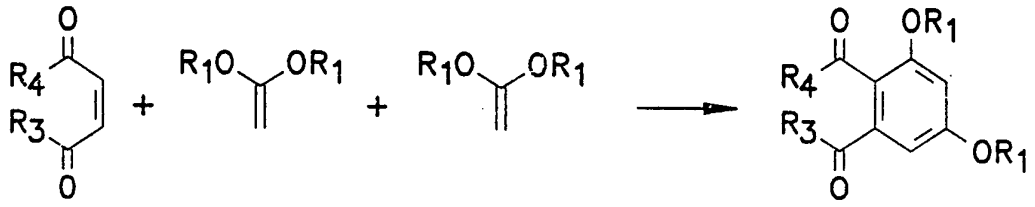


FIG. 2C

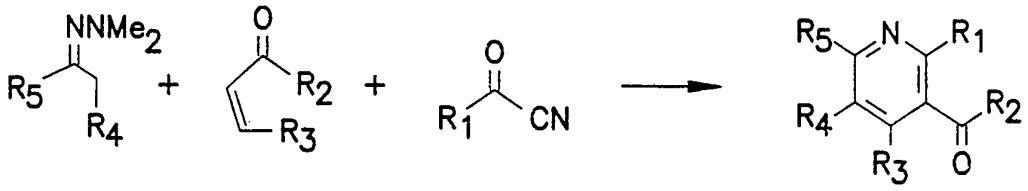


FIG. 2D

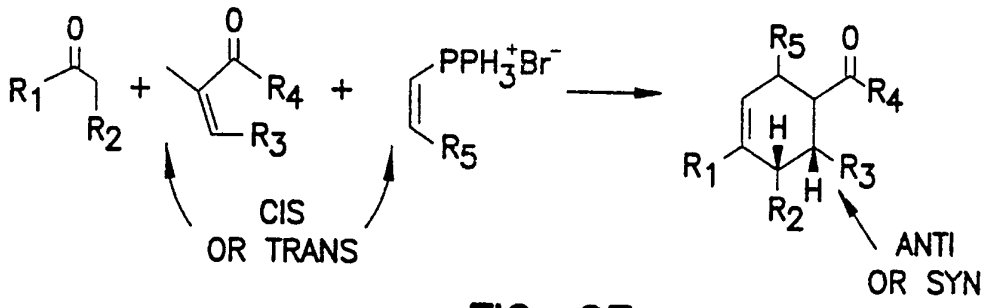


FIG. 2E

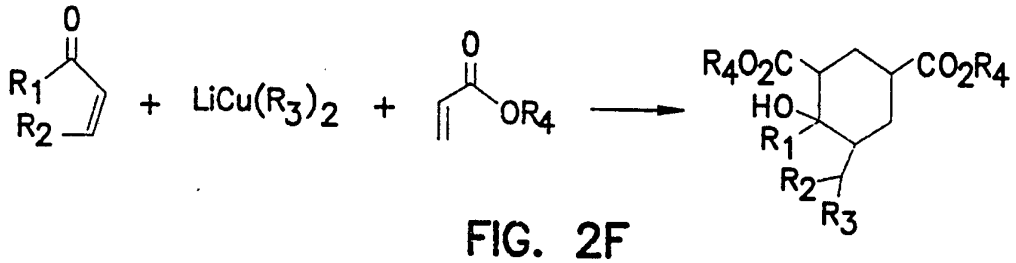


FIG. 2F

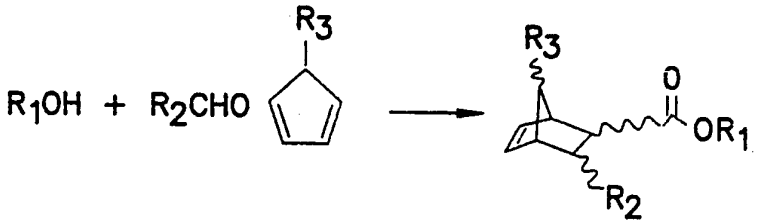


FIG. 2G

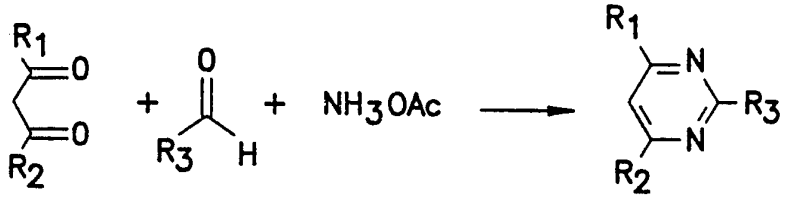


FIG. 2H

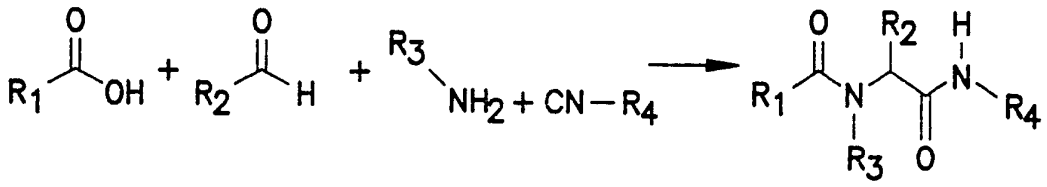


FIG. 2I

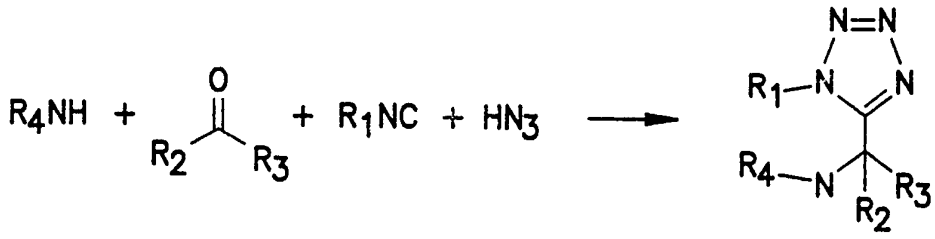


FIG. 2J

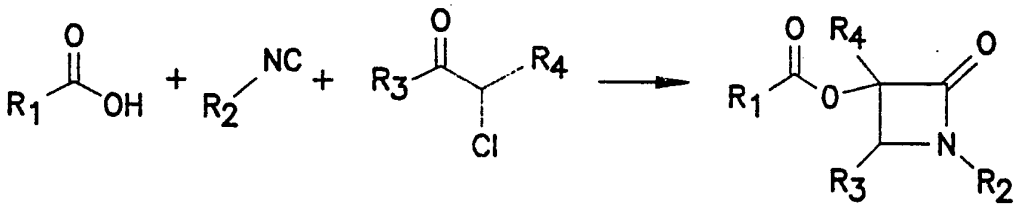


FIG. 2K

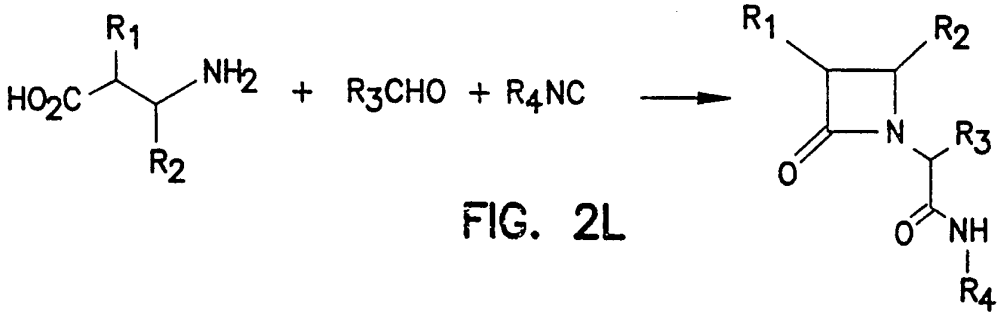


FIG. 2L

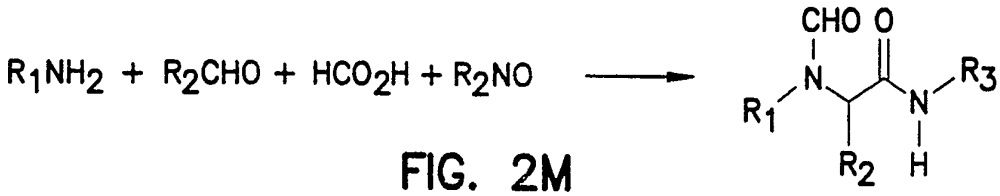


FIG. 2M

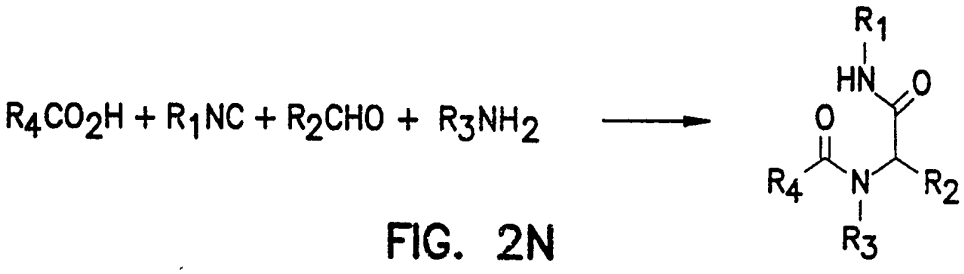


FIG. 2N

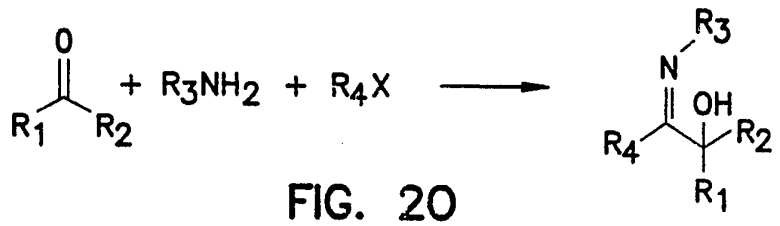


FIG. 2O

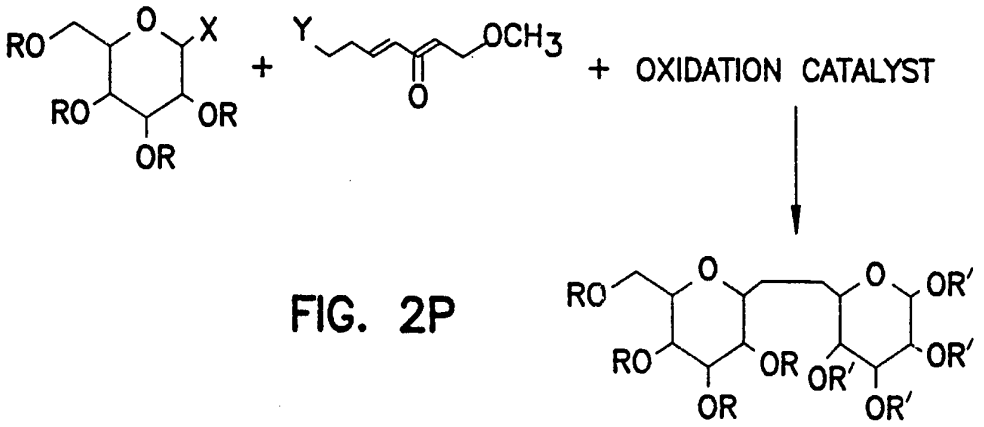
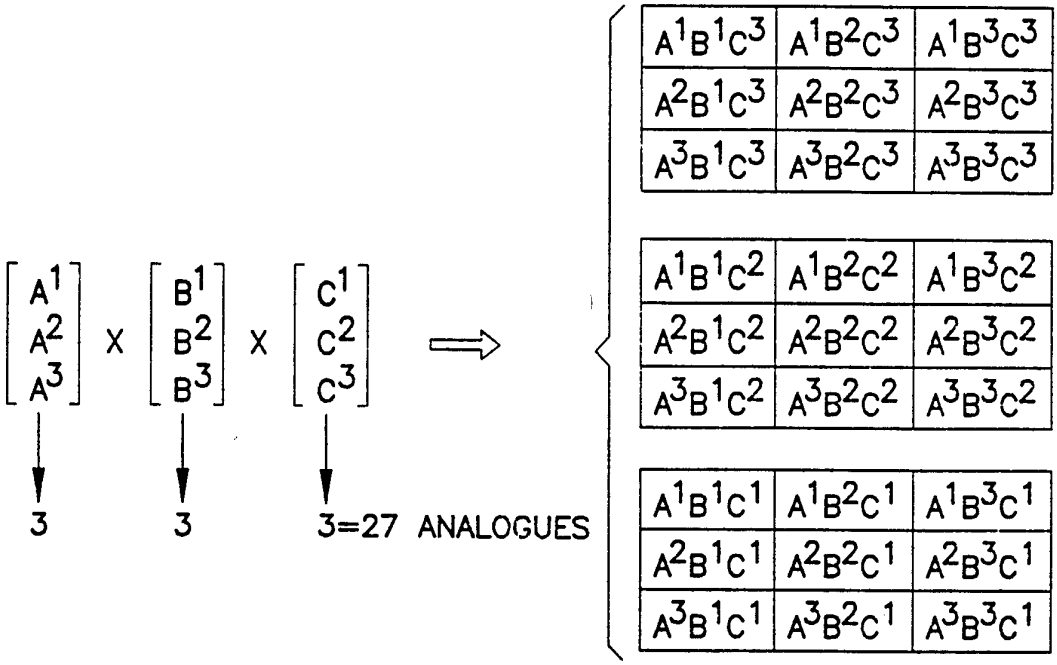


FIG. 2P



NUMBER OF REAGENTS PER COMPONENT A, B, C	NUMBER OF ANALOGUES PRODUCED
3, 3, 3	27
5, 5, 5	125
10, 10, 10	1000
20, 20, 20	8000
x, y, z	x*y*z

FIG. 3

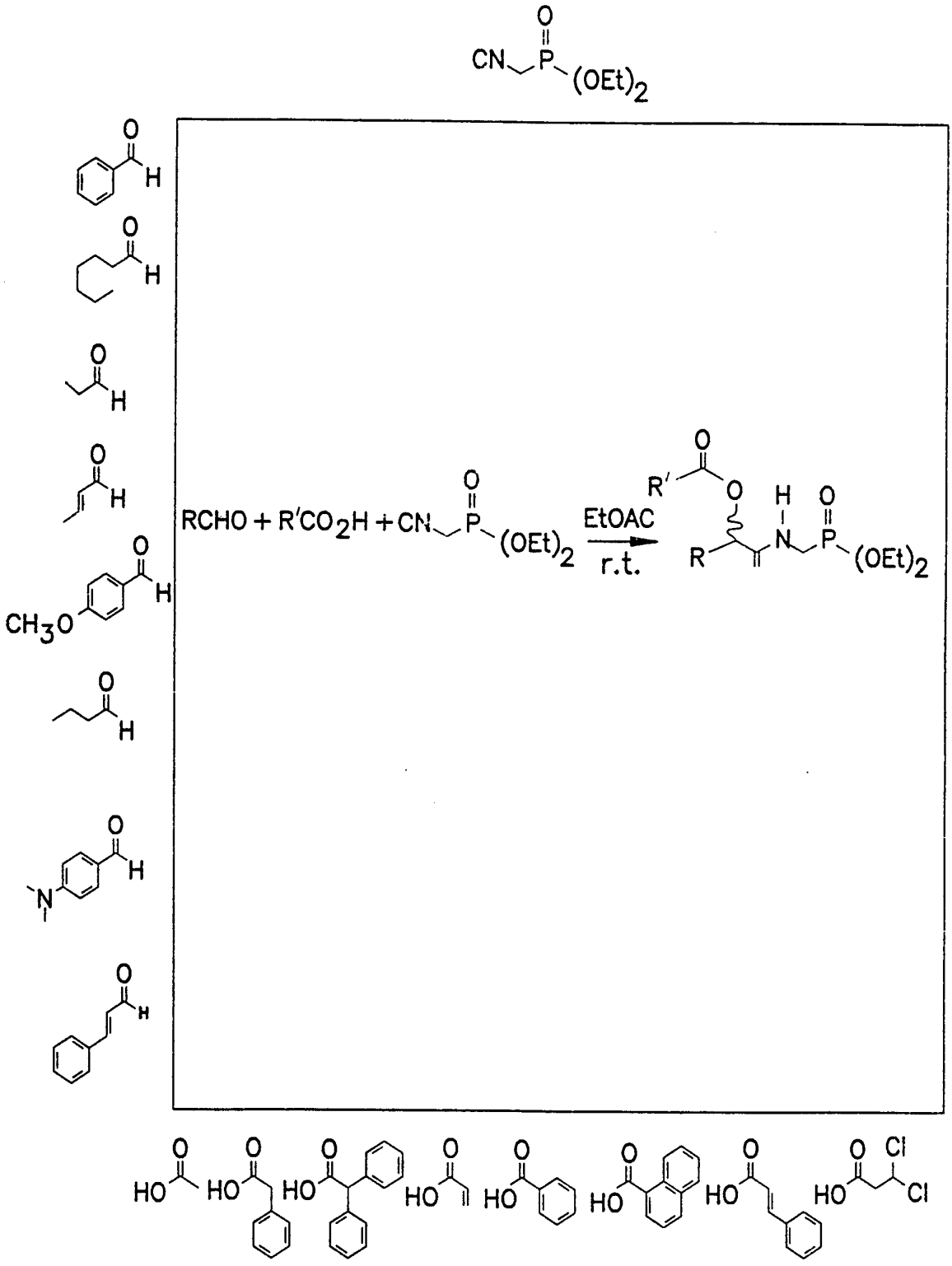


FIG. 4

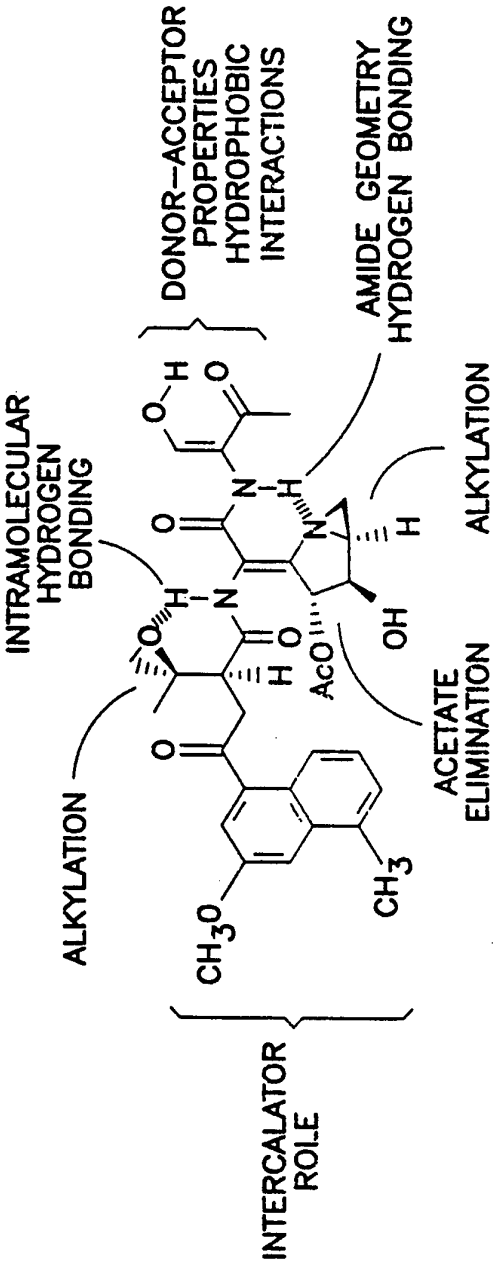

DONOR–ACCEPTOR PROPERTIES HYDROPHOBIC INTERACTIONS
AMIDE GEOMETRY HYDROGEN BONDING
INTRAMOLECULAR HYDROGEN BONDING
ALKYLATION
ALKYLATION
ACETATE ELIMINATION
INTERCALATOR ROLE

FIG. 5

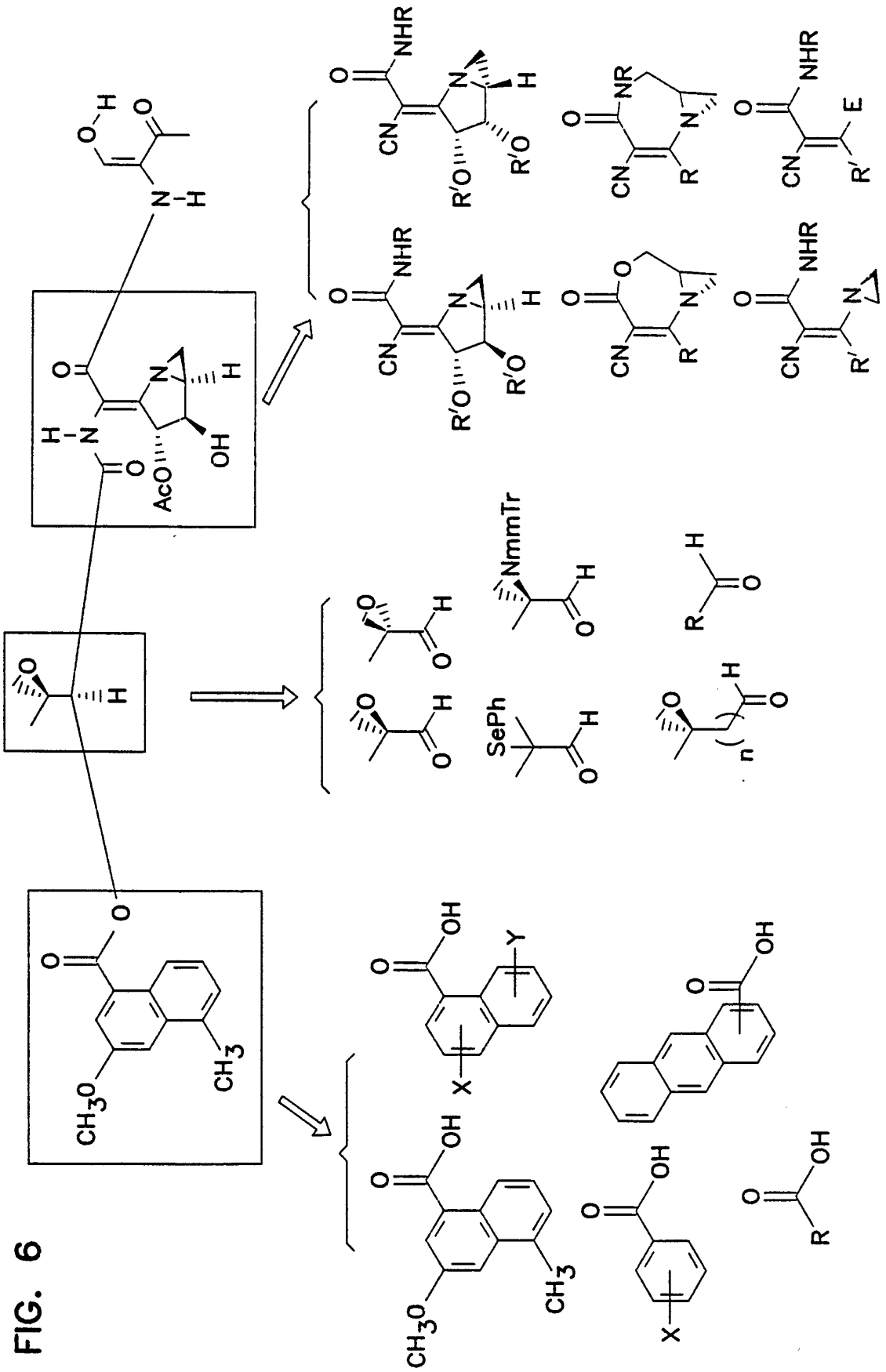


FIG. 6

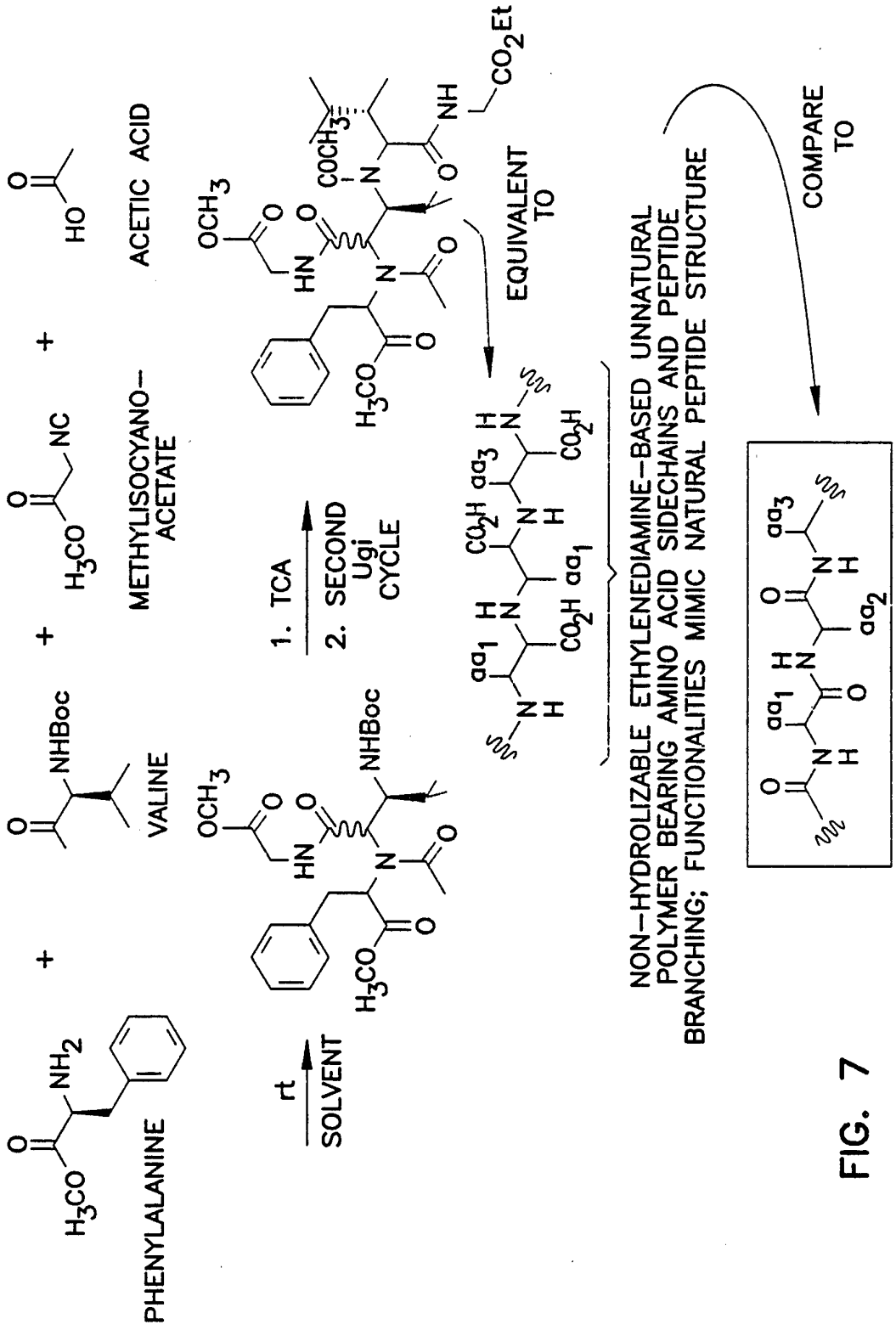

PHENYLALANINE
VALINE
METHYLISOCYANO-ACETATE
ACETIC ACID
rt
SOLVENT
1. TCA
2. SECOND Ugi CYCLE
EQUIVALENT TO
NON-HYDROLIZABLE ETHYLENEDIAMINE-BASED UNNATURAL POLYMER BEARING AMINO ACID SIDECHAINS AND PEPTIDE BRANCHING; FUNCTIONALITIES MIMIC NATURAL PEPTIDE STRUCTURE
COMPARE TO

FIG. 7

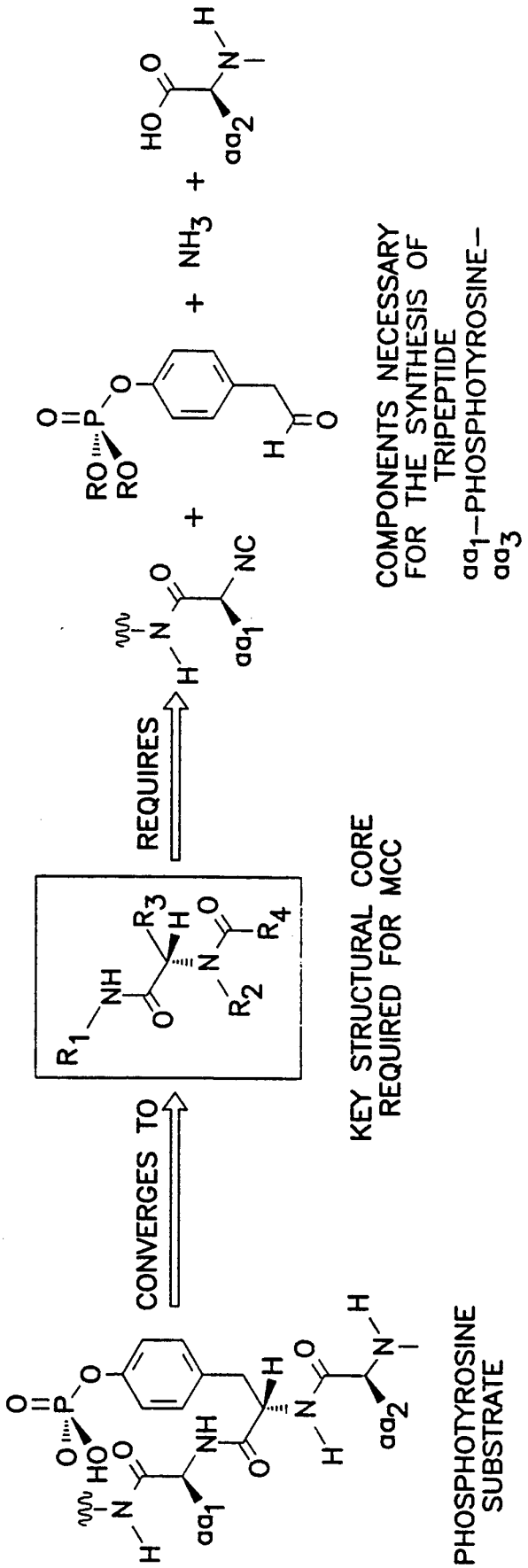

PHOSPHOTYROSINE SUBSTRATE
CONVERGES TO
KEY STRUCTURAL CORE REQUIRED FOR MCC
REQUIRES
COMPONENTS NECESSARY FOR THE SYNTHESIS OF TRIPEPTIDE aa1–PHOSPHOTYROSINE–aa3

FIG. 8

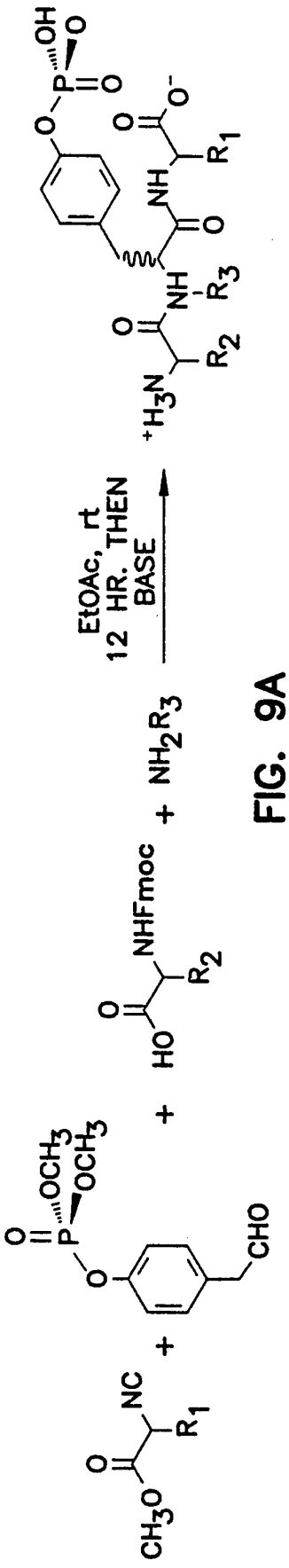

EtOAc, rt
12 HR. THEN
BASE
+ NH2R3
NHFmoc
R2
HO
+
OCH3
OCH3
CHO
NC
R1
CH3O

FIG. 9A

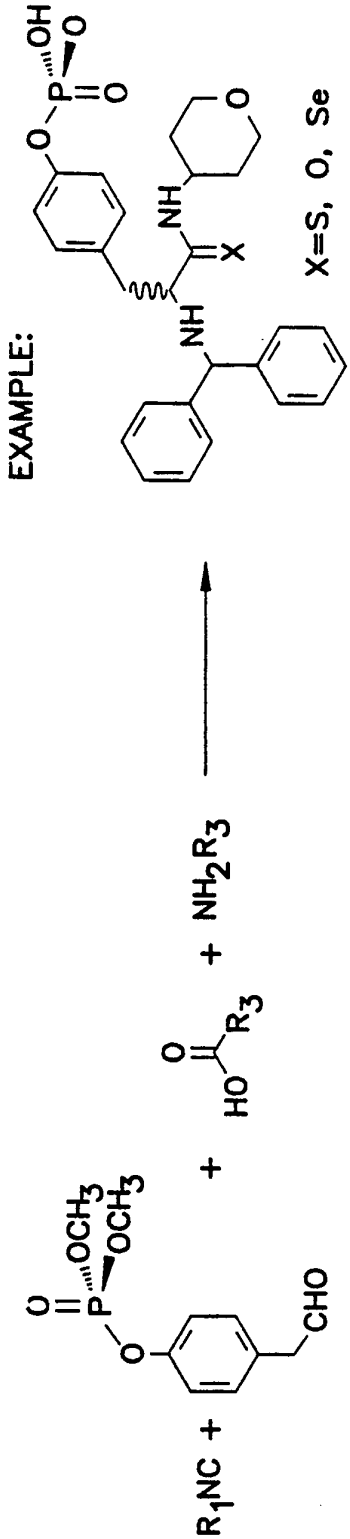
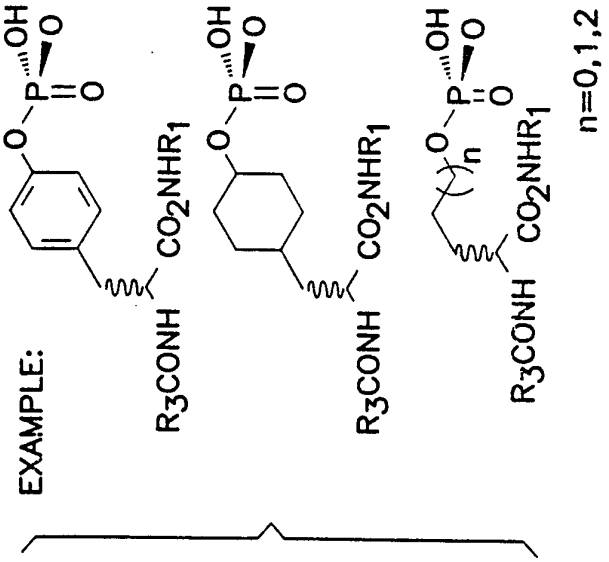

EXAMPLE:
X=S, O, Se
+ NH2R3
R3
HO
R2=ALKYL OR ACYL
R3=ALKYL OR ACYL
R1=ALKYL
R1NC +
CHO

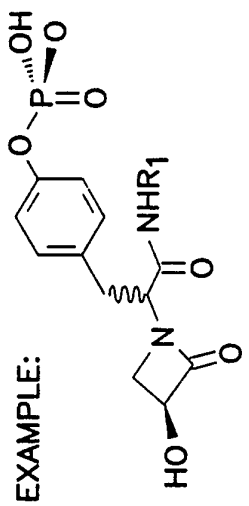
FIG. 9B



R_1NC + R_2CHO + $HO-C(=O)-CH(R^3)-NH_2$ + NH_3

R_1=ALKYL R_2=ALKYL, ARYL, ALPHATIC OR AROMATIC R_3=ALKYL OR ARYL,

FIG. 9C



R_1NC + R_2CHO + $HO-C(=O)-CH(OH)-CH_2-NH_2$

R_1=ALKYL R_2=ALKYL, ARYL, ALIPHATIC OR AROMATIC [WHERE THE AMIRE AND ACID ARE ON THE SAME MOLECULE]

FIG. 9D

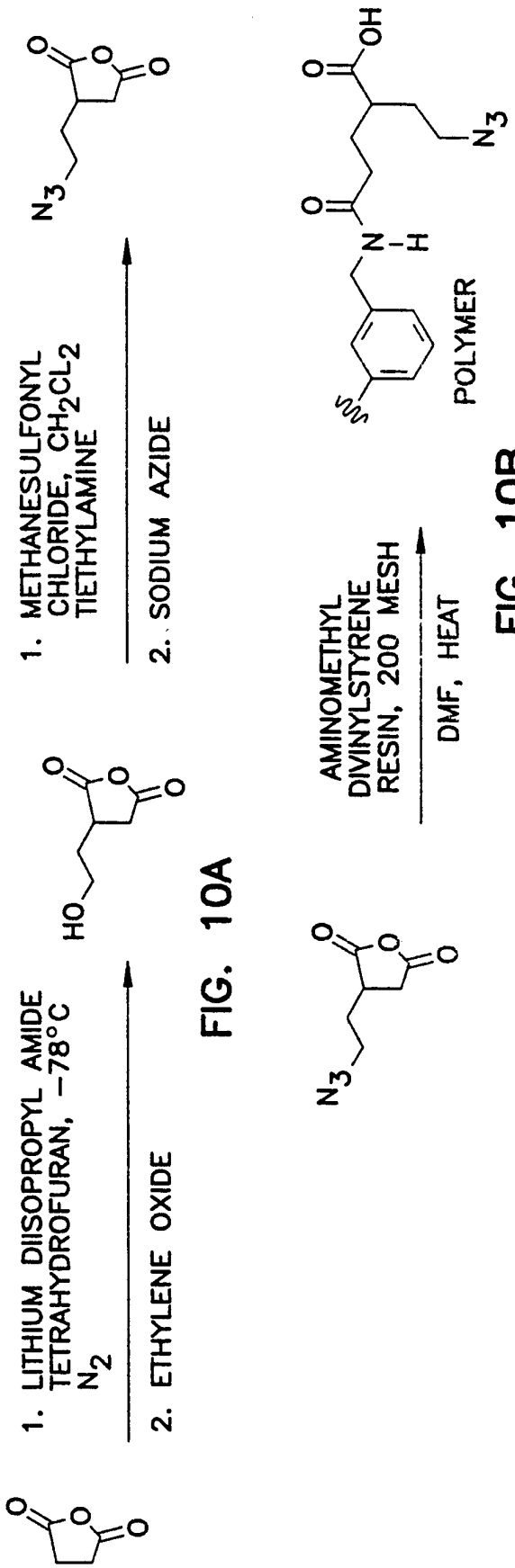


FIG. 10A

FIG. 10B

FIG. 10C

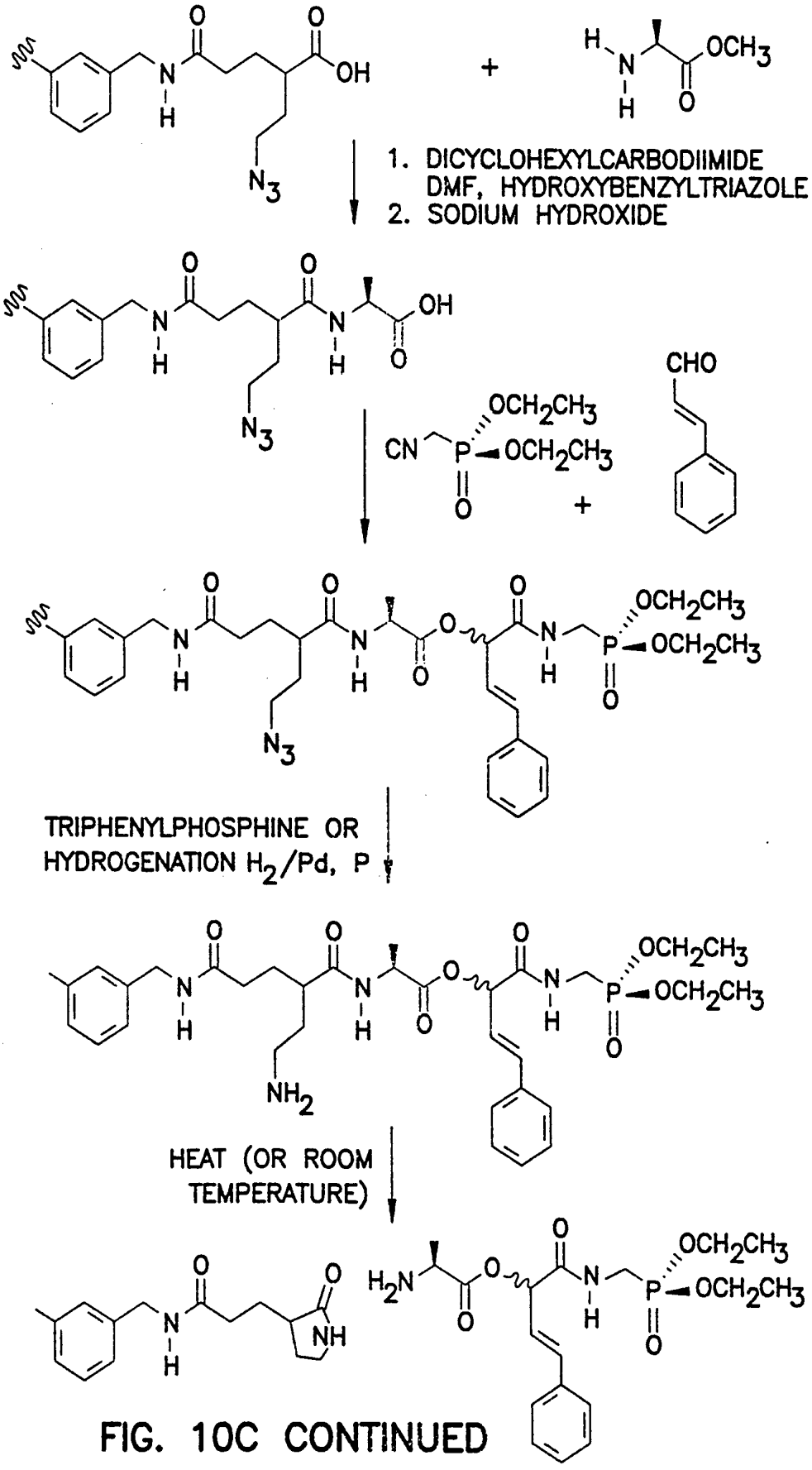


FIG. 10C CONTINUED

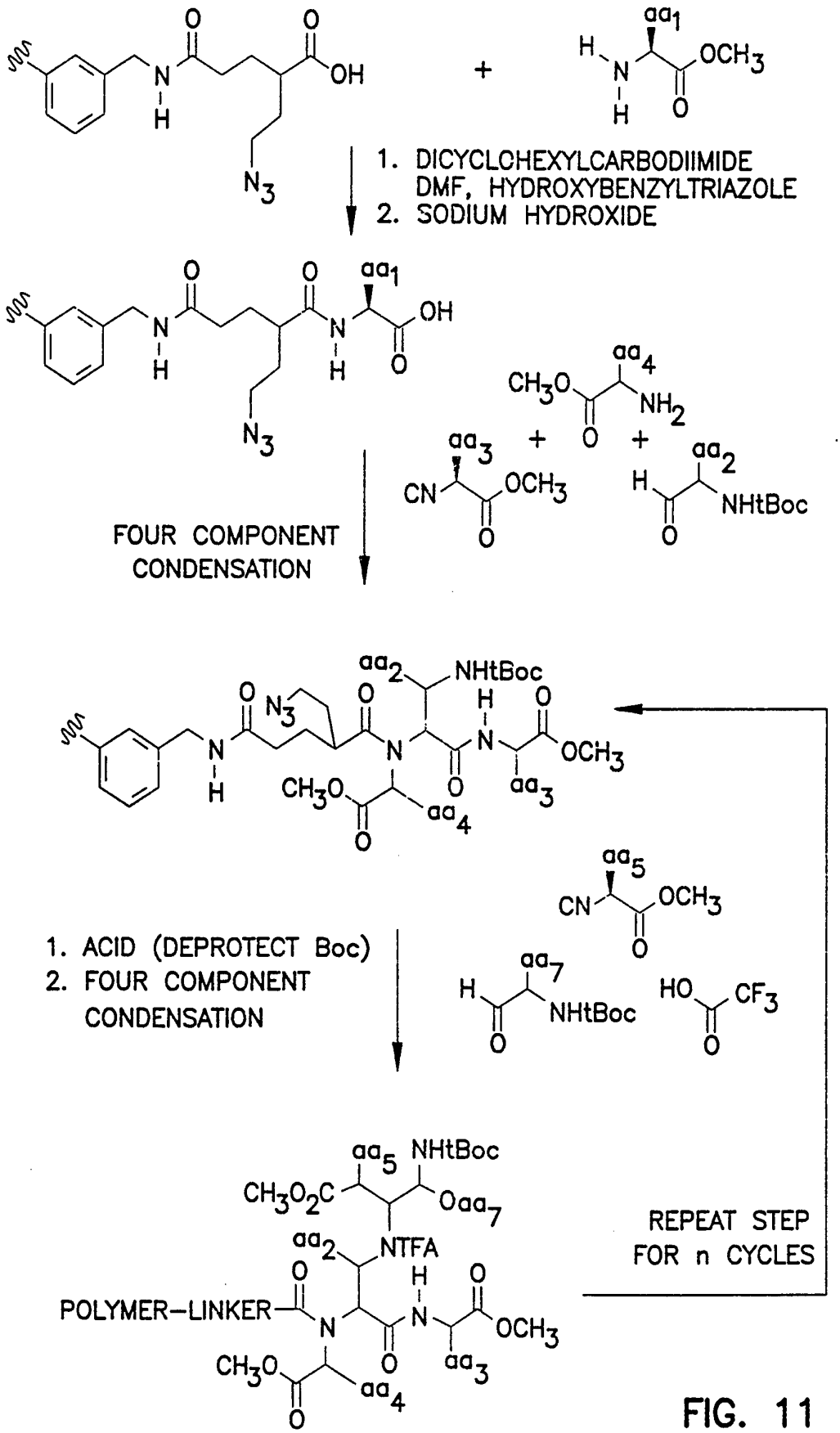

1. DICYCLOHEXYLCARBODIIMIDE
DMF, HYDROXYBENZYLTRIAZOLE
2. SODIUM HYDROXIDE
FOUR COMPONENT
CONDENSATION
1. ACID (DEPROTECT Boc)
2. FOUR COMPONENT
CONDENSATION
REPEAT STEP
FOR n CYCLES
POLYMER–LINKER

FIG. 11

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 94/08141

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07B43/06 B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol.114, 1992, WASHINGTON, DC US pages 10997 - 10998 J. A. ELLMAN, B. A. BUNIN 'A General and expedient method for the solid-phase synthesis of 1,4-Benzodiazepine derivatives' see the whole document ---	1,2,4-6, 13-15
A	PEPTIDE RESEARCH, vol.6, no.3, June 1993 pages 161 - 170 V. NIKOLAIEV ET AL. 'Peptide encoding for structure determination of nonsequenceable polymers within libraries synthesized and tested on solid-phase supports' --- -/--	1-17

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1994	25. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seufert, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol.354, 7 November 1991, LONDON GB pages 84 - 86 R. A. HOUGHTON ET AL. 'Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery' see the whole document	7,16
A	NATURE, vol.354, 7 November 1991, LONDON GB pages 82 - 83 K. S. LAM ET AL. 'A new type of synthetic peptide library for identifying ligand-binding activity' see the whole document	7,16
A	ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, vol.31, no.4, 1992, WEINHEIM DE pages 367 - 486 G. JUNG, A. G. BECK-SICKINGER 'Multiple peptide synthesis methods and their applications'	7,16
P,X	PROC. NATL. ACAD. SCI., vol.90, August 1993 pages 6909 - 6913 S. HOBBS DEWITT ET AL. 'Diversomers: an approach to nonpeptide, nonoligomeric chemical diversity'	1-16
T	JOURNAL OF MEDICINAL CHEMISTRY, vol.37, no.10, 13 May 1994, WASHINGTON US pages 1385 - 1401 E. M. GORDEN ET AL. 'Application of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions' see the whole document, especially page1390 - pqge 1392	

1