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DESCRIPTION

COMBINATORIAL LIBRARIES AND METHODS FOR THEIR USE

This application is a continuation-in-part of U.S. application Serial No. 08/168,966, filed December 15, 1993, which is a continuation of U.S. application Serial No. 07/978,646, filed November 19, 1992.

Background of the Invention

This invention relates to synthetic combinatorial libraries useful for drug discovery, and methods for their use in such drug discovery.

Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe the generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. These libraries are composed of mixtures of free peptides which form a heterogenous library. Systematic identification of optimal peptide ligands is achieved by screening a library followed by iterative selection and synthesis processes. For example, one library consisted of a series of six residue peptides having the first two positions specifically defined, and the last four positions consisting of a random mixture of 18 L-amino acids. This library was screened to determine which pair of defined peptides had optimum activity in an assay. A second library was then synthesized in which the optimal pair of peptides were included, the third position of each peptide individually synthesized, and the last three peptides consisted of a random mixture of 18 L-amino acids. This library was screened as before and the process repeated until the optimum six residue peptide was identified. Houghten et al. state:

> "A number of other libraries, such as one composed entirely of D-amino acids, have been prepared which in total permit the systematic screening of hundreds of millions of peptides.

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A fundamental feature of SPCLs [synthetic peptide combinatorial libraries] is that free peptides can be generated and used in solution in virtually all existing assay systems at a concentration of each peptide most applicable to the assay. This approach has also been successfully used in radio-receptor assays (opioid peptides) and plaque inhibition assays (human immunodeficiency virus (HIV-1) and Herpes Simplex Virus (HSV)). SPCLs, as described, greatly aid all areas of drug discovery and research involving peptides."

Lam et al., 354 *Nature* 82, 1991, and WO 92/00091 (PCT/US91/04666) and Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe systematic synthesis and screening of peptide and other libraries of defined structure. The method used by Lam et al. is based on a one bead one peptide approach in which a large peptide library consisting of millions of beads are screened. Each bead contains a single peptide. The authors state:

"It is clearly not enough to use a random mixture of activated amino acids in a peptide synthesis protocol, because the widely different coupling rates of different amino acids will lead to unequal representation and because each bead will contain a mixture of different peptides. Our solution was to use a 'split synthesis' approach. The first cycle consisted of distributing a pool of resin beads into separate reaction vessels each with a single amino acid, allowing the coupling reactions to go to completion, and then repooling the beads. The cycle was repeated several times to extend the peptide chain. In this fashion, each bead should contain only a single peptide species."

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The library of beads was screened by a staining procedure and stained beads visualized using a microscope, and removed. The structure of the peptide is obtained by a chemical analysis of the material on the single bead. Lam et al. indicate:

> "Additionally, our approach has far greater potential for applying the richness of well-established peptide chemistry to synthesize libraries incorporated D-amino acids or unnatural amino acids as well as specific secondary structures including cyclic peptides. All of this can be accomplished without need to keep records of the synthetic products as our interest is focused just on those peptides which provide a strong interaction signal with the acceptor."

Summary of the Invention

The present invention features an efficient method for the construction and decomposition of combinatorial libraries to allow identification of useful polysubunit compounds (made up from two or more subunits which may be the same or different, referred to sometimes as polymers). The method is not limited to identification of polypeptides; it can be applied to any combination of compounds made up of differing subunits (referred to sometimes as monomeric compounds) within one polysubunit compound. Synthesis of such polysubunits can be carried out chemically or with the use of catalysts, including enzymes. Thus, for example, the subunits may be selected from natural or unnatural moieties, including amino acids, nucleotides, sugars, lipids, and carbohydrates. In addition, the bond used to attach each subunit to the prior subunit can make any desired type of bond, and may include covalent, ionic or coordination bonds. The bonds may be selectively cleavable by enzymes or chemical treatments, as desired. Examples of such bonds include: peptide [R^1CONHR^2], ester [R^1COOR^2], sulfonamide [$R^1SO_2NR^2$],

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thioester [R^1COSR^2], phosphodiester [R^1OPOR^2], ether [R^1COCR^2], thioether [R^1CSCR^2], phosphamide [R^1PONH-], amine [-CH_2NHC-], and azo [-CNNC-] (where each R^1 and R^2 may be the same or different and may be cyclic or acyclic). In the method, a free or solid-phase-bound polysubunit can be used to allow ready screening of the synthesized polysubunits in any desired test or assay system. A significant advantage of this invention is that it provides a series of libraries or collections of polysubunits which can be reused for screening for any number of desired properties in any chosen assays.

The present method does not employ a mixed synthesis in which several different subunits are chemically incorporated within one vessel, but rather a synthesis in which a single subunit is incorporated at each step. See Figure 1.

Thus, in one example, the method involves a first step of attaching ten different subunits A, B, C . . . J, to a solid support in ten separate vessels or columns. In the second step, a portion or aliquot of the material synthesized at the first step is retained as separate columns, while the remainder (which is still attached to individual solid supports) is mixed or pooled, divided into ten new different columns, and ten further parallel syntheses carried out to provide the dimer XA^1, XB^1, XC^1 . . . XJ^1, where X is any one of the original A-J, and A^1, B^1, C^1 . . . J^1 are ten different subunits which may be the same or different from A-J. Of course, fewer or more than ten syntheses can be used in this second step. In the third step, a portion of the newly synthesized material of step two is again retained in separate columns, and the remainder mixed and divided into ten further columns so that the synthetic procedure can be repeated until the whole length of the desired polysubunit is synthesized. In this way a series of vessels is formed at each step, differing from those in prior steps by the presence of an extra subunit.

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The final ten columns in the above example (each having a variety of different polysubunits with a known subunit at their terminus) can be assayed using any standard assay format. That is, each of the ten mixtures is assayed to determine which mixture contains one or more active compounds. The column which is found to contain an active compound identifies the subunit required at the polysubunit terminus to be active in the assay. For example, the column containing polysubunits of sequence $XXXXJ^1$ may be active in the assay. This indicates that J^1 is required at the terminus of a polysubunit in this assay. This subunit is now bonded to each of the columns retained in the previous synthetic step (in the example, the columns XXXA, XXXB, . . . XXXJ). These ten newly synthesized series of compounds can then be assayed and the process repeated until the final polysubunit sequence is known. See Figures 2 and 3.

Thus, the method of the present invention features a set or kit of partial combinatorial libraries with each library containing one subunit more than the prior library. The final library is decomposed stepwise starting with the last subunit. This form of retroanalysis or antithesis allows definition of the synthesis of the desired product, which is accomplished in the final analytic step. Once constructed, the entire set of libraries can be used for many different projects.

Thus, in a first aspect, the invention features a kit which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds, and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits, but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at their terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each

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constructed from the known number minus one subunit, as described above.

This kit forms a combinatorial library which can be screened and decomposed as discussed above. The number of first and second pluralities of vessels can be the same or different, and generally the terminus of the polysubunits to which subunits are added is the same in each set of vessels.

In preferred embodiments, the kit may include further pluralities of vessels in which the number of subunits present in the polysubunit is the known number minus two, or three, or more. Thus, each plurality of vessels differs from other pluralities of vessels in the number of subunits present in the polysubunits. Most preferably, the number of subunits in each plurality of vessels differs by one and ranges from one to the known number. As discussed above, such a kit is useful for systematic identification of the sequence of a desired polysubunit, using the method of retroanalysis.

As noted above, the polysubunits may be formed from any series of subunits desired, and the number of vessels containing these polysubunits at each step can be different for each subunit added. Thus, as described above, ten amino acids may be incorporated in one step, two or three carbohydrates may be incorporated in another step, and twenty amino acids in the following step. There is no limit to the steps of addition of subunits, nor to the choice of subunits which can be added at each step. All that is necessary for the kit to be useful is that the kit include a series of vessels which contain polysubunits differing sequentially in the number of subunits present in those polysubunits. The final plurality of vessels in the kit will contain only the first subunit free in solution, or bound to a solid support.

In a related aspect the invention features, a method for construction of a kit, following the steps

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described above, and a method for screening such kits as described above.

Specifically, the invention features a method for construction of a kit useful for identification of a desired compound, by the steps of: (a) providing a first plurality of vessels containing different polysubunits each constructed from a known number of subunits joined together by one or more bonds. Each subunit and bond can be the same or different, and each different polysubunit has a different sequence of subunits but the same known subunit at one terminus of the polysubunit. Each vessel includes polysubunits having a different known subunit at their terminus; (b) combining aliquots of the different polysubunits from each vessel to form a mixture; wherein the aliquots are less than all of the contents of each vessel; (c) dividing the mixture into a second plurality of vessels; and (d) bonding a further subunit to each polysubunit in the second plurality of vessels; wherein the subunit is different for each second plurality of vessels.

In preferred embodiments, the method further includes repeating steps (a) through (d) for the second plurality of vessels; or repeating steps (a) through (d) a plurality of times.

In a related aspect, the invention features a method for identification of a desired compound, including the steps of: (a) providing a kit as described above, and screening the first plurality of vessels to identify a vessel having a desired activity in an assay, and thereby identifying a first known subunit; and (b) coupling the first known subunit to a terminus of an aliquot of the contents of each of the different polysubunits in the second plurality of vessels.

In preferred embodiments, the method further involves screening the different polysubunits having the first known subunit for the desired activity in the assay,

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and thereby identifying a second known subunit; or repeating steps (a) and (b) a plurality of times.

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

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 illustrates split pool synthesis of a combinatorial library.

Figure 2 illustrates utilization of the deconvolution pathway to determine the sequence of a member of a combinatorial library.

Figure 3 illustrates the deconvolution of a pentapeptide library.

Figure 4 illustrates the corrected absorbances of pools of library products reacted with β-endorphin antibody to determine the sequence of the pentapeptide which bound best to the β-endorphin antibody.

Applicant has determined that a useful series, or kit, of libraries can be constructed by stepwise synthesis of a polysubunit from different subunits. While these libraries and methods for their synthesis and use will commonly be applied for use with peptides formed from standard L- or D-amino acids, those of ordinary skill in the art will recognize that other subunits and bonds other than covalent, ionic or coordination bonds can be used to form the final polysubunit, which can then be screened in any standard assay. These synthetic protocols and assays are well known to those of ordinary skill in the art and need not be repeated here. See, Lam et al., *supra*, and Houghten et al., *supra*, the whole of all of which are hereby incorporated by reference herein. As an illustrative example of a method and kit of this invention, the following is provided. This example is not limiting in the invention and those of ordinary skill will

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realize (as noted above) that other equivalent methods, syntheses, and analyses can be readily used.

Example

The method proposed here is based on a true combinatorial synthesis and makes efficient use of all of the materials. The first step is to attach ten subunits, A to J, to a solid support in ten columns. One-sixth of this material is retained in this form, giving A, B, . . . J; the remainder, still attached to the solid support, is pooled, divided into ten columns, and ten parallel syntheses are carried out, to provide XA, XB, . . . XJ. One-fifth of this material is retained in this form, and the combinatorial synthesis is repeated to give ten polysubunits, XXA, XXB, . . . XXJ. One-fourth is kept and the fourth residue is added to the remainder by combinatorial synthesis to give the polysubunits XXXA, XXXB, . . . XXXJ. After retaining one-third of these polysubunits the final combinatorial step of synthesis is completed, and the materials are removed from the column to provide ten solutions containing XXXXA, XXXXB, XXXXJ. These are now assayed.

If a positive assay result is obtained with one vessel containing polysubunits of the structure XXXXC (*i.e.*, C is the critical subunit at the fifth position in the pentasubunit), the experimenter returns to the columns retained at the previous step, and to an aliquot of each adds C, to provide ten solutions, XXXAC, XXXBC . . . XXXJC. Again, these are tested. If a positive assay result is obtained with XXXEC (*i.e.*, E is the critical subunit at position four in the pentasubunit), the experimenter returns to the previous partial library and completes it by adding E + C to all ten to give XXAEC, XXBEC . . . XXJEC. These steps are repeated until all of the positions are specified.

Thus, in essence a set of partial combinatorial libraries is made, each one residue longer than the last. The final library is decomposed stepwise, starting with

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the last residue. This form of retro-analysis allows definition of the synthesis of the desired product, which is indeed accomplished in the very last step. Once constructed, the entire set of libraries can be used again for different projects.

It is notable that the final synthetic step also provides a method for synthesis of the desired product.

Materials and Methods

Solvents. DMF (HPLC grade) was purchased from Baxter and was used without further purification. CH_2Cl_2 was purchased from Fisher Scientific and was distilled over CaH_2 before use.

Instrumentation. UV absorbances were measured on a Hewlett-Packard 8452A diode array spectrophotometer. All centrifugations were carried out on an Eppendorf 5415 C centrifuge for one minute at 10,000 rpm.

Formation of the Pentapeptide Library. The peptide libraries were synthesized manually by a solid phase method. Tentagel (TG) resin was the solid support for all synthesis and was 90 μm in diameter. The amino acids used for the formation of the libraries were Gly-FMOC, Leu-FMOC, Phe-FMOC and Tyr(tBu)-FMOC. All amino acids were coupled with the aid of HBTU and DIPEA. The Tentagel, HBTU, DIPEA and amino acids used were purchased from NovaBiochem. Tentagel was added to 4 glass fritted filter vials, shaken with DMF and filtered. To each vial was added one of the FMOC protected amino acid components of the library, HBTU, DMF and 20 μl DIPEA. All four vials were shaken for one hour, filtered and washed with DMF. A 5% solution (v/v) of acetic anhydride in DMF was added to each vial and shaken for 20 minutes to cap any uncoupled free amino acid groups. The beads were then filtered, washed with DMF (2x) and CH_2Cl_2 (2x) and dried for two hours in a vacuum oven (55°C at 20 mm Hg). Approximately, a 75 mg portion of the amino acid coupled Tentagel bead was taken from each vial and labeled in a manner describing the length of the peptide and the

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identity of the last amino acid coupled. Also, an approximately 5 mg portion of the resin was taken to do an FMOC deprotection test in order to quantitate the completeness of the previous amino acid coupling step. The remaining resin was combined and shaken in 3:1 (v/v) DMF/CH_2Cl_2 for 30 minutes to randomize the beads. The beads were quartered into equivalent weight (and therefore equivalent moles) and set into 4 glass fritted filter vials and subjected twice to 10 minute shakings with 20% Piperidine in DMF (v/v) in order to cleave the N-terminal FMOC protecting group. The beads were filtered, washed with DMF (3x) and coupled with one of the 4 amino acid components of the library to give the 4 dipeptide pools, TG-Xaa-Gly-FMOC, TG-Xaa-Leu-FMOC, TG-Xaa-Phe-FMOC, and TG-Xaa-Tyr(tBu)-FMOC. The steps of 1) capping the peptide with acetic anhydride, 2) saving and labeling a 75 mg portion of the resin, 3) combining, randomizing and evenly dividing the beads and 4) coupling each division with an FMOC protected amino acid was repeated until the 5th coupling. At this point there are 4 pentapeptide libraries. These pentapeptide libraries were cleaved of the t-butyl protecting group on the tyrosines by mixing the resin with TFA (Pierce) for 2 hours. The beads were filtered and washed with ethanol (2x) and DMF (2x). The beads were then shaken with 20% Piperidine in DMF (v/v) twice for 10 minutes to cleave the N-terminal FMOC group. The deprotected beads were then washed with DMF (3x), CH_2Cl_2 (2x) and set in a vacuum oven overnight (55°C at 20 mm Hg) to evaporate any residual solvent.

ELISA Procedure. The library containing Tentagel resin (~3 mg) was added to a polypropylene tube. The tube was coated with a 1:1 (v/v) blocking solution of 3% BSA in PBS (w/v) and .05% Tween-20 (Sigma) in PBS (v/v). The tube was incubated with 500 µl of a 1:1:1 (v/v/v) solution consisting of 1 µg/ml mouse anti-β-endorphin monoclonal antibody (Boehringer Mannheim) in PBS, 3% BSA in PBS and .05% Tween-20 in PBS at 37°C for

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one hour. The tube was centrifuged and the supernatant was decanted. The tube was washed 3 times by repetitively adding 0.05% Tween-20 in PBS, centrifuging the tube and decanting the supernatant. The tube was then incubated with 500 μl of goat anti-mouse antibody conjugated to glucose oxidase (from Cappel, diluted by a factor of 1000 with a 1:1 (v/v) solution of 3% BSA in PBS and .05% Tween-20 in PBS). The tube was washed 3 times by repetitively adding .05% Tween-20 in PBS, centrifuging the tube and decanting the supernatant and 2 times by adding PBS, centrifuging the tube and decanting the supernatant.

A developing solution containing 25 ml of 0.1M Na_3PO_4 (pH 6.0), 3 ml of 20% glucose in H_2O, 200 μl horseradish peroxidase (0.1% in .1M Na_3PO_4, pH 6.0) and 200 μl ABTS dye (45 mg/ml in 0.1M Na_3PO_4, pH 6.0) was prepared. A 500 μl aliquot of the developing solution was added to the tube and allowed one hour to react with the peptide library before UV readings were taken. For the UV measurements of the assay, 900 μl of .1M Na_3PO_4 (pH 6.0) was placed in a cuvette and measured as the blank, 100 μl of the developed solution was then introduced to the cuvette and the UV absorbance at 416 nm was monitored.

Data Analysis. for each ELISA, besides assaying the pentapeptide libraries, a positive and negative control were also simultaneously assayed. The positive control was the independently synthesized pentapeptide, TG-Leu-Phe-Gly-Gly-Tyr-NH_2. The negative control was acetylated Tentagel (TG-Ac). The measured UV absorbance was normalized, to take into account the weight differences of the TG-resin in each assay tube, to give a normalized absorbance. The normalization was calculated by multiplying the measured absorbance at 416 nm by the mass of the material in the TG-Ac (negative control) assay tube. This product was divided by the mass of the TG-resin in the pentapeptide library tube to give the normalized absorbance. The absorbance of the negative

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control was subtracted from the normalized absorbance to give a corrected absorbance.

In the early stages of the deconvolution, the negative control gave higher absorbance readings than some of the libraries. This accounts for the sub-zero absorbances seen on the graphs. As the library became more defined, fewer negative corrected absorbances were obtained.

Quantification of Amino Acid Coupling. A standard solution was prepared by adding DMF to a known amount of Gly-FMOC to a volume of 100 ml. 980 μl of 20% piperidine in DMF (v/v) was scanned as a blank. 20 μl of the Gly-FMOC solution was added to the blank, swirled for 5 minutes and scanned. The piperidine-benzofulvene complex that forms upon cleavage of the FMOC group by piperidine has a diagnostic UV absorbance peak at 302 nm. After the coupling, capping and drying steps of the combinatorial library synthesis, a determination of the completeness of each coupling was performed by taking a known mass of Tentagel resin (usually ~5 mg) from each reaction vial and placing the sample in a 1 dram vial. The vials containing the resin samples were shaken with 1000 μl of 20% piperidine in DMF for 15 minutes. The resin was allowed to settle to the bottom and 100 μl of the piperidine treated resin solution was added to 900 μl of 20% piperidine in DMF which was previously added to a cuvette and scanned as a blank. The UV absorbance at 302 nm was compared to the standard to quantitate the extent of the amino acid coupling.

As a simple test of our method of deconvolution, an experiment was devised which screened libraries containing the well studied pentapeptide sequence, Leu-Phe-Gly-Gly-Tyr-NH_2, which displays nanomolar binding to commercially available anti-β-endorphin monoclonal antibody. A synthetic combinatorial peptide library with a degree of 5 and an alphabet consisting of leucine, glycine, phenylalanine and tyrosine, was synthesized on

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Tentagel solid support. The total number of molecules in this library is 4^5 (1024 members).

The coupling of the FMOC protected amino acids to the solid support with HBTU proceeded smoothly and quantitatively, according to FMOC cleavage measurements taken after each coupling. The quantitativeness of the coupling is advantageous since we can assume that the loading capacity (and therefore the number of moles per gram of resin) is approximately constant, no matter at what stage we are at in the synthesis or deconvolution of the library.

The binding assays against the β-endorphin antibody were performed with the peptide libraries remaining attached to the Tentagel resin. The deconvolution sequence can be followed pictorially in Figure 3 showing Scheme 3 and the corrected absorbances for each round are depicted in Figure 4. In the 4 pools of the pentapeptide libraries, where only the N-terminal amino acid was defined, TG-Xaa-Xaa-Xaa-Xaa-Tyr clearly gave the strongest absorbance. From this point, the 4 tetrapeptide libraries, TG-Xaa-Xaa-Xaa-Gly-FMOC, TG-Xaa-Xaa-Xaa-Leu-FMOC, TG-Xaa-Xaa-Xaa-Phe-FMOC and TG-Xaa-Xaa-Xaa-Tyr-FMOC, saved and labeled during the formation of the pentapeptide combinatorial library, were all coupled with Tyr-FMOC. Of the pools TG-Xaa-Xaa-Xaa-Gly-Tyr, TG-Xaa-Xaa-Xaa-Leu-Tyr, TG-Xaa-Xaa-Xaa-Phe-Tyr, and TG-Xaa-Xaa-Xaa-Tyr-Tyr, TG-Xaa-Xaa-Xaa-Gly-Tyr, obviously gave the strongest binding. This method of deconvolution was repeated to deduce that TG-Xaa-Xaa-Gly-Gly-Tyr was the best binder to the β-endorphin antibody. Solving for the fourth amino acid did not give a clear winner. TG-Xaa-Phe-Gly-Gly-Tyr, the sequence leading to the native epitope to the antibody, was the strongest binder. However, TG-Xaa-Leu-Gly-Gly-Tyr also showed significant binding to the antibody. Significant enough, in fact, to warrant the coupling of Leu-Gly-Gly-Tyr as well as Phe-

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Gly-Gly-Tyr to the monocoupled Tentagel libraries saved from the first step of the combinatorial synthesis.

In the final analysis, TG-Leu-Phe-Gly-Gly-Tyr, the native epitope, was the most extensive binder. Other weaker binders were also deduced, TG-Phe-Phe-Gly-Gly-Tyr and TG-Leu-Leu-Gly-Gly-Tyr also showed significant binding to the β-endorphin antibody.

Iterative methods of deconvolution have a number of advantages. A variety of chemical libraries, not just peptide or oligonucleotide libraries, can be solved iteratively. An advantage of an iterative deconvolution over encoded libraries is that the use of a decoding tag is avoided. This averts the concern of whether the decoding tag is involved in the binding of a library member to a receptor. Also, an iterative strategy avoids the sensitivity concerns of solving for the active member of a library because iterative methods are not dependent upon sequencing or decoding for the library members bound to a receptor.

Making combinatorial libraries by the split pool method is a cumbersome process. By cataloguing sublibraries along each step of a combinatorial synthesis, only one library needs to be made by the split pool method. While this may not be a tremendous advantage in making libraries that are synthesized in a highly automated manner, like oligonucleotide libraries, in the synthesis of other libraries not yet amenable to automated processes, like small molecule libraries, this iterative method avoids much of the time and labor involved in making split pool libraries for the previous iterative approaches.

Other embodiments are within the following claims.

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Claims

1. A kit comprising:

a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said first plurality of vessels comprising polysubunits having a different known subunit at said terminus, and

a second plurality of vessels comprising different polysubunits each constructed from said known number minus one subunit joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said second plurality of vessels comprising polysubunits having a different known subunit at said terminus.

2. The kit of claim 1, wherein said kit further comprises a third plurality of vessels comprising different polysubunits constructed from said known number minus two subunits.

3. The kit of claim 1, wherein said kit further comprises a fourth plurality of vessels comprising different polysubunits constructed from said known number minus three subunits.

4. The kit of claim 1, wherein said kit comprises further pluralities of vessels comprising different polysubunits, each said plurality of vessels differing from said other plurality of vessels in the number of subunits present in a said polysubunit.

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5. The kit of claim 4, wherein said further pluralities of vessels include polysubunits differing from other pluralities of vessels by having from said known number minus two subunits to one subunit.

6. Method for construction of a kit useful for identification of a desired compound, comprising the steps of:

(a) providing a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each said vessel comprising polysubunits having a different known subunit at said terminus,

(b) combining aliquots of said different polysubunits from each said vessel to form a mixture, wherein said aliquots are less than all of the contents of each said vessel,

(c) dividing said mixture into a second plurality of vessels, and

(d) bonding a further subunit to each said polysubunit in said second plurality of vessels; wherein said subunit is different for each said second plurality of vessels.

7. The method of claim 6, wherein said method further comprises repeating said steps (a) through (d) for said second plurality of vessels.

8. The method of claim 7, comprising repeating said steps (a) through (d) a plurality of times.

9. Method for identification of a desired compound, comprising the steps of:

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(a) providing a kit of claim 1 and screening said first plurality of vessels to identify a said vessel having a desired activity in an assay, and thereby identifying a first known subunit; and

(b) coupling said first known subunit to a terminus of an aliquot of the contents of each of said different polysubunits in said second plurality of vessels.

10. The method of claim 9, wherein said method further comprises screening said different polysubunits comprising said first known subunit for said desired activity in said assay, and thereby identifying a second known subunit.

11. The method of claim 9, wherein said steps (a) and (b) are repeated a plurality of times.

12. The method of claim 10, wherein said steps (a) and (b) are repeated a plurality of times.

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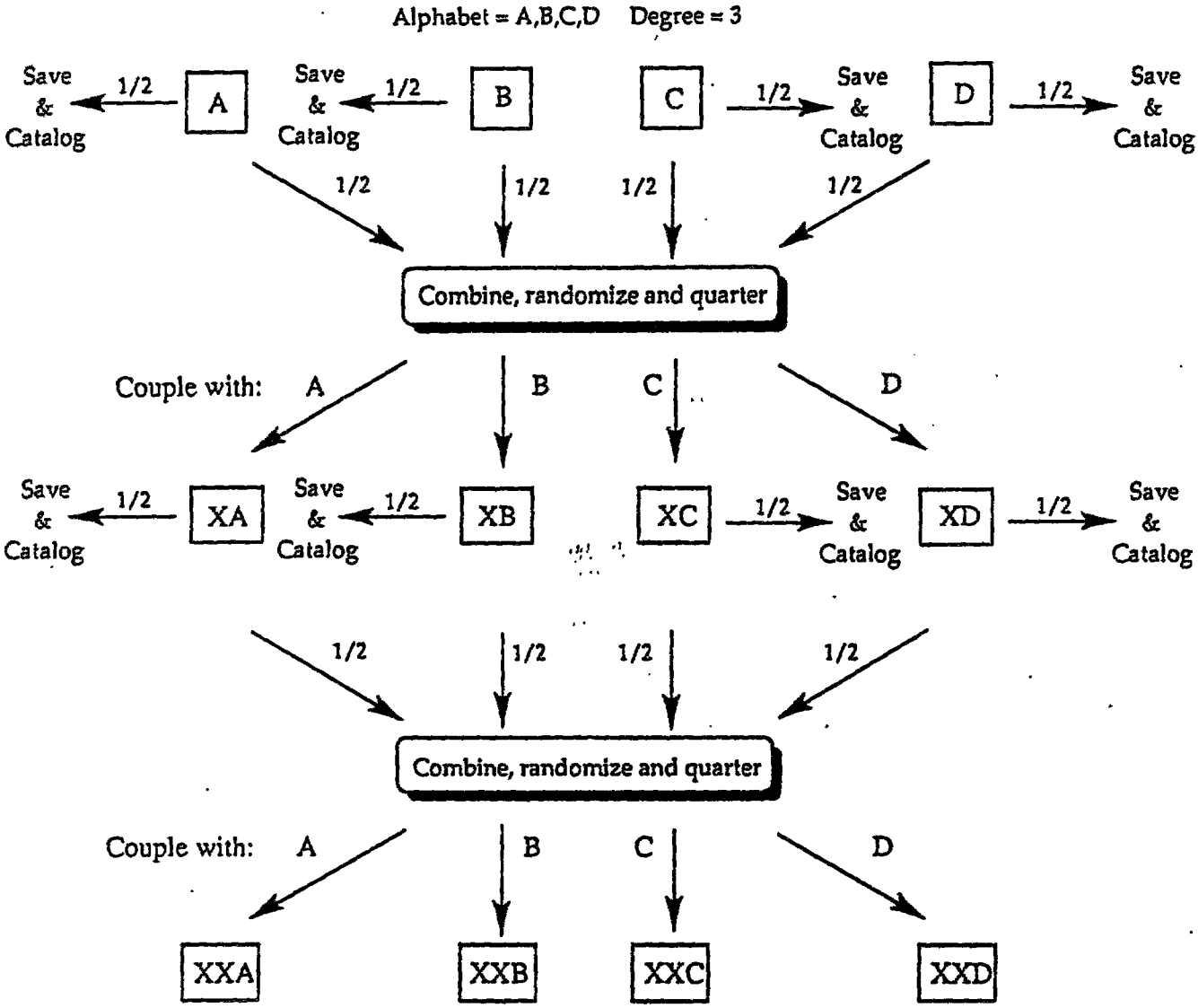
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Abstract

Kit, and method for its use and construction, which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at one terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each constructed from the known number minus one subunit, as described above.

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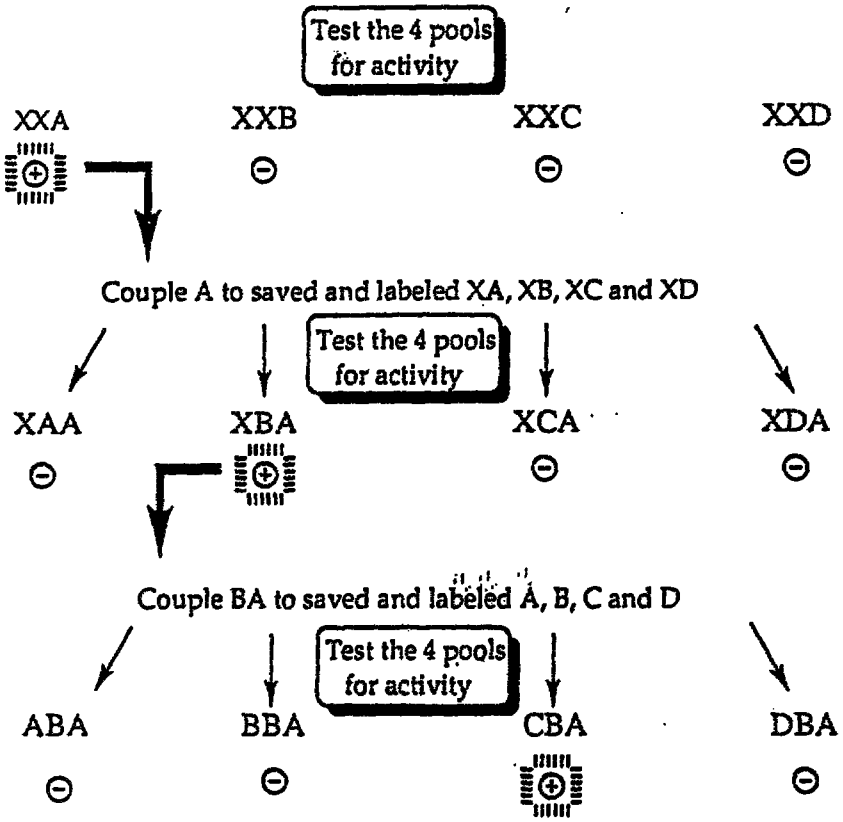


Each pool contains 16 molecules. There are a total of 64 unique molecules.

Scheme 1. A depiction of the split pool synthesis of a combinatorial library with an alphabet of 4 and a degree of 3. After each step of the synthesis, a portion of each sublibrary is saved and catalogued before the randomization step.

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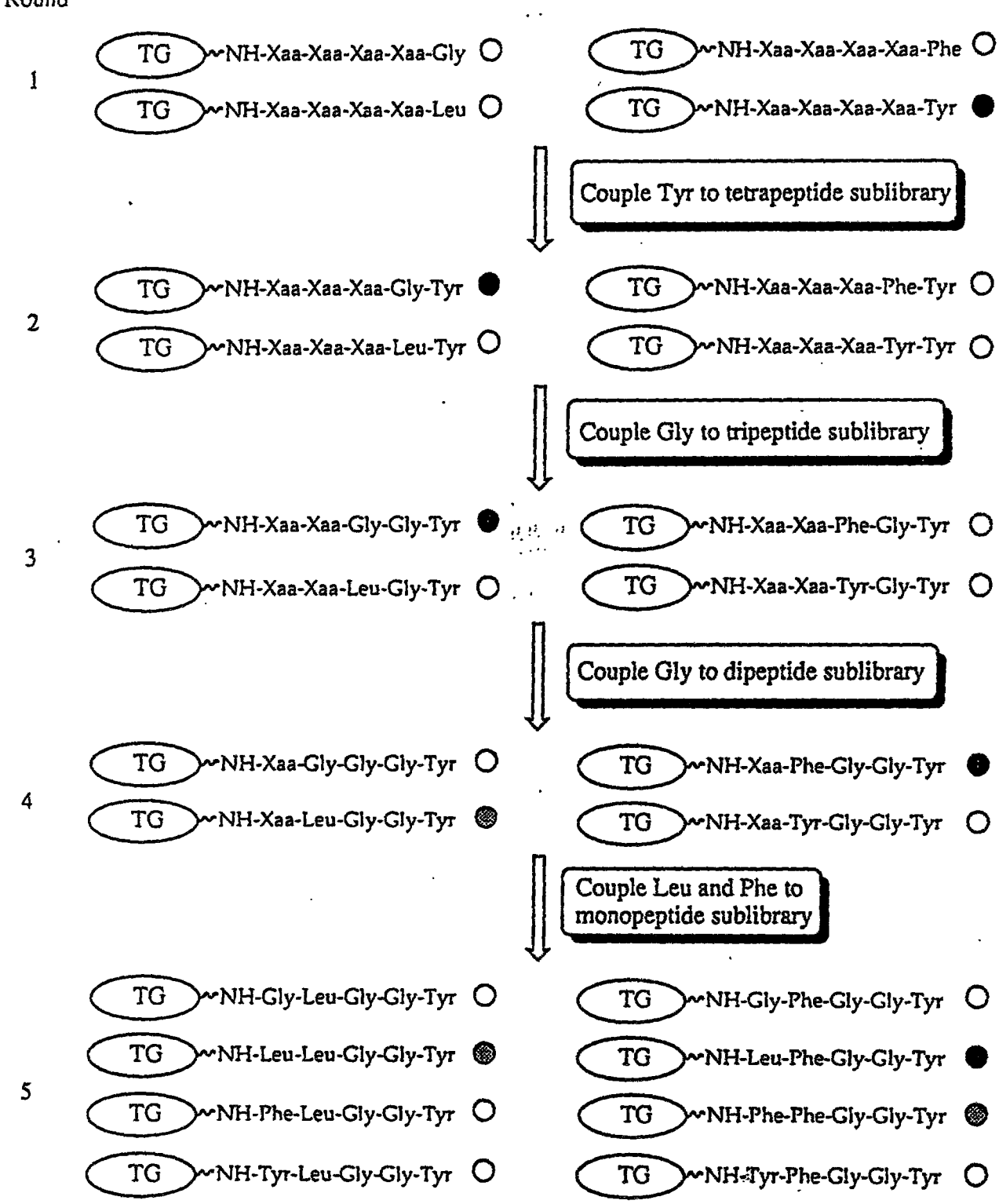


Scheme 2. A deconvulatory pathway for solving the active member of a combinatorial library.

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FIGURE 2

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Scheme 3. Route of deconvolution of the Tentagel bound combinatorial pentapeptide library. Each peptide pool of a round was tested against the anti-β-endorphin antibody. The defined positions of the pool with the strongest and significant binding were noted and coupled to the appropriate sublibrary for the next round.

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FIGURE 3

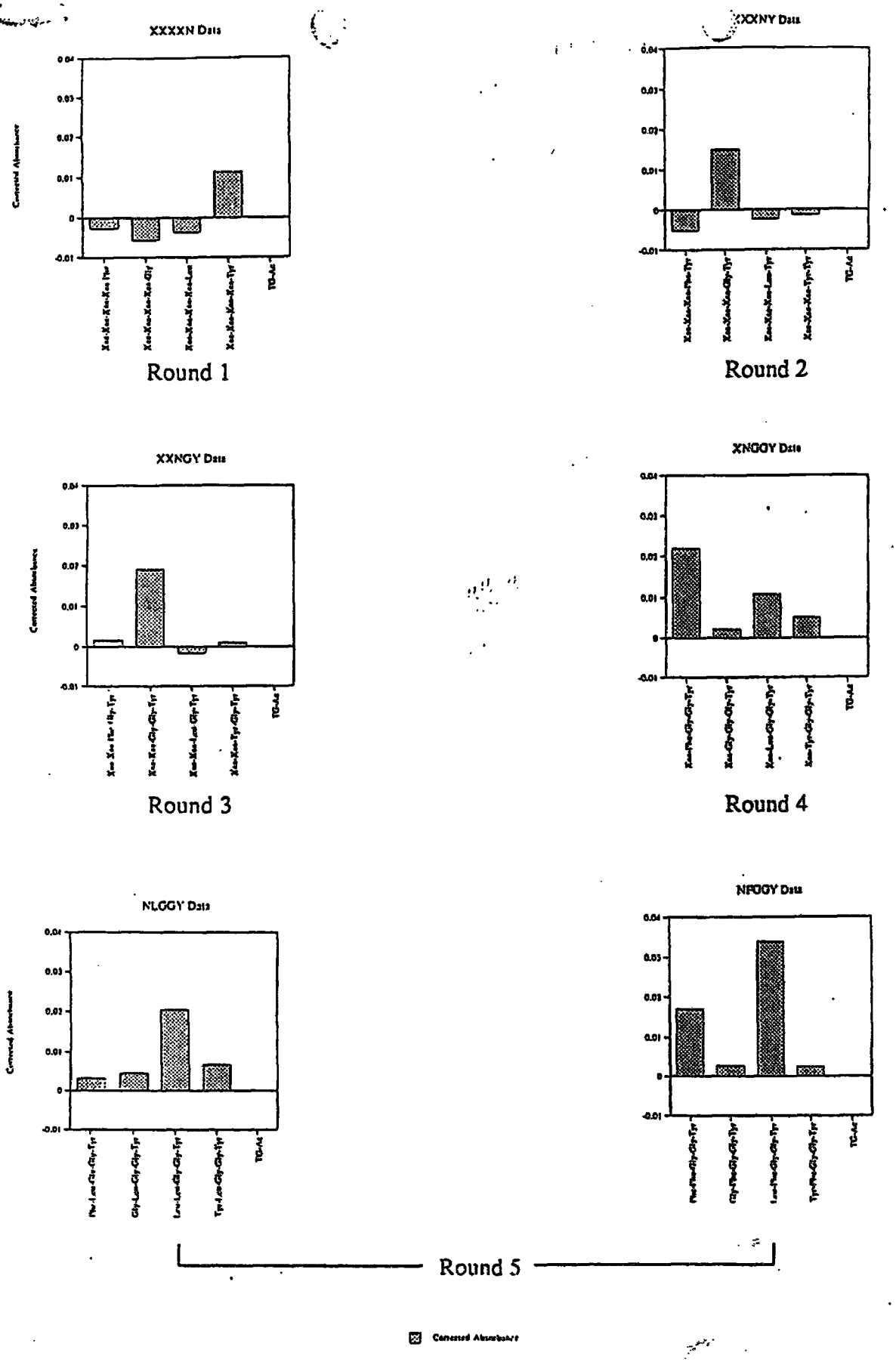


Figure 1. Corrected absorbances of each pool in comparison to the acetylated TentaGel control pool. N denotes the amino acid to be defined in that round. Xaa is a randomized position.

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FIGURE 4

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N-SUBSTITUTED AZABICYCLOHEPTANE DERIVATIVES, THE PREPARATION AND USE THEREOF

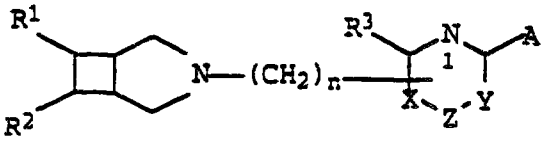
The present invention relates to novel N-substituted azabicycloheptane derivatives, the preparation and use thereof for preparing pharmaceutical agents.

It is known that 5- or 6-membered heterocyclic nitrogen derivatives with basic substituents have neuroleptic effects (EP 196 132, EP 70 053, EP 110 435).

It appears that the high affinities for serotonin receptors, in addition to the dopamine affinities, are particularly important for this.

We have now found that N-substituted 3-azabicyclo[3.2.0]heptane derivatives of the formula I

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I

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where

R^1 is hydrogen or phenyl, pyridyl, thienyl or pyrrole [sic], each of which is unsubstituted or mono- or disubstituted by halogen, C_1-C_4-alkyl, trifluoromethyl, hydroxyl, C_1-C_4-alkoxy, amino, monomethylamino, dimethylamino, cyano or nitro,

R^2 is hydrogen or phenyl which is unsubstituted or substituted by halogen, methoxy, hydroxyl or amino,

n is 0, 1, 2, 3 or 4,

R^3 is hydrogen, hydroxyl, C_1-C_4-alkyl or C_1-C_4-alkoxy, or together with the adjacent carbon atom is C=O or C=S,

X and Y are each carbon atoms, CH, CH_2, NH or C_1-C_4-alkyl-N groups or nitrogen atoms,

Z is a direct linkage, CO, CS or CH or CH_2 in which one hydrogen can be replaced by hydroxyl, amino, C_1-C_4-alkoxy or halogen, and

A is hydrogen, hydroxyl, amino, mercapto, C_1-C_4-alkyl-

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amino, di-C_1-C_4-alkylamino, C_1-C_4-alkylthio or C_1-C_4-alkoxy, or together with the adjacent carbon is C=O, or

A is C_3-C_4-alkylene which is attached to Y and which can contain one or two non-cumulative double bonds and in which one CH or CH_2 can be replaced by a nitrogen or sulfur atom or a NH or N-CH_3 group, and where the ring can be either monosubstituted by fluorine, chlorine, methyl, methoxy, nitro or amino or, in the case of a benzene ring, mono-, di- or trisubstituted by fluorine, chlorine, methyl, trifluoromethyl, nitro, hydroxyl, methoxy, amino, monomethylamino or dimethylamino,

and in which the ring on the right in formula I can have C_1-C_4-alkyl, allyl or benzyl attached to N-1 and can contain 1-3 non-cumulative double bonds,

and the salts thereof with physiologically tolerated acids have valuable pharmacological properties.

The following meanings may be particularly mentioned for R^1, R^2, R^3 and n:

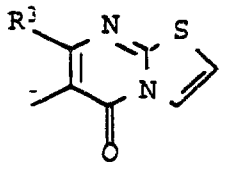
R^1: phenyl which is unsubstituted or substituted by fluorine, chlorine, iodine, methoxy, nitro, trifluoromethyl, hydroxyl or amino,

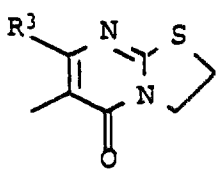
R^2: hydrogen,

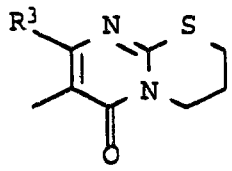
R^3: methyl and hydroxyl,

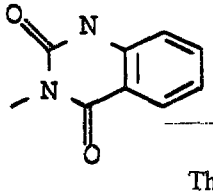
n: 2.

The ring system on the right in formula I is, in particular,





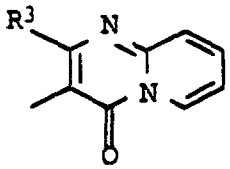


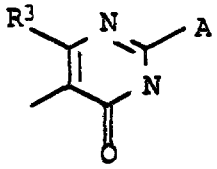


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Particularly preferred compounds are those where

R^1 is phenyl which is preferably substituted in the p position by fluorine and chlorine or in the m position by fluorine or chlorine

R^2 is hydrogen and

R^3 is methyl and hydroxyl

and the ring system on the right in the molecule is derived from

7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one,
7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidin-5-one,
8-methyl-2H,6H-pyrimido[2,1-b][1,3]thiazin-6-one,
2,4(1H,3H)-quinazolinedione,
2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one,
6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one or
2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone.

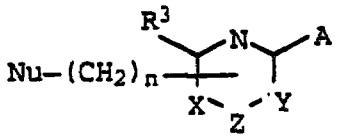
The following compounds are particularly preferred:

6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one,
6-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one,
6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]-pyrimidin-5-one,
6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-8-methyl-2H,6H-pyrimido[2,1-b][1,3]thiazin-6-one,
6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-2,4-(1H,3H)-quinazolinedione,

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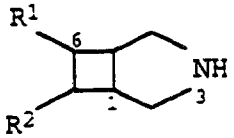
3-β-[exo-6-p-trifluoromethylphenyl-3-azabicyclo[3.2.0]-heptan-3-yl]-ethyl-2,4-(1H,3H)-quinazolinedione,
3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-2-methyl-4H-pyrido-[1,2-a]pyrimidin-4-one,
3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]-pyrimidin-4-one,
5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone,
5-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]heptan-3-yl]-ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone.

The compounds of the formula I according to the invention can be prepared by reacting a compound of the formula II



II

where n, R^3, X, Y, Z and A have the abovementioned meanings, and Nu is a nucleofugic leaving group, with a 3-azabicyclo[3.2.0]heptane derivative of the formula III



III

where R^1 and R^2 have the abovementioned meanings, and converting the resulting compound where appropriate into the addition salt with a physiologically tolerated acid.

Suitable and preferred for the nucleofugic leaving group Nu are halogens, especially bromine or chlorine.

The reaction is expediently carried out in the presence of an inert base such as triethylamine or potassium carbonate to bind the acid and in an inert solvent such as a cyclic saturated ether, especially tetrahydrofuran or dioxane, or an aromatic hydrocarbon

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such as toluene or xylene.

The reaction is usually carried out at from 20 to 150°C, in particular from 80 to 140°C, and is generally complete after from 1 to 10 hours.

The compounds of the formula I according to the invention can be purified either by recrystallization from conventional organic solvents, preferably from a lower alcohol such as ethanol, or by column chromatography.

Racemates can be resolved into the enantiomers in a straightforward way by classical methods using optically active carboxylic acids, eg. tartaric acid derivatives, in an inert solvent, eg. lower alcohols.

The free 3-azabicyclo[3.2.0]heptane derivatives of the formula I can be converted in a conventional way into the addition salt with a pharmacologically acceptable acid, preferably by adding one equivalent of the appropriate acid to a solution thereof. Examples of pharmaceutically acceptable acids are hydrochloric acid, phosphoric acid, sulfuric acid, methanesulfonic acid, sulfamic acid, maleic acid, fumaric acid, oxalic acid, tartaric acid or citric acid.

The compounds according to the invention have valuable pharmacological properties. They can be used as neuroleptics (especially atypical), antidepressants, sedatives, hypnotics, CNS protectives or muscle relaxants. It is possible for more than one of the said effects to occur together in a compound according to the invention. The pharmacological effects are demonstrated both in vivo and in vitro, and the substances can be characterized in particular by the affinity, which is in some cases very high and selective, for receptor subtypes such as dopamine D_1, D_2, D_3 and D_4 receptors, serotonin 1A, 1D and 2 receptors, alpha 1 and 2 receptors, histamine 1 and muscarine receptors.

The following methods are used for the in vivo characterization:

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a) Influence on orientation motility

Mice in a new environment show exploratory behavior which is manifested by increased motor activity. This motor activity is measured in a cage with a photoelectric detector for 30 min after the animals (female NMRI mice) have been placed in the cage.

ED50: dose which reduces the motor activity by 50% from that of placebo-treated controls.

b) Apomorphine antagonism

Female NMRI mice receive 1.21 mg/kg apomorphine s.c. At this dose, apomorphine leads to motor activation which is manifested by continuous climbing when the animals are placed in wire mesh cages. The climbing is scored in the following way (every 2 min for 30 min):

0: animal has four paws on the floor

1: animal has two paws on the wire

2: animal has four paws on the wire (is climbing).

The climbing behavior is inhibited by pretreatment with antipsychotics.

ED50: dose which inhibits the climbing activity of the animals by 50% from that of placebo-treated controls.

c) Methamphetamine antagonism

Female NMRI mice receive 1 mg/kg methamphetamine orally and, after 30 min, are placed in a cage with a photoelectric detector to measure the motor activity (2 animals/cage, 4 cages/dose). The test substances are given orally 30 min before the methamphetamine. The increase in activity caused by methamphetamine for the period from 15 to 60 min after the animals are placed in the cage is calculated as the difference between methamphetamine controls and placebo controls and set equal to 100%. The ED100 is the dose of test substance which completely abolishes the increase in activity.

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d) L-5-HTP antagonism

Female Sprague-Dawley rats receive L-5-HTP in a dose of 316 mg/kg i.p. The animals then develop an excitation state, and the following symptoms of this
- for paw [sic] treading and
- tremor

are scored (0 = absent, 1 = moderate, 2 = marked) every 10 min from 20 to 60 min after L-5-HTP administration. The average score after L-5-HTP administration is 17. The test substances are administered orally 60 min before L-5-HTP. The ED50 is the dosage which reduces the average score by 50% from that of the controls.

The stated methods are suitable for characterizing substances as antipsychotics; in particular, the inhibition of the methamphetamine-induced motor stimulation is regarded as predictive of an antipsychotic effect. Inhibition of the L-5-HTP excitation indicates a serotonin-antagonistic effect, which is characteristic of atypical neuroleptics.

The novel compounds show a good effect in these tests.

The present invention accordingly also relates to a therapeutic composition which has a content of a compound of the formula I or the pharmacologically acceptable acid addition salt thereof as active ingredient in addition to conventional excipients and diluents, as well as to the use of the novel compounds for controlling diseases.

The compounds according to the invention can be administered orally or parenterally, intravenously or intramuscularly, in a conventional way.

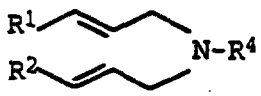
The dosage depends on the age, condition and weight of the patient and on the mode of administration. The daily dose of active ingredient is normally about 1-100 mg/kg of body weight on oral administration and 0.1-10 mg/kg of body weight on parenteral administration.

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The novel compounds can be used in conventional solid or liquid pharmaceutical forms, eg. as uncoated or (film-)coated tablets, capsules, powders, granules, suppositories, solutions, ointments, creams or sprays. These are produced in a conventional way. The active ingredients can for this purpose be processed with conventional pharmaceutical aids such as tablet binders, fillers, preservatives, tablet disintegrants, flow regulators, plasticizers, wetting agents, dispersants, emulsifiers, solvents, retarding agents, antioxidants and/or propellent gases (cf. H. Sucker et al.: Pharmazeutische Technologie, Thieme-Verlag, Stuttgart, 1978). The forms obtained in this way normally contain from 1 to 99% by weight of the active ingredient.

The substances of the formula II which are required as starting materials for synthesizing the novel compounds are known or can be synthesized from appropriate starting materials by preparation methods described in the literature.

The substances of the formula III can be prepared by subjecting an amine of the formula



IV

where R^1 and R^2 have the abovementioned meanings, and R^4 is hydrogen, acetyl, benzyl or trifluoroacetyl, to a photochemical 2+2 cycloaddition and, where appropriate, eliminating an acyl or benzyl group.

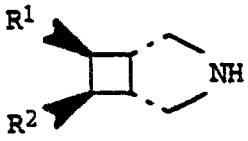
The photoreaction takes place satisfactorily in an inert solvent, preferably acetone, at from 20 to 80°C. A particularly suitable light source is a high-pressure mercury lamp. It is advantageous where appropriate to carry out the photocycloaddition in a silica apparatus under a nitrogen atmosphere with or without the addition of about 1 mol of hydrochloric acid per mol of amine.

In most cases, the photocycloaddition takes place highly diastereoselectively to give the bicyclic com-

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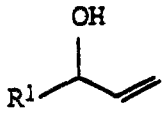
pounds III with R^1 and R^2 in the exo configuration:



The two enantiomers can be isolated pure by racemate resolution, eg. using optically active tartaric acid derivatives.

An acyl group is eliminated by conventional methods, as is a benzyl group.

The amines of the formula IV are disclosed in the literature or can be prepared by either reacting an aldehyde R^1-CHO with vinylmagnesium chloride to give the allyl alcohol V



V

subsequently rearranging with hydrogen chloride to give the allyl chloride VI



VI

and finally substituting with the appropriate allylamine VII



VII

or subjecting a cinnamaldehyde VIII



VIII

directly to reductive amination with the allylamine VII where R^4 is hydrogen.

The following examples illustrate the invention:

A Preparation of the starting materials

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aa) 1-(4-Fluorophenyl)allyl alcohol

1550 ml (2.0 M [sic]) of a 1.29 M solution of vinylmagnesium chloride in tetrahydrofuran were introduced under nitrogen to a 4 l stirred flask. Subsequently, while stirring under nitrogen, a solution of 222.0 g (1.764 M [sic]) of 4-fluorobenzaldehyde in tetrahydrofuran was added at 30-35°C in the course of 30 min while cooling the mixture in ice. The mixture was then stirred at room temperature under nitrogen for 2.5 h. Subsequently, while stirring and cooling with ice, 180 ml of water were added, the mixture was filtered with suction, and the residue on the filter was washed three times with 150 ml of tetrahydrofuran. The filtrates were combined, dried with sodium sulfate and concentrated to yield 265.7 g (99%) of product as a yellowish brown oil.

ab) 3-(4-Fluorophenyl)allyl chloride

273.6 g (1.798 M [sic]) of 1-(4-fluorophenyl)-allyl alcohol were dissolved by stirring in 2000 ml of methanol. Subsequently, 101.0 g (2.770 M [sic]) of hydrogen chloride were passed in over the course of 3 h, during which the temperature rose to 37°C. The mixture was then stirred for 1 h, washed with 600 ml of ice-cold water and a mixture of 150 ml of saturated brine and 150 ml of water and then dried over sodium sulfate and concentrated to yield 294.6 g (98%) of a brown oil.

ac) N-Allyl-N-[3-(4-fluorophenyl)allyl]amine

231.8 g (1.359 M [sic]) of 3-(4-fluorophenyl)-allyl chloride were added over the course of 25 min to a refluxing solution of 795.0 g (13.92 M [sic]) of allylamine in 360 ml of toluene, and then refluxing was continued for 1 h. Subsequently, 1000 ml were distilled out

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through a 10 cm column (5 mm glass rings) with the bath at up to 125°C. 1000 ml of water were added to the residue, and the pH was adjusted to 0.7 with 38% strength hydrochloric acid. The organic phase was separated off and discarded. The aqueous phase was adjusted to pH 12.7 with 50% strength sodium hydroxide solution and was extracted with toluene and concentrated. The residue was distilled through a column under 0.7-1 mbar to yield, with the bath at 120-160°C, 191.8 g (74%) of a pale yellow oil.

ad) exo-6-(p-Fluorophenyl)-3-azabicyclo[3.2.0]heptane

130 ml of 10% strength hydrochloric acid and 600 mg of Michler's ketone were added to 19.4 g (102 mM [sic]) of N-allyl-N-[3-(4-fluorophenyl)allyl]amine, and the mixture was exposed under nitrogen to a 150 watt high-pressure mercury lamp in a silica apparatus at room temperature for 55 h. The mixture was then evaporated and the residue was partitioned between methylene chloride and water. The aqueous phase was made alkaline with aqueous ammonia solution and extracted twice more with methylene chloride. The combined organic phases were dried with sodium sulfate and concentrated.

Yield 19.3 g (99%), melting point: 165-166°C (maleate).

The antipodes were separated by adding 15.0 g (78.5 mM [sic]) of the racemate to a solution of 31.7 g (78.5 mM [sic]) of (-)-di-O-toluoyl-L-tartaric acid in 300 ml of boiling ethanol. The crystals which separated out on cooling (13.8 g) were filtered off with suction, washing with ethanol and recrystallized from 200 ml of ethanol with the addition of 200 ml of water.

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Liberation of the base provided the (+) antipode (5.5 g) with $[\alpha]_D$ = + 970° (EtOH, c = 0.969). 14.2 g of salt crystallized out of the mother liquor overnight and were recrystallized from 400 ml of ethanol (insolubles filtered off at the boiling point and solution concentrated to 300 ml). Liberation of the base yielded 4.0 g of the (-) antipode with $[\alpha]_D$ = -96.0°. EtOH, c = 0.940).

The exo-phenyl configurations were demonstrated by X-ray structural analysis.

ae) exo-6-Phenyl-3-azabicyclo[3.2.0]heptane

300 ml of 10% strength hydrochloric acid were added to 50.0 g (28.9 mM [sic]) of N-cinnamyl-N-allylamine in 1600 ml of acetone and the mixture was exposed under nitrogen to a 150 watt high-pressure mercury lamp in a silica apparatus at room temperature for 48 h. The mixture was then concentrated, and the residue was partitioned between methylene chloride and water. The aqueous solution was made alkaline with aqueous ammonia solution and then extracted twice more with methylene chloride. The combined organic phases were dried with sodium sulfate and concentrated.

Yield 49.0 g (98%) of viscous oil, melting point 177-178°C (maleate).

af) exo-6,7-Diphenyl-3-azabicyclo[3.2.0]heptane

16.0 g (254 mM [sic]) of ammonium formate and 2.0 g of palladium (10%) on carbon were added to 12.0 g (35.4 mM [sic]) of exo-6,7-diphenyl-3-benzyl-3-azabicyclo[3.2.0]heptane in a mixture of 300 ml of n-propanol and 16 ml of water, and the mixture was refluxed for 4 h (evolution of carbon dioxide). After cooling, the catalyst was filtered off by suction, washing with propanol and methylene chloride,

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and the filtrate was concentrated. The residue was partitioned between methylene chloride and water, and the aqueous phase was made alkaline with aqueous ammonia solution and extracted twice more with methylene chloride. The combined organic phases were dried with sodium sulfate and concentrated to yield 8.1 g (92%) of product, melting point 140-142°C (maleate).

ag) N-Allyl-N-3-(3,5-dichlorophenyl)allylamine

4.5 ml (60 mM [sic], 3.4 g) of allylamine and 17.0 g of sodium sulfate were added to 12.0 g (59.7 mM [sic]) of 3,5-dichlorocinnamaldehyde in 180 ml of methylene chloride, and the mixture was stirred at room temperature for 24 h. The sodium sulfate was then filtered off and washed with methylene chloride, and the filtrate was evaporated to dryness. The resulting yellow oil was dissolved in 200 ml of absolute methanol and, under nitrogen, 2.5 g (66.0 mM [sic]) of sodium borohydride were added a little at a time. The mixture became slightly warm and was then stirred for 1 h and subsequently neutralized (pH = 7) with 10% strength hydrochloric acid. The solvent was removed under reduced pressure, and the residue was taken up in methylene chloride. The organic phase was washed twice with water, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (silica gel, methylene chloride + 5% methanol).

Yield: 9.2 g (63%) of yellow oil.

ah) N-Allyl-2,2,2-trifluoro-N-[3-(3-pyridyl)allyl]-acetamide

16.1 g (76.6 mM [sic]) of trifluoroacetic anhydride were slowly added dropwise to a solution of 10.0 g (57.5 mM [sic]) of N-allyl-N-3-(3-pyridyl)allylamine and 10.7 ml of tri-

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ethylamine in 100 ml of tetrahydrofuran at 0°C. The mixture was then stirred at room temperature for 2 h, poured into 250 ml of ice-water and extracted three times with 150 ml of methyl tert.-butyl ether each time. The combined organic phases were dried over sodium sulfate and concentrated. Yield: 14.3 g (92%) of dark brown oil.

ai) 2,2,2-Trifluoro-1-[exo-6-(3-pyridyl)-3-azabicyclo[3.2.0]hept-3-yl]ethanone

14.0 g (51.8 mM [sic]) of N-allyl-2,2,2-trifluoro-N-[3-(3-pyridyl)allyl]acetamide were dissolved in 140 ml of acetone, 30 ml of a 10% strength aqueous hydrochloric acid were added, and the mixture was exposed to a 150 watt high-pressure mercury lamp in a Duran glass apparatus at room temperature under nitrogen for 48 h. The solution was then concentrated, the residue was taken up in 150 ml of water, and the solution was adjusted to pH 8-9 with an aqueous ammonia solution. The aqueous phase was extracted twice with methyl tert.-butyl ether, and the combined organic phases were dried over sodium sulfate and concentrated. The residue was fractionated by column chromatography (silica gel, methylene chloride + 2% methanol) to yield 6.2 g (42%) of unchanged N-allyl-2,2,2-trifluoro-N-[3-(3-pyridyl)allyl]acetamide and 3.7 g (26%) of 2,2,2-trifluoro-1-[exo-6-(3-pyridyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethanone as dark oil.

ak) exo-6-(3-Pyridyl)-3-azabicyclo[3.2.0]heptane

2.5 g of potassium hydroxide pellets were added to a solution of 3.7 g (13.7 mM [sic]) of 2,2,2-trifluoro-1-[exo-6-(3-pyridyl)-3-azabicyclo[3.2.0]hept-3-yl]ethanone in 50 ml of ethanol, and the solution was then stirred at

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room temperature for 2 h and subsequently poured into 100 ml of ice-water. The aqueous phase was extracted three times with methyl tert.-butyl ether, and the combined organic phases were dried over sodium sulfate and concentrated. Yield 2.3 g (96%) of yellow oil, melting point 202-205°C (hydrochloride).

The following substances can be prepared in a similar way:

al) exo-6-(m-fluorophenyl)-3-azabicyclo[3.2.0]-heptane

am) exo-6-(o-fluorophenyl)-3-azabicyclo[3.2.0]-heptane, melting point 118-120°C (maleate)

an) exo-6-(p-chlorophenyl)-3-azabicyclo[3.2.0]-heptane, melting point 152-154°C (maleate)

ao) exo-6-(m-chlorophenyl)-3-azabicyclo[3.2.0]-heptane, melting point 130-132°C (maleate)

ap) exo-6-(p-methoxyphenyl)-3-azabicyclo[3.2.0]-heptane

aq) exo-6-(m-methoxyphenyl)-3-azabicyclo[3.2.0]-heptane

ar) exo-6-(p-nitrophenyl)-3-azabicyclo[3.2.0]heptane, melting point 158-160°C (maleate)

as) exo-6-(m-nitrophenyl)-3-azabicyclo[3.2.0]heptane

at) exo-6-(p-trifluoromethylphenyl)-3-azabicyclo-[3.2.0]heptane, melting point 155-156°C (maleate)

au) exo-6-(m-trifluoromethylphenyl)-3-azabicyclo-[3.2.0]heptane

av) exo-6-(3,4-dichlorophenyl)-3-azabicyclo[3.2.0]-heptane

aw) exo-6-(3,5-dichlorophenyl)-3-azabicyclo[3.2.0]-heptane, melting point >250°C (hydrochloride)

ax) exo-6-(3,4-dimethoxyphenyl)-3-azabicyclo-[3.2.0]heptane

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ay) exo-6-(m-hydroxyphenyl)-3-azabicyclo[3.2.0]-heptane

az) exo-6-(p-hydroxyphenyl)-3-azabicyclo[3.2.0]-heptane

ba) exo-6-(3,4-dihydroxyphenyl)-3-azabicyclo-[3.2.0]heptane

bb) exo-6-(p-methylphenyl)-3-azabicyclo[3.2.0]-heptane

bc) exo-6-(m-methylphenyl)-3-azabicyclo[3.2.0]-heptane

bd) exo-6-(p-t-butylphenyl)-3-azabicyclo[3.2.0]-heptane, melting point >255°C (hydrochloride)

be) exo-6-(m-aminophenyl)-3-azabicyclo[3.2.0]-heptane

bf) exo-6-(p-aminophenyl)-3-azabicyclo[3.2.0]-heptane

bg) exo-6-(p-cyanophenyl)-3-azabicyclo[3.2.0]-heptane, melting point 168-178°C (maleate)

bh) exo-6-thien-2-yl-3-azabicyclo[3.2.0]heptane, melting point 180-182°C (hydrochloride)

bi) exo-6-thien-2-yl-3-azabicyclo[3.2.0]heptane, melting point 143-145°C (hydrochloride)

bk) exo-6-(5-chlorothien-2-yl)-3-azabicyclo[3.2.0]-heptane, melting point 156-157°C (maleate)

bl) exo-6-pyrrol-2-yl-3-azabicyclo[3.2.0]heptane

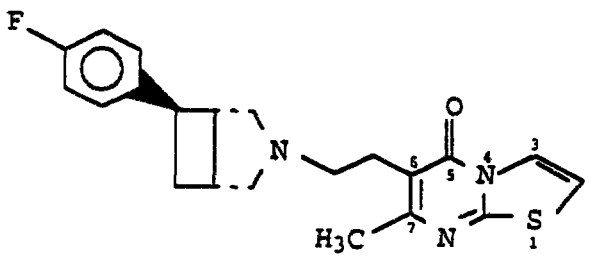
bm) exo-6-pyrid-4-yl-3-azabicyclo[3.2.0]heptane

bn) exo-6-pyrid-2-yl-3-azabicyclo[3.2.0]heptane

B Preparation of the final products

EXAMPLE 1

6-β-[exo-6-p-Fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3.2-a]pyrimidin-5-one dihydrochloride



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3.6 g (26 mM [sic]) of 6-(2-chloroethyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one and 5.6 g (40 mM [sic]) of finely powdered calcium carbonate and 0.5 g of potassium iodide were added to 2.5 g (13.1 mM [sic]) of exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptane in 40 ml of xylene, and the mixture was refluxed while stirring vigorously for 11 h.

After cooling, the mixture was concentrated in a rotary evaporator, and the residue was partitioned between methylene chloride and water.

The aqueous phase was extracted twice with methylene chloride, and then the organic phase was dried with sodium sulfate and concentrated. The crude product (7.7 g) was purified by column chromatography (silica gel, methylene chloride/methanol 96/4).

The free base (3.5 g) was taken up in 150 ml of ether, the mixture was filtered to remove insolubles, and excess ethereal hydrochloric acid was added to the solution. The solid was then filtered off with suction under nitrogen in the cold, washed with a large amount of ether and dried on the funnel under nitrogen. 3.5 g (60%) of product x 2 HCl, melting point 222-224°C, were isolated. The maleate melts at 133-135°C.

The following can be prepared in a similar way:

1a. (+)-6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3.2-a]pyrimidin-5-one maleate, melting point 158-160°C, $[\alpha]_D$ = +56.2° (EtOH)

1b. (-)-6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3.2-a]pyrimidin-5-one maleate, melting point 147-149°C, $[\alpha]_D$ = -52.8° (EtOH)

2. 6-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 167-168°C (maleate)

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3. 6-β-[exo-6,7-diphenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 154-156°C
4. 6-β-[exo-6,7-bis-(p-fluorophenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]-pyrimidin-5-one
5. 6-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 162-164°C (maleate)
6. 6-β-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 149-152°C (maleate)
7. 6-β-[exo-6-m-hydroxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (see also Example 49), melting point 76-78°C
8. 6-β-[exo-6-p-aminophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (see also Example 50)
9. 6-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 155-157°C (maleate)
10. 6-β-[exo-6-p-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 168-170°C (maleate)
11. 6-β-[exo-6-p-nitrophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
12. 6-β-[exo-6-m-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
13. 6-β-[exo-6-p-hydroxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (see also Example 49)
14. 6-β-[exo-6-p-trifluoromethylphenyl-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]-pyrimidin-5-one, melting point 187-189°C (maleate)
15. 6-β-[exo-6-(p-t-butylphenyl)-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyr-imidin-5-one, decomposition point 207-209°C

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(maleate)

16. 6-β-[endo-6-(p-t-butylphenyl)-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 131-133°C
17. 6-β-[exo-6-(3,4-dichlorophenyl)-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
18. 6-β-[exo-6-(3,4-dimethoxyphenyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]-pyrimidin-5-one, decomposition point 210-212°C (dihydrochloride)
19. 6-β-[exo-6-p-cyanophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 130-132°C
20. 6-β-[exo-6-(3,4-dihydroxyphenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (see also Example 49)
21. 6-β-[exo-6-(o-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 164-165°C (maleate)
22. 6-β-[exo-6-thien-3-yl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
23. 6-β-[exo-6-(5-chlorothien-2-yl)-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
24. 6-β-[exo-6-pyrrol-2-yl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
25. 6-β-[exo-6-pyrid-4-yl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
26. 6-β-[exo-6-pyrid-3-yl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one
27. 6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 253-255°C
28. 6-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidin-5-one

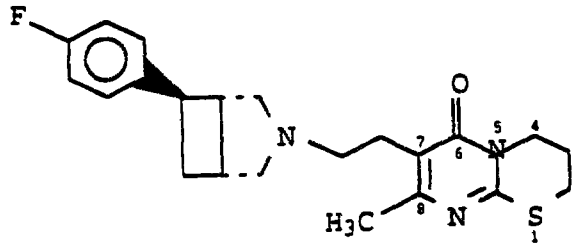
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29. 6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,3,7-trimethyl-5H-thiazolo[3,2-a]pyrimidin-5-one
30. 6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-5H-thiazolo[3,2-a]pyrimidin-5-one, melting point 164-166°C (dihydrochloride x H_2O)
31. 6-β-[exo-6-phenylphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-5H-thiazolo[3,2-a]pyrimidin-5-one.

EXAMPLE 32

7-β-[exo-6-p-Fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one dihydrochloride



3.3 g (13.6 mM [sic]) of 7-chloroethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one and 5.0 g (36 mM [sic]) of finely powdered potassium carbonate and 0.5 g of potassium iodide were added to 2.5 g (13.1 mM [sic]) of exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptane in 40 ml of xylene, and the mixture was refluxed while stirring vigorously for 12 h.

After cooling, the mixture was concentrated in a rotary evaporator, and the residue was partitioned between methylene chloride and water.

The aqueous phase was then extracted twice with methylene chloride, and the organic phase was dried with sodium sulfate and concentrated. The crude product (5.6 g) was purified by column chromatography (silica gel, methylene chloride/methanol 93/7).

The free base was taken up in 200 ml of ether,

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the mixture was filtered to remove insolubles, and excess ethereal hydrochloric acid was added to the solution. The solid was then filtered off with suction under nitrogen in the cold, washed with a large amount of ether and dried on the funnel under nitrogen. 3.2 g (52%) of product x 2 HCl, melting point 120-121°C, were isolated.

The following can be prepared in a similar way:

33. 7-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one,

34. 7-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one,

35. 7-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one,

36. 7-β-[exo-6-p-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one,

37. 7-β-[exo-6-m-hydroxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2H,6H-pyrimido[2.1-b][1.3]thiazin-6-one (see also Example 49),

38. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, melting point 158-160°C,

38a. (+)-3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, melting point 160-162°C, $[\alpha]_D$ = +88.6° (CH_2Cl_2)

38b. (-)-3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, melting point 161-162°C, $[\alpha]_D$ = -87.5° (CH_2Cl_2)

39. 3-β-[3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 158-160°C,

40. 3-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-2,4(1H,3H)-quinazolinedione, melting point 144-146°C,

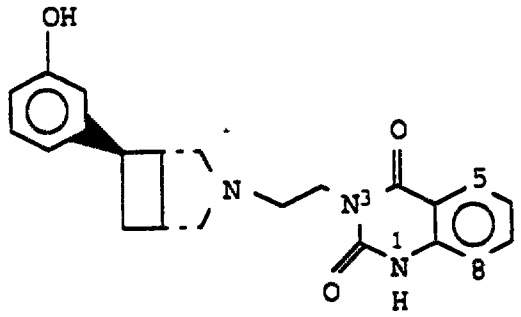
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41. 3-β-[exo-6-m-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione,
42. 3-β-[exo-6-p-cyanophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 230-232°C,
43. 3-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 183-185°C,
44. 3-β-[exo-6-p-hydroxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 220-223°C (see also Example 49),
45. 3-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 187-189°C,
46. 3-β-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 113-115°C,
47. 3-β-[exo-6-p-nitrophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 209-211°C,
48. 3-β-[endo-6-p-nitrophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 212-214°C.

EXAMPLE 49

3-β-[exo-6-m-Hydroxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione



28.5 ml (28.5 mM [sic]) of boron tribromide (1 M solution in methylene chloride) were added dropwise to

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4.6 g (11.8 mM [sic]) of 3-β-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione in 120 ml of methylene chloride at room temperature, and the mixture was stirred overnight. After cooling, 100 ml of 2 N sodium hydroxide solution were added, the organic phase was separated off, and the aqueous phase was extracted with methylene chloride. Drying and concentration resulted in 4.7 g of crude product which was purified by column chromatography (silica gel, methylene chloride/methanol 96/4). Yield: 2.8 g (61%), melting point 149-151°C (hydrochloride).

EXAMPLE 50

3-β-[exo-6-p-Aminophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione

16.3 g (40.1 mM [sic]) of 3-β-[exo-6-p-nitrophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione were dissolved in 300 ml of glacial acetic acid, 1.7 g of palladium on carbon (10%) were added, and the mixture was hydrogenated at room temperature under atmospheric pressure for 4 h. The catalyst was filtered off with suction, the mother liquor was concentrated, the residue was taken up in 400 ml of water, the solution was made alkaline by stirring with concentrated ammonia, and the precipitated solid was filtered off with suction, washing with water. The crude product (15.3 g) was purified by column chromatography (silica gel, methylene chloride/methanol 95/5). Yield: 12.4 g (76%) with melting point 196-198°C.

EXAMPLE 51

3-β-[exo-6-p-Iodophenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-2,4(1H,3H)-quinazolinedione

5.6 g (14.9 mM [sic]) of 3-β-[exo-6-p-aminophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione were dissolved in 100 ml of 50% concentrated hydrochloric acid. At 0-5°C, a solution of 1.05 g (15.0 mM [sic]) of sodium nitrite in 6 ml of water was added dropwise, and the mixture was then stirred at the

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same temperature for 20 min. Subsequently a solution of 2.5 g (15.0 mM [sic]) of potassium iodide in 12 ml of water was added, the ice bath was removed, and the mixture was slowly heated while stirring vigorously to 85-90°C. After 40 min, the mixture was allowed to cool, ice/water were added, the mixture was made alkaline with concentrated ammonia, 300 ml of methylene chloride were added, and the mixture was then stirred vigorously. After phase separation, the aqueous phase was extracted with methylene chloride, and the combined organic phases were dried and concentrated. The crude product (6.0 g) was purified by column chromatography (silica gel, methylene chloride/ methanol 95/5). Yield: 3.2 g (57%), melting point 162-164°C.

52. 3-β-[exo-6-p-trifluormethylphenyl-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 190-192°C,
53. 3-β-[exo-6-(3,4-dichlorophenyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione,
54. 3-β-[exo-6-(3,4-dihydroxyphenyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione (as Example 49),
55. 3-β-[exo-6-(3,5-dichlorophenyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione, melting point 189-192°C,
56. 3-β-[exo-6-o-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)-quinazolinedione,
57. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methyl-2,4(1H,3H)-quinazolinedione, melting point 170-173°C,
58. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-chloro-2,4(1H,3H)-quinazolinedione, melting point 214-216°C,
59. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-fluoro-2,4(1H,3H)-quinazolinedione,
60. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-5-chloro-2,4(1H,3H)-quinazolinedione,

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melting point

61. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-fluoro-2,4(1H,3H)-quinazolinedione, melting point 186-188°C,
62. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-methyl-2,4(1H,3H)-quinazolinedione, melting point 166-168°C,
63. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-8-methoxy-2,4(1H,3H)-quinazolinedione,
64. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-trifluoromethyl-2,4(1H,3H)-quinazolinedione,
65. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-nitro-2,4(1H,3H)-quinazolinedione,
66. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-nitro-2,4(1H,3H)-quinazolinedione,
67. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-amino-2,4(1H,3H)-quinazolinedione,
68. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-amino-2,4(1H,3H)-quinazolinedione,
69. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6-hydroxy-2,4(1H,3H)-quinazolinedione,
70. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-5-chloro-2,4(1H,3H)-quinazolinedione, melting point 194-196°C (maleate),
71. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,7-dimethoxy-2,4(1H,3H)-quinazolinedione, melting point 203-205°C,
72. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,8-dichloro-2,4(1H,3H)-quinazolinedione,
73. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,7,8-trimethoxy-2,4(1H,3H)-quinazolinedione,
74. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1-methyl-2,4-(1H,3H)quinazolinedione, melting point 89-90°C,

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75. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1-ethyl-2,4(1H,3H)-quinazolinedione, melting point 92-95°C (hydrochloride),
76. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1-allyl-2,4(1H,3H)-quinazolinedione,
77. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1-benzyl-2,4(1H,3H)-quinazolinedione, melting point 133-135°C,
78. 3-γ-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]propyl-2,4(1H,3H)quinazolinedione, melting point 75-77°C,
79. 3-δ-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]butyl-2,4(1H,3H)quinazolinedione,
80. 3-β-[exo-6-(2-thienyl)-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, melting point 171-173°C,
81. 3-β-[exo-6-(5-chloro-2-thienyl)-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, decomposition above 176°C,
82. 3-β-[exo-6-(3-thienyl)-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4(1H,3H)quinazolinedione, melting point 158-159°C,
83. 3-β-[exo-6-(3-pyridyl)-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,4-(1H,3H)quinazolinedione, decomposition above 84°C,
84. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1H-thieno[3.2-d]pyrimidine-2,4-dione, melting point 230-232°C,
85. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1H-thieno[2.3-d]pyrimidine-2,4-dione,
86. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1H-thieno[3.4-d]pyrimidine-2,4-dione,
87. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1-methyl-1H,3H-pyrido[2.3-d]pyrimidine-2,4-dione,

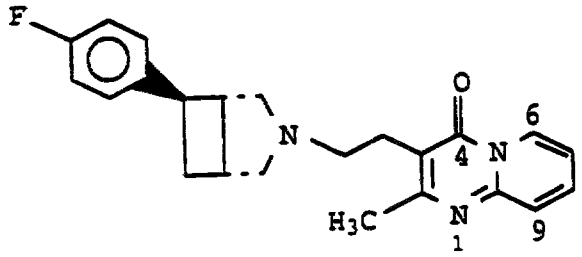
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88. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-3H-quinazolin-4-one, decomposition above 225°C (hydrochloride),

89. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methoxy-3H-quinazolin-4-one.

EXAMPLE 90

3-β-[exo-6-p-Fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one dihydrochloride



3.8 g (17 mM [sic]) of 3-(2-chloroethyl)-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one and 4.2 g (30 mM [sic]) of finely powdered potassium carbonate and 0.5 g of potassium iodide were added to 3.0 g (15.7 mM [sic]) of exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]heptane in 60 ml of xylene, and the mixture was refluxed with vigorous stirring for 11 h. After cooling, the mixture was concentrated in a rotary evaporator and the residue was partitioned between methylene chloride and water. The aqueous phase was extracted twice with methylene chloride and then the organic phase was dried with sodium sulfate and concentrated. The crude product (7.8 g) was purified by column chromatography (silica gel, methylene chloride/methanol 94/4). The free base (3.4 g) was taken up in 200 ml of ether, the mixture was filtered to remove insolubles, and excess ethereal hydrochloric acid was added to this solution. The solid was then filtered off with suction under nitrogen in the cold, washed with a large quantity of ether and dried on the funnel under nitrogen. 3.8 g (54%) of product x 2 HCl, melting point

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>250°C, were isolated.

The following can be prepared in a similar way:

91. 3-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one,
92. 3-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one,
93. 3-β-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one,
94. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,6-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point 59-61°C (dihydrochloride),
95. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,7-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point 247-249°C (dihydrochloride),
96. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,8-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point >250°C (dihydrochloride),
97. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,9-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, decomposition >208°C (dihydrochloride),
98. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,6,8-trimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point >260°C (dihydrochloride),
99. 3-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,7-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point >250°C (dihydrochloride),
100. 3-β-[exo-6-o-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,7-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point 262-264°C (dihydrochloride),
101. 3-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]-ethyl-2,8-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, melting point >250°C (dihydrochloride),
102. 3-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,9-dimethyl-4H-pyrido[1.2-a]pyrimidin-4-one, decomposition >213°C (dihydrochloride),

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103. 3-β-[exo-6-(5-chlorothien-2-yl)-3-azabicyclo[3.2.0]-hept-3-yl]ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one,
104. 3-β-[exo-6-pyrid-4-yl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-4H-pyrido[1.2-a]pyrimidin-4-one,
105. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,7,8,9-tetrahydro-2-methyl-4H-pyrido-[1.2-a]pyrimidin-4-one, melting point 151-153°C (maleate),
106. 3-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,7,8,9-tetrahydro-2-methyl-4H-pyrido-[1.2-a]pyrimidin-4-one,
107. 3-β-[exo-6-p-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-6,7,8,9-tetrahydro-2-methyl-4H-pyrido-[1.2-a]pyrimidin-4-one,
108. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-4H-pyrimido[1.2-a]pyrimidin-4-one,
109. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-7-chloro-4H-pyrimido[1.2-a]pyrimidin-4-one,
110. 6-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-7-methyl-1H,5H-imidazo[1.2-a]pyrimidin-4-one,
111. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methyl-7,8-dihydro-4H,6H-pyrrolo[1.2-a]-pyrimidin-4-one,
112. 2-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methylbenzimidazole, melting point 166-168°C,
113. 1-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethylbenzimidazole, melting point 94-96°C, (hydrochloride),
114. 1-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2,3-dihydrobenzimidazol-2-one,
115. 2-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]methylbenzimidazole,

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116. 3-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethylindole, melting point 193-195°C (hydrochloride),
117. 3-β-2-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethylindole,
118. 3-β-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]ethylindole, melting point 104-105°C (hydrochloride),
119. 3-β-[exo-6-m-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]ethylindole,
120. 2-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-3,4-dihydro-4-quinazolinone, melting point 152-154°C,
121. 2-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-3,4-dihydro-4-quinazolinethione,
122. 2-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-3,4-dihydro-4-quinazolinone, melting point 147-149°C,
123. 2-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-3,4-dihydro-4-quinazolinone,
124. 2-[exo-6-o-methoxyphenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-3,4-dihydro-4-quinazolinone,
125. 2-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-4-hydroxy-6-methylpyrimidine, melting point 174-175°C,
126. 6-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyl-2-methyl-4-hydroxypyrimidine, melting point 147-149°C (dihydrochloride),
127. 6-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]methyluracil, melting point 201-203°C,
128. 6-[exo-6-phenyl-3-azabicyclo[3.2.0]hept-3-yl]methyluracil, melting point 183-184°C,
129. 6-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimidine, melting point 108-110°C (hydrochloride),

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130. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-6-methyl-2,4-dioxopyrimidine, melting point 197-199°C,
131. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-1,6-dimethyl-2,4-dioxopyrimidine, melting point 186-188°C,
132. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-3,6-dimethyl-2,4-dioxopyrimidine,
133. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-3,6-dimethyl-2,4-dithiopyrimidine,
134. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-1,3,6-trimethyl-2,4-dioxopyrimidine, melting point 90-93°C (hydrochloride),
135. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-thiomethyl-6-methyl-4(3H)-pyrimidinone,
136. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-mercapto-6-methyl-4(3H)-pyrimidinone,
137. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-thiomethyl-3,6-dimethyl-4(3H)-pyrimidinone,
138. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-1,2,3,4-tetrahydro-6-amino-1-methyl-2,4-dioxopyrimidine
139. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-amino-6-methyl-4(3H)-pyrimidinone,
140. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-amino-3,6-dimethyl-4(3H)-pyrimidinone, melting point 78-80°C,
141. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone, melting point 163-165°C (dihydrochloride),
142. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-dimethylamino-4(3H)-pyrimidinone,

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143. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3-ethyl-6-methyl-4(3H)-pyrimidinone,

144. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-amino-3-ethyl-6-methyl-4(3H)-pyrimidinone, melting point 77-80°C (hydrochloride),

145. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-ethylamino-3,6-dimethyl-4(3H)-pyrimidinone,

146. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-n-propylamino-3,6-dimethyl-4(3H)-pyrimidinone,

147. 5-β-[exo-6-p-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone, decomposition above 144°C (dihydrochloride x 2 H_2O),

148. 5-β-[exo-6-m-chlorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone, melting point 147-149°C (dihydrochloride),

149. 5-β-[exo-6-o-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone, melting point 173-175°C (dihydrochloride),

150. 5-β-[exo-6-p-trifluoromethylphenyl-3-azabicyclo-[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone,

151. 5-β-[exo-6-p-cyanophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone,

152. 5-β-[exo-6-m-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-3,6-dimethyl-4(3H)-pyrimidinone,

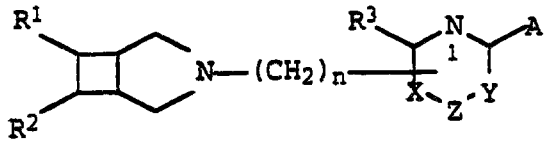
153. 5-β-[exo-6-p-fluorophenyl-3-azabicyclo[3.2.0]hept-3-yl]ethyl-2-methylamino-4-methoxy-6-methylpyrimidine.

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We claim:

1. An N-substituted 3-azabicyclo[3.2.0]heptane derivative of the formula I



I

where

R^1 is hydrogen or phenyl, pyridyl, thienyl or pyrrole [sic], each of which is unsubstituted or mono- or disubstituted by halogen, C_1-C_4-alkyl, trifluoromethyl, hydroxyl, C_1-C_4-alkoxy, amino, monomethylamino, dimethylamino, cyano or nitro,

R^2 is hydrogen or phenyl which is unsubstituted or substituted by halogen, methoxy, hydroxyl or amino,

n is 0, 1, 2, 3 or 4,

R^3 is hydrogen, hydroxyl, C_1-C_4-alkyl or C_1-C_4-alkoxy, or together with the adjacent carbon atom is C=O or C=S,

X and Y are each carbon atoms, CH, CH_2, NH or C_1-C_4-alkyl-N groups or nitrogen atoms,

Z is a direct linkage, CO, CS or CH or CH_2 in which one hydrogen can be replaced by hydroxyl, amino, C_1-C_4-alkoxy or halogen, and

A is hydrogen, hydroxyl, amino, mercapto, C_1-C_4-alkylamino, di-C_1-C_4-alkylamino, C_1-C_4-alkylthio or C_1-C_4-alkoxy, or together with the adjacent carbon is C=O, or

A is C_3-C_4-alkylene which is attached to Y and which can contain one or two non-cumulative double bonds and in which one CH or CH_2 can be replaced by a nitrogen or sulfur atom or a NH or N-CH_3 group, and where the ring can be either monosubstituted by fluorine, chlorine, methyl, methoxy, nitro or amino or, in the case of a benzene ring, mono-, di- or trisubstituted by fluorine, chlorine, methyl, tri-

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fluoromethyl, nitro, hydroxyl, methoxy, amino, monomethylamino or dimethylamino,

and in which the ring on the right in formula I can have C_1-C_4-alkyl, allyl or benzyl attached to N-1 and can contain 1-3 non-cumulative double bonds,

and the salts thereof with physiologically tolerated acids.

2. A compound of the formula I as claimed in claim 1 for use for controlling diseases.

* C L M S *

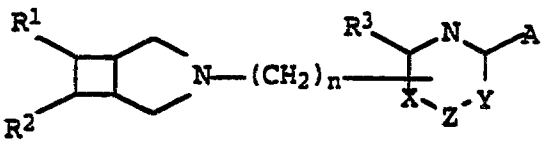
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N-SUBSTITUTED AZABICYCLOHEPTANE DERIVATIVES, THE PREPARATION AND USE THEREOF

Abstract of the Disclosure: Compounds of the formula I



I

where R^1, R^2, n, R^3, A, X, Y and Z have the meanings stated in the description, and the preparation thereof are described. The novel compounds are suitable for controlling diseases.

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☑ Description : paged ...1... to paged ...15...
☑ Claim(s) : paged ...16... to paged ...18...
☑ Drawing(s) : paged ...1/5... to paged ...5/5...
☑ Abstract : paged ...19... to paged ...-...
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DESCRIPTION

COMBINATORIAL LIBRARIES AND METHODS FOR THEIR USE

This application is a continuation-in-part of U.S. application Serial No. 08/168,966, filed December 15, 1993, which is a continuation of U.S. application Serial No. 07/978,646, filed November 19, 1992.

Background of the Invention

This invention relates to synthetic combinatorial libraries useful for drug discovery, and methods for their use in such drug discovery.

Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe the generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. These libraries are composed of mixtures of free peptides which form a heterogenous library. Systematic identification of optimal peptide ligands is achieved by screening a library followed by iterative selection and synthesis processes. For example, one library consisted of a series of six residue peptides having the first two positions specifically defined, and the last four positions consisting of a random mixture of 18 L-amino acids. This library was screened to determine which pair of defined peptides had optimum activity in an assay. A second library was then synthesized in which the optimal pair of peptides were included, the third position of each peptide individually synthesized, and the last three peptides consisted of a random mixture of 18 L-amino acids. This library was screened as before and the process repeated until the optimum six residue peptide was identified. Houghten et al. state:

> "A number of other libraries, such as one composed entirely of D-amino acids, have been prepared which in total permit the systematic screening of hundreds of millions of peptides.

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> A fundamental feature of SPCLs [synthetic peptide combinatorial libraries] is that free peptides can be generated and used in solution in virtually all existing assay systems at a concentration of each peptide most applicable to the assay. This approach has also been successfully used in radio-receptor assays (opioid peptides) and plaque inhibition assays (human immunodeficiency virus (HIV-1) and Herpes Simplex Virus (HSV)). SPCLs, as described, greatly aid all areas of drug discovery and research involving peptides."

Lam et al., 354 *Nature* 82, 1991, and WO 92/00091 (PCT/US91/04666) and Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe systematic synthesis and screening of peptide and other libraries of defined structure. The method used by Lam et al. is based on a one bead one peptide approach in which a large peptide library consisting of millions of beads are screened. Each bead contains a single peptide. The authors state:

> "It is clearly not enough to use a random mixture of activated amino acids in a peptide synthesis protocol, because the widely different coupling rates of different amino acids will lead to unequal representation and because each bead will contain a mixture of different peptides. Our solution was to use a 'split synthesis' approach. The first cycle consisted of distributing a pool of resin beads into separate reaction vessels each with a single amino acid, allowing the coupling reactions to go to completion, and then repooling the beads. The cycle was repeated several times to extend the peptide chain. In this fashion, each bead should contain only a single peptide species."

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The library of beads was screened by a staining procedure and stained beads visualized using a microscope, and removed. The structure of the peptide is obtained by a chemical analysis of the material on the single bead. Lam et al. indicate:

> "Additionally, our approach has far greater potential for applying the richness of well-established peptide chemistry to synthesize libraries incorporated D-amino acids or unnatural amino acids as well as specific secondary structures including cyclic peptides. All of this can be accomplished without need to keep records of the synthetic products as our interest is focused just on those peptides which provide a strong interaction signal with the acceptor."

Summary of the Invention

The present invention features an efficient method for the construction and decomposition of combinatorial libraries to allow identification of useful polysubunit compounds (made up from two or more subunits which may be the same or different, referred to sometimes as polymers). The method is not limited to identification of polypeptides; it can be applied to any combination of compounds made up of differing subunits (referred to sometimes as monomeric compounds) within one polysubunit compound. Synthesis of such polysubunits can be carried out chemically or with the use of catalysts, including enzymes. Thus, for example, the subunits may be selected from natural or unnatural moieties, including amino acids, nucleotides, sugars, lipids, and carbohydrates. In addition, the bond used to attach each subunit to the prior subunit can make any desired type of bond, and may include covalent, ionic or coordination bonds. The bonds may be selectively cleavable by enzymes or chemical treatments, as desired. Examples of such bonds include: peptide [R^1CONHR^2], ester [R^1COOR^2], sulfonamide [$R^1SO_2NR^2$],

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thioester [R^1COSR^2], phosphodiester [R^1OPOR^2], ether [R^1COCR^2], thioether [R^1CSCR^2], phosphamide [R^1PONH-], amine [$-CH_2NHC-$], and azo [-CNNC-] (where each R^1 and R^2 may be the same or different and may be cyclic or acyclic). In the method, a free or solid-phase-bound polysubunit can be used to allow ready screening of the synthesized polysubunits in any desired test or assay system. A significant advantage of this invention is that it provides a series of libraries or collections of polysubunits which can be reused for screening for any number of desired properties in any chosen assays.

The present method does not employ a mixed synthesis in which several different subunits are chemically incorporated within one vessel, but rather a synthesis in which a single subunit is incorporated at each step. See Figure 1.

Thus, in one example, the method involves a first step of attaching ten different subunits A, B, C . . . J, to a solid support in ten separate vessels or columns. In the second step, a portion or aliquot of the material synthesized at the first step is retained as separate columns, while the remainder (which is still attached to individual solid supports) is mixed or pooled, divided into ten new different columns, and ten further parallel syntheses carried out to provide the dimer XA^1, XB^1, XC^1 . . . XJ^1, where X is any one of the original A-J, and A^1, B^1, C^1 . . . J^1 are ten different subunits which may be the same or different from A-J. Of course, fewer or more than ten syntheses can be used in this second step. In the third step, a portion of the newly synthesized material of step two is again retained in separate columns, and the remainder mixed and divided into ten further columns so that the synthetic procedure can be repeated until the whole length of the desired polysubunit is synthesized. In this way a series of vessels is formed at each step, differing from those in prior steps by the presence of an extra subunit.

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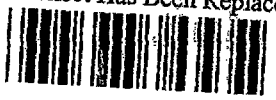
The final ten columns in the above example (each having a variety of different polysubunits with a known subunit at their terminus) can be assayed using any standard assay format. That is, each of the ten mixtures is assayed to determine which mixture contains one or more active compounds. The column which is found to contain an active compound identifies the subunit required at the polysubunit terminus to be active in the assay. For example, the column containing polysubunits of sequence XXXXJ^1 may be active in the assay. This indicates that J^1 is required at the terminus of a polysubunit in this assay. This subunit is now bonded to each of the columns retained in the previous synthetic step (in the example, the columns XXXA, XXXB, . . . XXXJ). These ten newly synthesized series of compounds can then be assayed and the process repeated until the final polysubunit sequence is known. See Figures 2 and 3.

Thus, the method of the present invention features a set or kit of partial combinatorial libraries with each library containing one subunit more than the prior library. The final library is decomposed stepwise starting with the last subunit. This form of retroanalysis or antithesis allows definition of the synthesis of the desired product, which is accomplished in the final analytic step. Once constructed, the entire set of libraries can be used for many different projects.

Thus, in a first aspect, the invention features a kit which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds, and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits, but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at their terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each

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constructed from the known number minus one subunit, as described above.

This kit forms a combinatorial library which can be screened and decomposed as discussed above. The number of first and second pluralities of vessels can be the same or different, and generally the terminus of the polysubunits to which subunits are added is the same in each set of vessels.

In preferred embodiments, the kit may include further pluralities of vessels in which the number of subunits present in the polysubunit is the known number minus two, or three, or more. Thus, each plurality of vessels differs from other pluralities of vessels in the number of subunits present in the polysubunits. Most preferably, the number of subunits in each plurality of vessels differs by one and ranges from one to the known number. As discussed above, such a kit is useful for systematic identification of the sequence of a desired polysubunit, using the method of retroanalysis.

As noted above, the polysubunits may be formed from any series of subunits desired, and the number of vessels containing these polysubunits at each step can be different for each subunit added. Thus, as described above, ten amino acids may be incorporated in one step, two or three carbohydrates may be incorporated in another step, and twenty amino acids in the following step. There is no limit to the steps of addition of subunits, nor to the choice of subunits which can be added at each step. All that is necessary for the kit to be useful is that the kit include a series of vessels which contain polysubunits differing sequentially in the number of subunits present in those polysubunits. The final plurality of vessels in the kit will contain only the first subunit free in solution, or bound to a solid support.

In a related aspect the invention features, a method for construction of a kit, following the steps

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described above, and a method for screening ~~such kits as~~ described above.

Specifically, the invention features a method for construction of a kit useful for identification of a desired compound, by the steps of: (a) providing a first plurality of vessels containing different polysubunits each constructed from a known number of subunits joined together by one or more bonds. Each subunit and bond can be the same or different, and each different polysubunit has a different sequence of subunits but the same known subunit at one terminus of the polysubunit. Each vessel includes polysubunits having a different known subunit at their terminus; (b) combining aliquots of the different polysubunits from each vessel to form a mixture; wherein the aliquots are less than all of the contents of each vessel; (c) dividing the mixture into a second plurality of vessels; and (d) bonding a further subunit to each polysubunit in the second plurality of vessels; wherein the subunit is different for each second plurality of vessels.

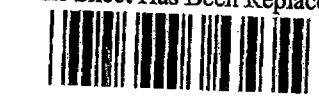
In preferred embodiments, the method further includes repeating steps (a) through (d) for the second plurality of vessels; or repeating steps (a) through (d) a plurality of times.

In a related aspect, the invention features a method for identification of a desired compound, including the steps of: (a) providing a kit as described above, and screening the first plurality of vessels to identify a vessel having a desired activity in an assay, and thereby identifying a first known subunit; and (b) coupling the first known subunit to a terminus of an aliquot of the contents of each of the different polysubunits in the second plurality of vessels.

In preferred embodiments, the method further involves screening the different polysubunits having the first known subunit for the desired activity in the assay,

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and thereby identifying a second known subunit; or repeating steps (a) and (b) a plurality of times.

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

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 illustrates split pool synthesis of a combinatorial library.

Figure 2 illustrates utilization of the deconvolution pathway to determine the sequence of a member of a combinatorial library.

Figure 3 illustrates the deconvolution of a pentapeptide library.

Figure 4 illustrates the corrected absorbances of pools of library products reacted with β-endorphin antibody to determine the sequence of the pentapeptide which bound best to the β-endorphin antibody.

Applicant has determined that a useful series, or kit, of libraries can be constructed by stepwise synthesis of a polysubunit from different subunits. While these libraries and methods for their synthesis and use will commonly be applied for use with peptides formed from standard L- or D-amino acids, those of ordinary skill in the art will recognize that other subunits and bonds other than covalent, ionic or coordination bonds can be used to form the final polysubunit, which can then be screened in any standard assay. These synthetic protocols and assays are well known to those of ordinary skill in the art and need not be repeated here. See, Lam et al., *supra*, and Houghten et al., *supra*, the whole of all of which are hereby incorporated by reference herein. As an illustrative example of a method and kit of this invention, the following is provided. This example is not limiting in the invention and those of ordinary skill will

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realize (as noted above) that other equivalent methods, syntheses, and analyses can be readily used.

Example

The method proposed here is based on a true combinatorial synthesis and makes efficient use of all of the materials. The first step is to attach ten subunits, A to J, to a solid support in ten columns. One-sixth of this material is retained in this form, giving A, B, . . . J; the remainder, still attached to the solid support, is pooled, divided into ten columns, and ten parallel syntheses are carried out, to provide XA, XB, . . . XJ. One-fifth of this material is retained in this form, and the combinatorial synthesis is repeated to give ten polysubunits, XXA, XXB, . . . XXJ. One-fourth is kept and the fourth residue is added to the remainder by combinatorial synthesis to give the polysubunits XXXA, XXXB, . . . XXXJ. After retaining one-third of these polysubunits the final combinatorial step of synthesis is completed, and the materials are removed from the column to provide ten solutions containing XXXXA, XXXXB, XXXXJ. These are now assayed.

If a positive assay result is obtained with one vessel containing polysubunits of the structure XXXXC (*i.e.*, C is the critical subunit at the fifth position in the pentasubunit), the experimenter returns to the columns retained at the previous step, and to an aliquot of each adds C, to provide ten solutions, XXXAC, XXXBC . . . XXXJC. Again, these are tested. If a positive assay result is obtained with XXXEC (*i.e.*, E is the critical subunit at position four in the pentasubunit), the experimenter returns to the previous partial library and completes it by adding E + C to all ten to give XXAEC, XXBEC . . . XXJEC. These steps are repeated until all of the positions are specified.

Thus, in essence a set of partial combinatorial libraries is made, each one residue longer than the last. The final library is decomposed stepwise, starting with

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the last residue. This form of retro-analysis allows definition of the synthesis of the desired product, which is indeed accomplished in the very last step. Once constructed, the entire set of libraries can be used again for different projects.

It is notable that the final synthetic step also provides a method for synthesis of the desired product.

Materials and Methods

Solvents. DMF (HPLC grade) was purchased from Baxter and was used without further purification. CH_2Cl_2 was purchased from Fisher Scientific and was distilled over CaH_2 before use.

Instrumentation. UV absorbances were measured on a Hewlett-Packard 8452A diode array spectrophotometer. All centrifugations were carried out on an Eppendorf 5415 C centrifuge for one minute at 10,000 rpm.

Formation of the Pentapeptide Library. The peptide libraries were synthesized manually by a solid phase method. Tentagel (TG) resin was the solid support for all synthesis and was 90 μm in diameter. The amino acids used for the formation of the libraries were Gly-FMOC, Leu-FMOC, Phe-FMOC and Tyr(tBu)-FMOC. All amino acids were coupled with the aid of HBTU and DIPEA. The Tentagel, HBTU, DIPEA and amino acids used were purchased from NovaBiochem. Tentagel was added to 4 glass fritted filter vials, shaken with DMF and filtered. To each vial was added one of the FMOC protected amino acid components of the library, HBTU, DMF and 20 μl DIPEA. All four vials were shaken for one hour, filtered and washed with DMF. A 5% solution (v/v) of acetic anhydride in DMF was added to each vial and shaken for 20 minutes to cap any uncoupled free amino acid groups. The beads were then filtered, washed with DMF (2x) and CH_2Cl_2 (2x) and dried for two hours in a vacuum oven (55°C at 20 mm Hg). Approximately, a 75 mg portion of the amino acid coupled Tentagel bead was taken from each vial and labeled in a manner describing the length of the peptide and the

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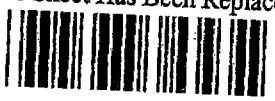
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identity of the last amino acid coupled. Also, an approximately 5 mg portion of the resin was taken to do an FMOC deprotection test in order to quantitate the completeness of the previous amino acid coupling step. The remaining resin was combined and shaken in 3:1 (v/v) DMF/CH_2Cl_2 for 30 minutes to randomize the beads. The beads were quartered into equivalent weight (and therefore equivalent moles) and set into 4 glass fritted filter vials and subjected twice to 10 minute shakings with 20% Piperidine in DMF (v/v) in order to cleave the N-terminal FMOC protecting group. The beads were filtered, washed with DMF (3x) and coupled with one of the 4 amino acid components of the library to give the 4 dipeptide pools, TG-Xaa-Gly-FMOC, TG-Xaa-Leu-FMOC, TG-Xaa-Phe-FMOC, and TG-Xaa-Tyr(tBu)-FMOC. The steps of 1) capping the peptide with acetic anhydride, 2) saving and labeling a 75 mg portion of the resin, 3) combining, randomizing and evenly dividing the beads and 4) coupling each division with an FMOC protected amino acid was repeated until the 5th coupling. At this point there are 4 pentapeptide libraries. These pentapeptide libraries were cleaved of the t-butyl protecting group on the tyrosines by mixing the resin with TFA (Pierce) for 2 hours. The beads were filtered and washed with ethanol (2x) and DMF (2x). The beads were then shaken with 20% Piperidine in DMF (v/v) twice for 10 minutes to cleave the N-terminal FMOC group. The deprotected beads were then washed with DMF (3x), CH_2Cl_2 (2x) and set in a vacuum oven overnight (55°C at 20 mm Hg) to evaporate any residual solvent.

ELISA Procedure. The library containing Tentagel resin (~3 mg) was added to a polypropylene tube. The tube was coated with a 1:1 (v/v) blocking solution of 3% BSA in PBS (w/v) and .05% Tween-20 (Sigma) in PBS (v/v). The tube was incubated with 500 μl of a 1:1:1 (v/v/v) solution consisting of 1 μg/ml mouse anti-β-endorphin monoclonal antibody (Boehringer Mannheim) in PBS, 3% BSA in PBS and .05% Tween-20 in PBS at 37°C for

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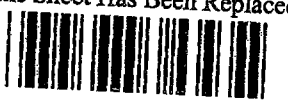
one hour. The tube was centrifuged and the supernatant was decanted. The tube was washed 3 times by repetitively adding 0.05% Tween-20 in PBS, centrifuging the tube and decanting the supernatant. The tube was then incubated with 500 μl of goat anti-mouse antibody conjugated to glucose oxidase (from Cappel, diluted by a factor of 1000 with a 1:1 (v/v) solution of 3% BSA in PBS and .05% Tween-20 in PBS). The tube was washed 3 times by repetitively adding .05% Tween-20 in PBS, centrifuging the tube and decanting the supernatant and 2 times by adding PBS, centrifuging the tube and decanting the supernatant.

A developing solution containing 25 ml of 0.1M Na_3PO_4 (pH 6.0), 3 ml of 20% glucose in H_2O, 200 μl horseradish peroxidase (0.1% in .1M Na_3PO_4, pH 6.0) and 200 μl ABTS dye (45 mg/ml in 0.1M Na_3PO_4, pH 6.0) was prepared. A 500 μl aliquot of the developing solution was added to the tube and allowed one hour to react with the peptide library before UV readings were taken. For the UV measurements of the assay, 900 μl of .1M Na_3PO_4 (pH 6.0) was placed in a cuvette and measured as the blank, 100 μl of the developed solution was then introduced to the cuvette and the UV absorbance at 416 nm was monitored.

Data Analysis. for each ELISA, besides assaying the pentapeptide libraries, a positive and negative control were also simultaneously assayed. The positive control was the independently synthesized pentapeptide, TG-Leu-Phe-Gly-Gly-Tyr-NH_2. The negative control was acetylated Tentagel (TG-Ac). The measured UV absorbance was normalized, to take into account the weight differences of the TG-resin in each assay tube, to give a normalized absorbance. The normalization was calculated by multiplying the measured absorbance at 416 nm by the mass of the material in the TG-Ac (negative control) assay tube. This product was divided by the mass of the TG-resin in the pentapeptide library tube to give the normalized absorbance. The absorbance of the negative

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control was subtracted from the normalized absorbance to give a corrected absorbance.

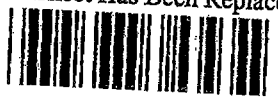
In the early stages of the deconvolution, the negative control gave higher absorbance readings than some of the libraries. This accounts for the sub-zero absorbances seen on the graphs. As the library became more defined, fewer negative corrected absorbances were obtained.

Quantification of Amino Acid Coupling. A standard solution was prepared by adding DMF to a known amount of Gly-FMOC to a volume of 100 ml. 980 μl of 20% piperidine in DMF (v/v) was scanned as a blank. 20 μl of the Gly-FMOC solution was added to the blank, swirled for 5 minutes and scanned. The piperidine-benzofulvene complex that forms upon cleavage of the FMOC group by piperidine has a diagnostic UV absorbance peak at 302 nm. After the coupling, capping and drying steps of the combinatorial library synthesis, a determination of the completeness of each coupling was performed by taking a known mass of Tentagel resin (usually ~5 mg) from each reaction vial and placing the sample in a 1 dram vial. The vials containing the resin samples were shaken with 1000 μl of 20% piperidine in DMF for 15 minutes. The resin was allowed to settle to the bottom and 100 μl of the piperidine treated resin solution was added to 900 μl of 20% piperidine in DMF which was previously added to a cuvette and scanned as a blank. The UV absorbance at 302 nm was compared to the standard to quantitate the extent of the amino acid coupling.

As a simple test of our method of deconvolution, an experiment was devised which screened libraries containing the well studied pentapeptide sequence, Leu-Phe-Gly-Gly-Tyr-NH_2, which displays nanomolar binding to commercially available anti-β-endorphin monoclonal antibody. A synthetic combinatorial peptide library with a degree of 5 and an alphabet consisting of leucine, glycine, phenylalanine and tyrosine, was synthesized on

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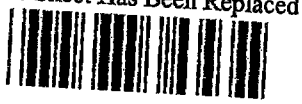
Tentagel solid support. The total number of molecules in this library is 4^5 (1024 members).

The coupling of the FMOC protected amino acids to the solid support with HBTU proceeded smoothly and quantitatively, according to FMOC cleavage measurements taken after each coupling. The quantitativeness of the coupling is advantageous since we can assume that the loading capacity (and therefore the number of moles per gram of resin) is approximately constant, no matter at what stage we are at in the synthesis or deconvolution of the library.

The binding assays against the β-endorphin antibody were performed with the peptide libraries remaining attached to the Tentagel resin. The deconvolution sequence can be followed pictorially in Figure 3 showing Scheme 3 and the corrected absorbances for each round are depicted in Figure 4. In the 4 pools of the pentapeptide libraries, where only the N-terminal amino acid was defined, TG-Xaa-Xaa-Xaa-Xaa-Tyr clearly gave the strongest absorbance. From this point, the 4 tetrapeptide libraries, TG-Xaa-Xaa-Xaa-Gly-FMOC, TG-Xaa-Xaa-Xaa-Leu-FMOC, TG-Xaa-Xaa-Xaa-Phe-FMOC and TG-Xaa-Xaa-Xaa-Tyr-FMOC, saved and labeled during the formation of the pentapeptide combinatorial library, were all coupled with Tyr-FMOC. Of the pools TG-Xaa-Xaa-Xaa-Gly-Tyr, TG-Xaa-Xaa-Xaa-Leu-Tyr, TG-Xaa-Xaa-Xaa-Phe-Tyr, and TG-Xaa-Xaa-Xaa-Tyr-Tyr, TG-Xaa-Xaa-Xaa-Gly-Tyr, obviously gave the strongest binding. This method of deconvolution was repeated to deduce that TG-Xaa-Xaa-Gly-Gly-Tyr was the best binder to the β-endorphin antibody. Solving for the fourth amino acid did not give a clear winner. TG-Xaa-Phe-Gly-Gly-Tyr, the sequence leading to the native epitope to the antibody, was the strongest binder. However, TG-Xaa-Leu-Gly-Gly-Tyr also showed significant binding to the antibody. Significant enough, in fact, to warrant the coupling of Leu-Gly-Gly-Tyr as well as Phe-

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Gly-Gly-Tyr to the monocoupled Tentagel libraries saved from the first step of the combinatorial synthesis.

In the final analysis, TG-Leu-Phe-Gly-Gly-Tyr, the native epitope, was the most extensive binder. Other weaker binders were also deduced, TG-Phe-Phe-Gly-Gly-Tyr and TG-Leu-Leu-Gly-Gly-Tyr also showed significant binding to the β-endorphin antibody.

Iterative methods of deconvolution have a number of advantages. A variety of chemical libraries, not just peptide or oligonucleotide libraries, can be solved iteratively. An advantage of an iterative deconvolution over encoded libraries is that the use of a decoding tag is avoided. This averts the concern of whether the decoding tag is involved in the binding of a library member to a receptor. Also, an iterative strategy avoids the sensitivity concerns of solving for the active member of a library because iterative methods are not dependent upon sequencing or decoding for the library members bound to a receptor.

Making combinatorial libraries by the split pool method is a cumbersome process. By cataloguing sublibraries along each step of a combinatorial synthesis, only one library needs to be made by the split pool method. While this may not be a tremendous advantage in making libraries that are synthesized in a highly automated manner, like oligonucleotide libraries, in the synthesis of other libraries not yet amenable to automated processes, like small molecule libraries, this iterative method avoids much of the time and labor involved in making split pool libraries for the previous iterative approaches.

Other embodiments are within the following claims.

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Claims

1. A kit comprising:

a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said first plurality of vessels comprising polysubunits having a different known subunit at said terminus, and

a second plurality of vessels comprising different polysubunits each constructed from said known number minus one subunit joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said second plurality of vessels comprising polysubunits having a different known subunit at said terminus.

2. The kit of claim 1, wherein said kit further comprises a third plurality of vessels comprising different polysubunits constructed from said known number minus two subunits.

3. The kit of claim 1, wherein said kit further comprises a fourth plurality of vessels comprising different polysubunits constructed from said known number minus three subunits.

4. The kit of claim 1, wherein said kit comprises further pluralities of vessels comprising different polysubunits, each said plurality of vessels differing from said other plurality of vessels in the number of subunits present in a said polysubunit.

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5. The kit of claim 4, wherein said further pluralities of vessels include polysubunits differing from other pluralities of vessels by having from said known number minus two subunits to one subunit.

6. Method for construction of a kit useful for identification of a desired compound, comprising the steps of:

(a) providing a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each said vessel comprising polysubunits having a different known subunit at said terminus,

(b) combining aliquots of said different polysubunits from each said vessel to form a mixture, wherein said aliquots are less than all of the contents of each said vessel,

(c) dividing said mixture into a second plurality of vessels, and

(d) bonding a further subunit to each said polysubunit in said second plurality of vessels; wherein said subunit is different for each said second plurality of vessels.

7. The method of claim 6, wherein said method further comprises repeating said steps (a) through (d) for said second plurality of vessels.

8. The method of claim 7, comprising repeating said steps (a) through (d) a plurality of times.

9. Method for identification of a desired compound, comprising the steps of:



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(a) providing a kit of claim 1 and screening said first plurality of vessels to identify a said vessel having a desired activity in an assay, and thereby identifying a first known subunit; and

(b) coupling said first known subunit to a terminus of an aliquot of the contents of each of said different polysubunits in said second plurality of vessels.

10. The method of claim 9, wherein said method further comprises screening said different polysubunits comprising said first known subunit for said desired activity in said assay, and thereby identifying a second known subunit.

11. The method of claim 9, wherein said steps (a) and (b) are repeated a plurality of times.

12. The method of claim 10, wherein said steps (a) and (b) are repeated a plurality of times.



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COMBINATORIAL LIBRARIES AND METHODS FOR THEIR USE

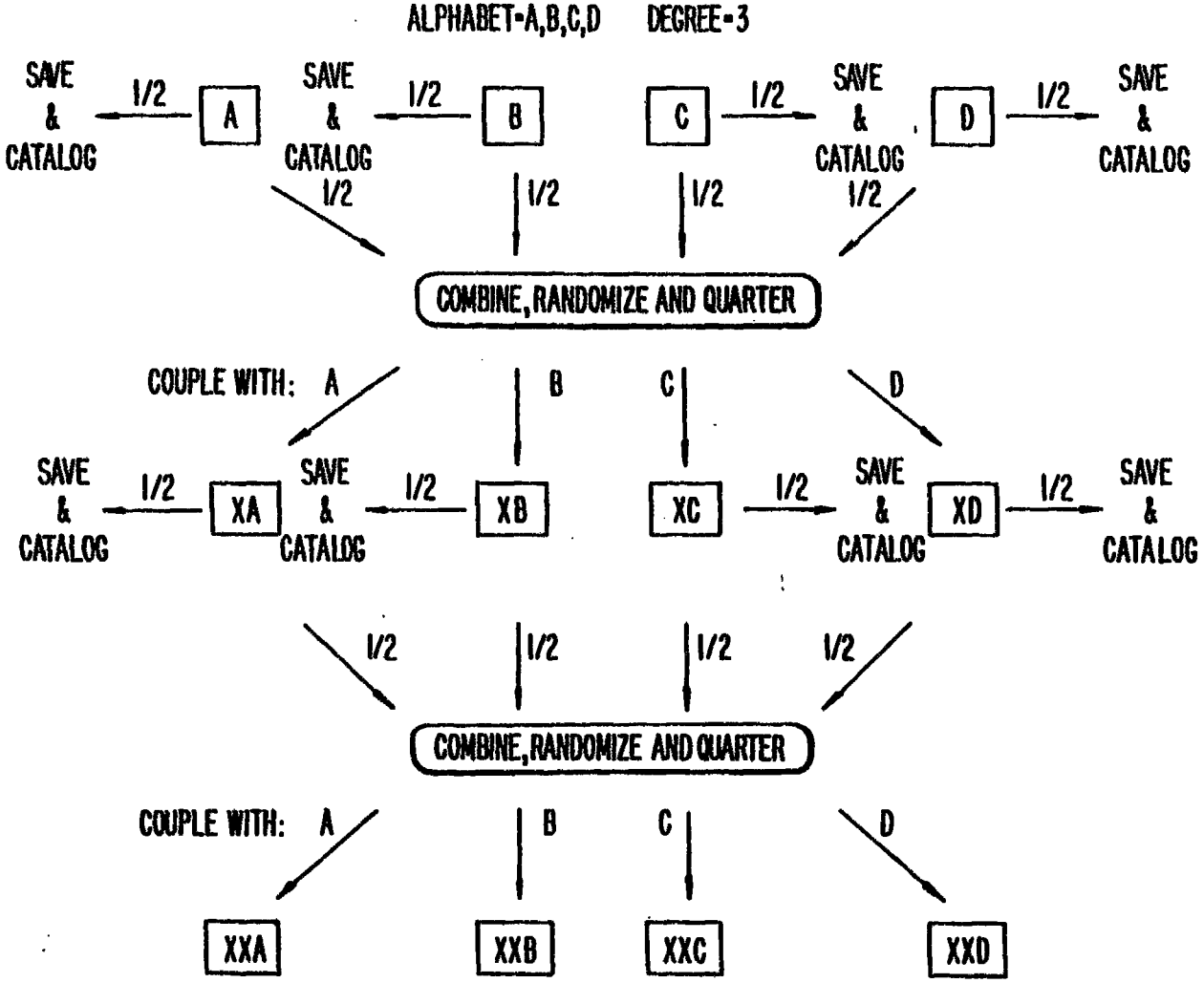
Abstract

Kit, and method for its use and construction, which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at one terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each constructed from the known number minus one subunit, as described above.

FIGURE 1.

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FIG. 1.



SCHEME 1. A DEPICTION OF THE SPLIT POOL SYNTHESIS OF A COMBINATORIAL LIBRARY WITH AN ALPHABET OF 4 AND A DEGREE OF 3. AFTER EACH STEP OF THE SYNTHESIS, A PORTION OF EACH SUBLIBRARY IS SAVED AND CATALOGUED BEFORE THE RANDOMIZATION STEP.

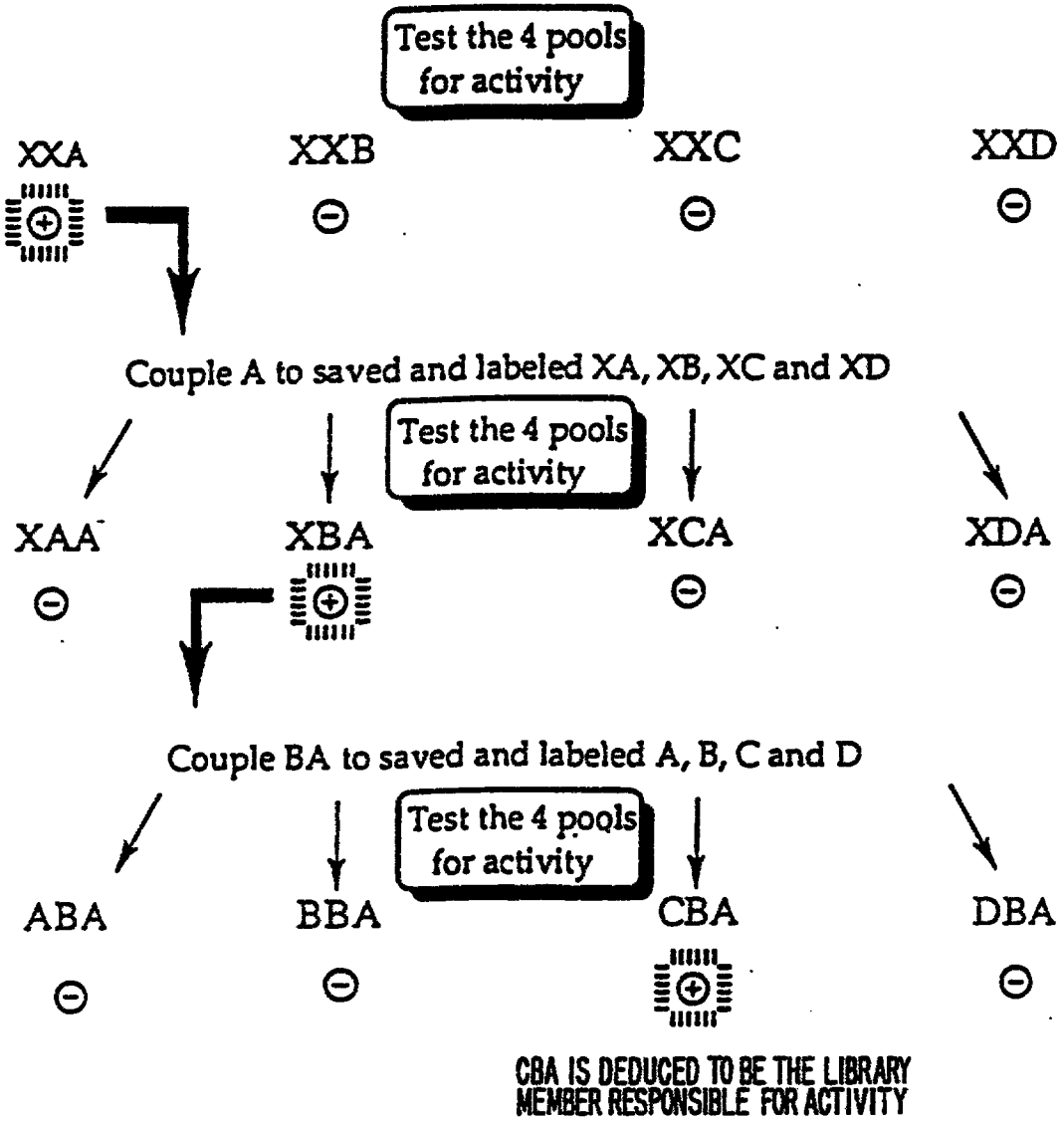
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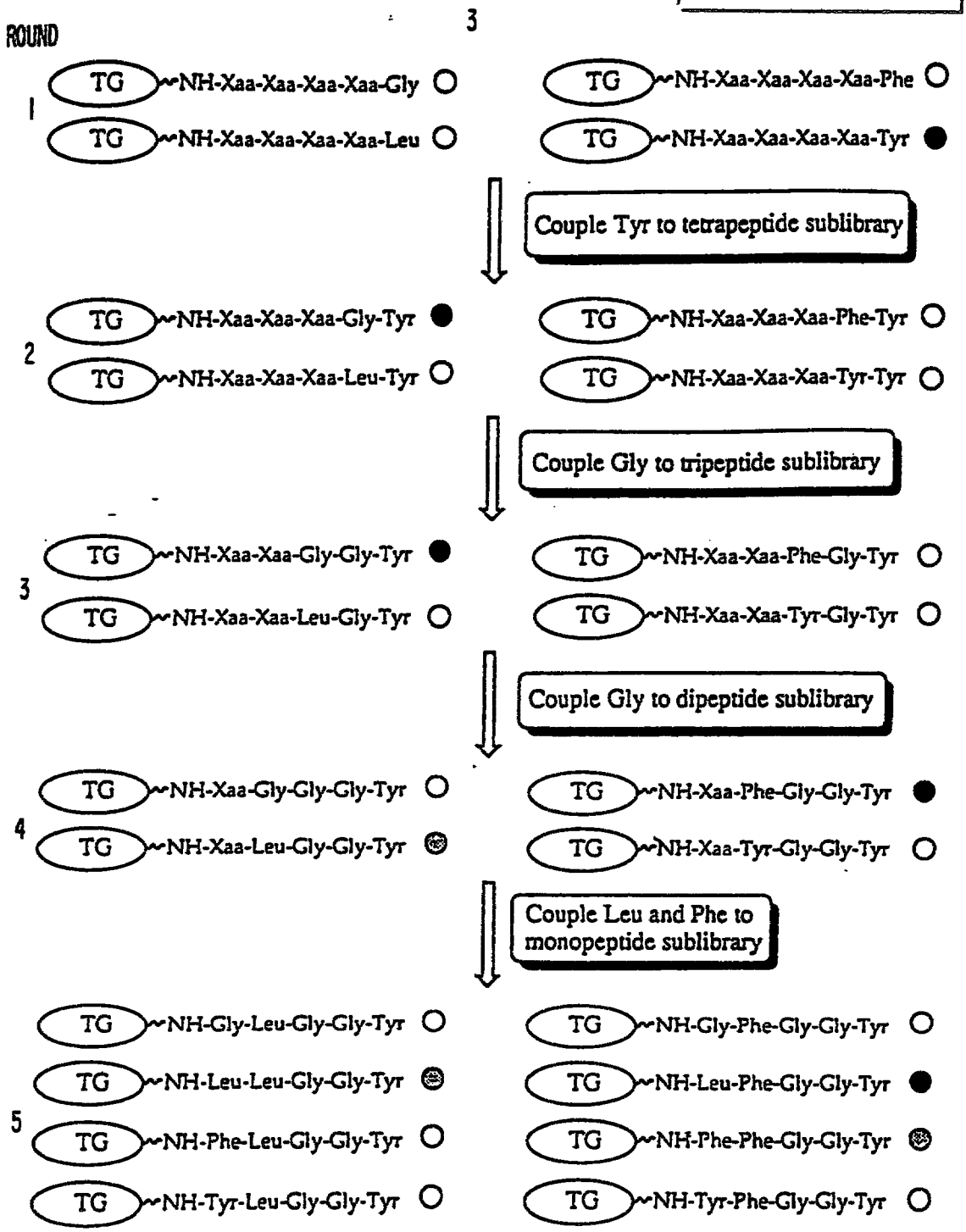
SCHEME 2. A DECONVULATORY PATHWAY FOR SOLVING THE ACTIVE MEMBER OF A COMBINATORIAL LIBRARY.

FIG. 2.

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● = STRONGEST BINDER OF POOL ◎ = SIGNIFICANT BINDING ○ = WEAK OR NEGLIGIBLE BINDING

SCHEME 3. ROUTE OF DECONVOLUTION OF THE TENTAGEL BOUND COMBINATORIAL PENTAPEPTIDE LIBRARY. EACH PEPTIDE POOL OF A ROUND WAS TESTED AGAINST THE ANTI-β-ENDORPHIN ANTIBODY. THE DEFINED POSITIONS OF THE POOL WITH THE STRONGEST AND SIGNIFICANT BINDING WERE NOTED AND COUPLED TO THE APPROPRIATE SUBLIBRARY FOR THE NEXT ROUND.

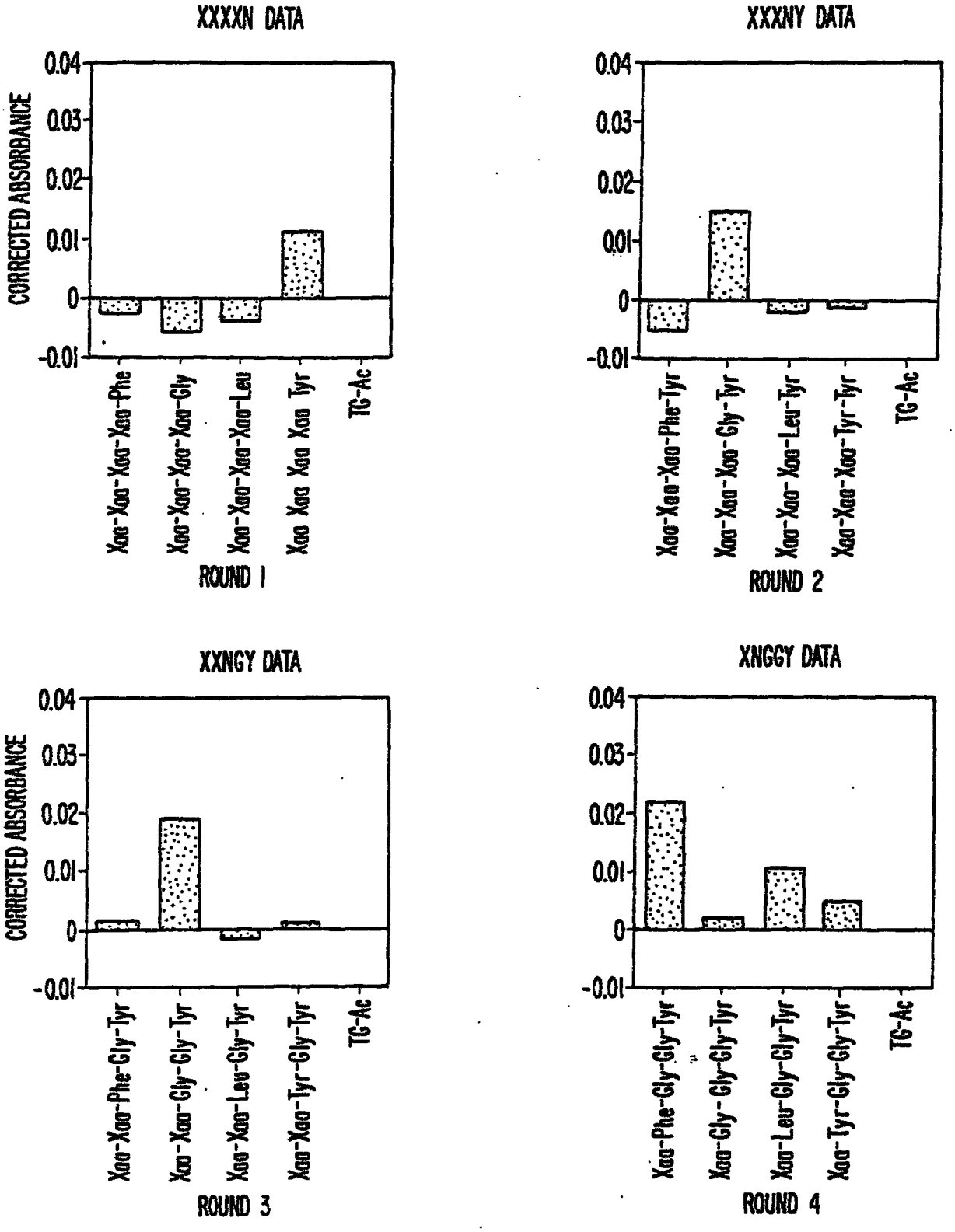
FIG. 3.

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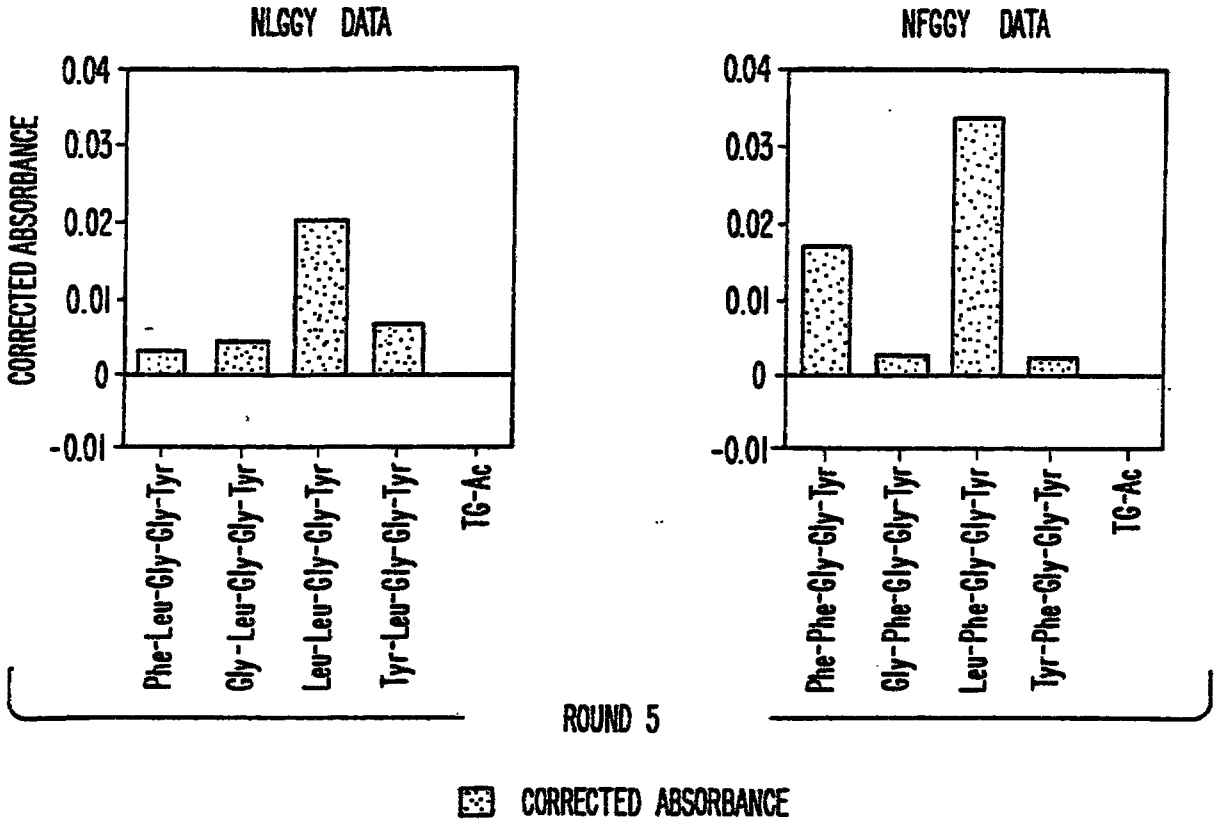
FIG. 4.



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CORRECTED ABSORBANCES OF EACH POOL IN COMPARISON TO THE ACETYLATED TentoGel CONTROL POOL. N DENOTES THE AMINO ACID TO BE DEFINED IN THAT ROUND. Xaa IS A RANDOMIZED POSITION.

FIG. 4 cont.

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DATE OF RECEIPT 6/2/98

~~DESCRIPTION~~

COMBINATORIAL LIBRARIES AND METHODS FOR THEIR USE

~~This~~ application is a continuation-in-part of U.S. application Serial No. 08/168,966, filed December 15, 1993, which is a continuation of U.S. application Serial No. 07/978,646, filed November 19, 1992.

Background of the Invention

This invention relates to synthetic combinatorial libraries useful for drug discovery, and methods for their use in such drug discovery.

Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe the generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. These libraries are composed of mixtures of free peptides which form a heterogenous library. Systematic identification of optimal peptide ligands is achieved by screening a library followed by iterative selection and synthesis processes. For example, one library consisted of a series of six residue peptides having the first two positions specifically defined, and the last four positions consisting of a random mixture of 18 L-amino acids. This library was screened to determine which pair of defined peptides had optimum activity in an assay. A second library was then synthesized in which the optimal pair of peptides were included, the third position of each peptide individually synthesized, and the last three peptides consisted of a random mixture of 18 L-amino acids. This library was screened as before and the process repeated until the optimum six residue peptide was identified. Houghten et al. state:

> "A number of other libraries, such as one composed entirely of D-amino acids, have been prepared which in total permit the systematic screening of hundreds of millions of peptides.

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> A fundamental feature of SPCLs [synthetic peptide combinatorial libraries] is that free peptides can be generated and used in solution in virtually all existing assay systems at a concentration of each peptide most applicable to the assay. This approach has also been successfully used in radio-receptor assays (opioid peptides) and plaque inhibition assays (human immunodeficiency virus (HIV-1) and Herpes Simplex Virus (HSV)). SPCLs, as described, greatly aid all areas of drug discovery and research involving peptides."

Lam et al., 354 *Nature* 82, 1991, and WO 92/00091 (PCT/US91/04666) and Houghten et al., 354 *Nature* 84, 1991 and WO 92/09300 (PCT/US91/08694), describe systematic synthesis and screening of peptide and other libraries of defined structure. The method used by Lam et al. is based on a one bead one peptide approach in which a large peptide library consisting of millions of beads are screened. Each bead contains a single peptide. The authors state:

> "It is clearly not enough to use a random mixture of activated amino acids in a peptide synthesis protocol, because the widely different coupling rates of different amino acids will lead to unequal representation and because each bead will contain a mixture of different peptides. Our solution was to use a 'split synthesis' approach. The first cycle consisted of distributing a pool of resin beads into separate reaction vessels each with a single amino acid, allowing the coupling reactions to go to completion, and then repooling the beads. The cycle was repeated several times to extend the peptide chain. In this fashion, each bead should contain only a single peptide species."

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The library of beads was screened by a staining procedure and stained beads visualized using a microscope, and removed. The structure of the peptide is obtained by a chemical analysis of the material on the single bead. Lam et al. indicate:

> "Additionally, our approach has far greater potential for applying the richness of well-established peptide chemistry to synthesize libraries incorporated D-amino acids or unnatural amino acids as well as specific secondary structures including cyclic peptides. All of this can be accomplished without need to keep records of the synthetic products as our interest is focused just on those peptides which provide a strong interaction signal with the acceptor."

Summary of the Invention

The present invention features an efficient method for the construction and decomposition of combinatorial libraries to allow identification of useful polysubunit compounds (made up from two or more subunits which may be the same or different, referred to sometimes as polymers). The method is not limited to identification of polypeptides; it can be applied to any combination of compounds made up of differing subunits (referred to sometimes as monomeric compounds) within one polysubunit compound. Synthesis of such polysubunits can be carried out chemically or with the use of catalysts, including enzymes. Thus, for example, the subunits may be selected from natural or unnatural moieties, including amino acids, nucleotides, sugars, lipids, and carbohydrates. In addition, the bond used to attach each subunit to the prior subunit can make any desired type of bond, and may include covalent, ionic or coordination bonds. The bonds may be selectively cleavable by enzymes or chemical treatments, as desired. Examples of such bonds include: peptide [R^1CONHR^2], ester [R^1COOR^2], sulfonamide [$R^1SO_2NR^2$],

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thioester [R^1COSR^2], phosphodiester [R^1OPOR^2], ether [R^1COCR^2], thioether [R^1CSCR^2], phosphamide [R^1PONH-], amine [-CH_2NHC-], and azo [-CNNC-] (where each R^1 and R^2 may be the same or different and may be cyclic or acyclic). In the method, a free or solid-phase-bound polysubunit can be used to allow ready screening of the synthesized polysubunits in any desired test or assay system. A significant advantage of this invention is that it provides a series of libraries or collections of polysubunits which can be reused for screening for any number of desired properties in any chosen assays.

The present method does not employ a mixed synthesis in which several different subunits are chemically incorporated within one vessel, but rather a synthesis in which a single subunit is incorporated at each step. See Figure 1.

Thus, in one example, the method involves a first step of attaching ten different subunits A, B, C . . . J, to a solid support in ten separate vessels or columns. In the second step, a portion or aliquot of the material synthesized at the first step is retained as separate columns, while the remainder (which is still attached to individual solid supports) is mixed or pooled, divided into ten new different columns, and ten further parallel syntheses carried out to provide the dimer XA^1, XB^1, XC^1 . . . XJ^1, where X is any one of the original A-J, and A^1, B^1, C^1 . . . J^1 are ten different subunits which may be the same or different from A-J. Of course, fewer or more than ten syntheses can be used in this second step. In the third step, a portion of the newly synthesized material of step two is again retained in separate columns, and the remainder mixed and divided into ten further columns so that the synthetic procedure can be repeated until the whole length of the desired polysubunit is synthesized. In this way a series of vessels is formed at each step, differing from those in prior steps by the presence of an extra subunit.

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The final ten columns in the above example (each having a variety of different polysubunits with a known subunit at their terminus) can be assayed using any standard assay format. That is, each of the ten mixtures is assayed to determine which mixture contains one or more active compounds. The column which is found to contain an active compound identifies the subunit required at the polysubunit terminus to be active in the assay. For example, the column containing polysubunits of sequence XXXXJ^{1} may be active in the assay. This indicates that J^{1} is required at the terminus of a polysubunit in this assay. This subunit is now bonded to each of the columns retained in the previous synthetic step (in the example, the columns XXXA, XXXB, . . . XXXJ). These ten newly synthesized series of compounds can then be assayed and the process repeated until the final polysubunit sequence is known. See Figures 2 and 3.

Thus, the method of the present invention features a set or kit of partial combinatorial libraries with each library containing one subunit more than the prior library. The final library is decomposed stepwise starting with the last subunit. This form of retroanalysis or antithesis allows definition of the synthesis of the desired product, which is accomplished in the final analytic step. Once constructed, the entire set of libraries can be used for many different projects.

Thus, in a first aspect, the invention features a kit which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds, and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits, but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at their terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each

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constructed from the known number minus one subunit, as described above.

This kit forms a combinatorial library which can be screened and decomposed as discussed above. The number of first and second pluralities of vessels can be the same or different, and generally the terminus of the polysubunits to which subunits are added is the same in each set of vessels.

In preferred embodiments, the kit may include further pluralities of vessels in which the number of subunits present in the polysubunit is the known number minus two, or three, or more. Thus, each plurality of vessels differs from other pluralities of vessels in the number of subunits present in the polysubunits. Most preferably, the number of subunits in each plurality of vessels differs by one and ranges from one to the known number. As discussed above, such a kit is useful for systematic identification of the sequence of a desired polysubunit, using the method of retroanalysis.

As noted above, the polysubunits may be formed from any series of subunits desired, and the number of vessels containing these polysubunits at each step can be different for each subunit added. Thus, as described above, ten amino acids may be incorporated in one step, two or three carbohydrates may be incorporated in another step, and twenty amino acids in the following step. There is no limit to the steps of addition of subunits, nor to the choice of subunits which can be added at each step. All that is necessary for the kit to be useful is that the kit include a series of vessels which contain polysubunits differing sequentially in the number of subunits present in those polysubunits. The final plurality of vessels in the kit will contain only the first subunit free in solution, or bound to a solid support.

In a related aspect the invention features, a method for construction of a kit, following the steps

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described above, and a method for screening such kits as described above.

Specifically, the invention features a method for construction of a kit useful for identification of a desired compound, by the steps of: (a) providing a first plurality of vessels containing different polysubunits each constructed from a known number of subunits joined together by one or more bonds. Each subunit and bond can be the same or different, and each different polysubunit has a different sequence of subunits but the same known subunit at one terminus of the polysubunit. Each vessel includes polysubunits having a different known subunit at their terminus; (b) combining aliquots of the different polysubunits from each vessel to form a mixture; wherein the aliquots are less than all of the contents of each vessel; (c) dividing the mixture into a second plurality of vessels; and (d) bonding a further subunit to each polysubunit in the second plurality of vessels; wherein the subunit is different for each second plurality of vessels.

In preferred embodiments, the method further includes repeating steps (a) through (d) for the second plurality of vessels; or repeating steps (a) through (d) a plurality of times.

In a related aspect, the invention features a method for identification of a desired compound, including the steps of: (a) providing a kit as described above, and screening the first plurality of vessels to identify a vessel having a desired activity in an assay, and thereby identifying a first known subunit; and (b) coupling the first known subunit to a terminus of an aliquot of the contents of each of the different polysubunits in the second plurality of vessels.

In preferred embodiments, the method further involves screening the different polysubunits having the first known subunit for the desired activity in the assay,

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and thereby identifying a second known subunit; or repeating steps (a) and (b) a plurality of times.

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

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 illustrates split pool synthesis of a combinatorial library.

Figure 2 illustrates utilization of the deconvolution pathway to determine the sequence of a member of a combinatorial library.

Figure 3 illustrates the deconvolution of a pentapeptide library.

Figure 4 illustrates the corrected absorbances of pools of library products reacted with β-endorphin antibody to determine the sequence of the pentapeptide which bound best to the β-endorphin antibody.

Applicant has determined that a useful series, or kit, of libraries can be constructed by stepwise synthesis of a polysubunit from different subunits. While these libraries and methods for their synthesis and use will commonly be applied for use with peptides formed from standard L- or D-amino acids, those of ordinary skill in the art will recognize that other subunits and bonds other than covalent, ionic or coordination bonds can be used to form the final polysubunit, which can then be screened in any standard assay. These synthetic protocols and assays are well known to those of ordinary skill in the art and need not be repeated here. See, Lam et al., *supra*, and Houghten et al., *supra*, the whole of all of which are hereby incorporated by reference herein. As an illustrative example of a method and kit of this invention, the following is provided. This example is not limiting in the invention and those of ordinary skill will

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realize (as noted above) that other equivalent methods, syntheses, and analyses can be readily used.

Example

The method proposed here is based on a true combinatorial synthesis and makes efficient use of all of the materials. The first step is to attach ten subunits, A to J, to a solid support in ten columns. One-sixth of this material is retained in this form, giving A, B, . . . J; the remainder, still attached to the solid support, is pooled, divided into ten columns, and ten parallel syntheses are carried out, to provide XA, XB, . . . XJ. One-fifth of this material is retained in this form, and the combinatorial synthesis is repeated to give ten polysubunits, XXA, XXB, . . . XXJ. One-fourth is kept and the fourth residue is added to the remainder by combinatorial synthesis to give the polysubunits XXXA, XXXB, . . . XXXJ. After retaining one-third of these polysubunits the final combinatorial step of synthesis is completed, and the materials are removed from the column to provide ten solutions containing XXXXA, XXXXB, XXXXJ. These are now assayed.

If a positive assay result is obtained with one vessel containing polysubunits of the structure XXXXC (*i.e.*, C is the critical subunit at the fifth position in the pentasubunit), the experimenter returns to the columns retained at the previous step, and to an aliquot of each adds C, to provide ten solutions, XXXAC, XXXBC . . . XXXJC. Again, these are tested. If a positive assay result is obtained with XXXEC (*i.e.*, E is the critical subunit at position four in the pentasubunit), the experimenter returns to the previous partial library and completes it by adding E + C to all ten to give XXAEC, XXBEC . . . XXJEC. These steps are repeated until all of the positions are specified.

Thus, in essence a set of partial combinatorial libraries is made, each one residue longer than the last. The final library is decomposed stepwise, starting with

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the last residue. This form of retro-analysis allows definition of the synthesis of the desired product, which is indeed accomplished in the very last step. Once constructed, the entire set of libraries can be used again for different projects.

It is notable that the final synthetic step also provides a method for synthesis of the desired product.

Materials and Methods

Solvents. DMF (HPLC grade) was purchased from Baxter and was used without further purification. CH_2Cl_2 was purchased from Fisher Scientific and was distilled over CaH_2 before use.

Instrumentation. UV absorbances were measured on a Hewlett-Packard 8452A diode array spectrophotometer. All centrifugations were carried out on an Eppendorf 5415 C centrifuge for one minute at 10,000 rpm.

Formation of the Pentapeptide Library. The peptide libraries were synthesized manually by a solid phase method. Tentagel (TG) resin was the solid support for all synthesis and was 90 μm in diameter. The amino acids used for the formation of the libraries were Gly-FMOC, Leu-FMOC, Phe-FMOC and Tyr(tBu)-FMOC. All amino acids were coupled with the aid of HBTU and DIPEA. The Tentagel, HBTU, DIPEA and amino acids used were purchased from NovaBiochem. Tentagel was added to 4 glass fritted filter vials, shaken with DMF and filtered. To each vial was added one of the FMOC protected amino acid components of the library, HBTU, DMF and 20 μl DIPEA. All four vials were shaken for one hour, filtered and washed with DMF. A 5% solution (v/v) of acetic anhydride in DMF was added to each vial and shaken for 20 minutes to cap any uncoupled free amino acid groups. The beads were then filtered, washed with DMF (2x) and CH_2Cl_2 (2x) and dried for two hours in a vacuum oven (55°C at 20 mm Hg). Approximately, a 75 mg portion of the amino acid coupled Tentagel bead was taken from each vial and labeled in a manner describing the length of the peptide and the

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identity of the last amino acid coupled. Also, an approximately 5 mg portion of the resin was taken to do an FMOC deprotection test in order to quantitate the completeness of the previous amino acid coupling step. The remaining resin was combined and shaken in 3:1 (v/v) DMF/CH_2Cl_2 for 30 minutes to randomize the beads. The beads were quartered into equivalent weight (and therefore equivalent moles) and set into 4 glass fritted filter vials and subjected twice to 10 minute shakings with 20% Piperidine in DMF (v/v) in order to cleave the N-terminal FMOC protecting group. The beads were filtered, washed with DMF (3x) and coupled with one of the 4 amino acid components of the library to give the 4 dipeptide pools, TG-Xaa-Gly-FMOC, TG-Xaa-Leu-FMOC, TG-Xaa-Phe-FMOC, and TG-Xaa-Tyr(tBu)-FMOC. The steps of 1) capping the peptide with acetic anhydride, 2) saving and labeling a 75 mg portion of the resin, 3) combining, randomizing and evenly dividing the beads and 4) coupling each division with an FMOC protected amino acid was repeated until the 5th coupling. At this point there are 4 pentapeptide libraries. These pentapeptide libraries were cleaved of the t-butyl protecting group on the tyrosines by mixing the resin with TFA (Pierce) for 2 hours. The beads were filtered and washed with ethanol (2x) and DMF (2x). The beads were then shaken with 20% Piperidine in DMF (v/v) twice for 10 minutes to cleave the N-terminal FMOC group. The deprotected beads were then washed with DMF (3x), CH_2Cl_2 (2x) and set in a vacuum oven overnight (55°C at 20 mm Hg) to evaporate any residual solvent.

ELISA Procedure. The library containing Tentagel resin (~3 mg) was added to a polypropylene tube. The tube was coated with a 1:1 (v/v) blocking solution of 3% BSA in PBS (w/v) and .05% Tween®-20 (Sigma) in PBS (v/v). The tube was incubated with 500 µl of a 1:1:1 (v/v/v) solution consisting of 1 µg/ml mouse anti-β-endorphin monoclonal antibody (Boehringer Mannheim) in PBS, 3% BSA in PBS and .05% Tween®-20 in PBS at 37°C for

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one hour. The tube was centrifuged and the supernatant was decanted. The tube was washed 3 times by repetitively adding 0.05% Tween®-20 in PBS, centrifuging the tube and decanting the supernatant. The tube was then incubated with 500 µl of goat anti-mouse antibody conjugated to glucose oxidase (from Cappel, diluted by a factor of 1000 with a 1:1 (v/v) solution of 3% BSA in PBS and .05% Tween-20 in PBS). The tube was washed 3 times by repetitively adding .05% Tween®-20 in PBS, centrifuging the tube and decanting the supernatant and 2 times by adding PBS, centrifuging the tube and decanting the supernatant.

A developing solution containing 25 ml of 0.1M Na_3PO_4 (pH 6.0), 3 ml of 20% glucose in H_2O, 200 µl horseradish peroxidase (0.1% in .1M Na_3PO_4, pH 6.0) and 200 µl ABTS dye (45 mg/ml in 0.1M Na_3PO_4, pH 6.0) was prepared. A 500 µl aliquot of the developing solution was added to the tube and allowed one hour to react with the peptide library before UV readings were taken. For the UV measurements of the assay, 900 µl of .1M Na_3PO_4 (pH 6.0) was placed in a cuvette and measured as the blank, 100 µl of the developed solution was then introduced to the cuvette and the UV absorbance at 416 nm was monitored.

Data Analysis. for each ELISA, besides assaying the pentapeptide libraries, a positive and negative control were also simultaneously assayed. The positive control was the independently synthesized pentapeptide, TG-Leu-Phe-Gly-Gly-Tyr-NH_2. The negative control was acetylated Tentagel (TG-Ac). The measured UV absorbance was normalized, to take into account the weight differences of the TG-resin in each assay tube, to give a normalized absorbance. The normalization was calculated by multiplying the measured absorbance at 416 nm by the mass of the material in the TG-Ac (negative control) assay tube. This product was divided by the mass of the TG-resin in the pentapeptide library tube to give the normalized absorbance. The absorbance of the negative

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control was subtracted from the normalized absorbance to give a corrected absorbance.

In the early stages of the deconvolution, the negative control gave higher absorbance readings than some of the libraries. This accounts for the sub-zero absorbances seen on the graphs. As the library became more defined, fewer negative corrected absorbances were obtained.

Quantification of Amino Acid Coupling. A standard solution was prepared by adding DMF to a known amount of Gly-FMOC to a volume of 100 ml. 980 μl of 20% piperidine in DMF (v/v) was scanned as a blank. 20 μl of the Gly-FMOC solution was added to the blank, swirled for 5 minutes and scanned. The piperidine-benzofulvene complex that forms upon cleavage of the FMOC group by piperidine has a diagnostic UV absorbance peak at 302 nm. After the coupling, capping and drying steps of the combinatorial library synthesis, a determination of the completeness of each coupling was performed by taking a known mass of Tentagel resin (usually ~5 mg) from each reaction vial and placing the sample in a 1 dram vial. The vials containing the resin samples were shaken with 1000 μl of 20% piperidine in DMF for 15 minutes. The resin was allowed to settle to the bottom and 100 μl of the piperidine treated resin solution was added to 900 μl of 20% piperidine in DMF which was previously added to a cuvette and scanned as a blank. The UV absorbance at 302 nm was compared to the standard to quantitate the extent of the amino acid coupling.

As a simple test of our method of deconvolution, an experiment was devised which screened libraries containing the well studied pentapeptide sequence, Leu-Phe-Gly-Gly-Tyr-NH_2, which displays nanomolar binding to commercially available anti-β-endorphin monoclonal antibody. A synthetic combinatorial peptide library with a degree of 5 and an alphabet consisting of leucine, glycine, phenylalanine and tyrosine, was synthesized on

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Tentagel solid support. The total number of molecules in this library is 4^5 (1024 members).

The coupling of the FMOC protected amino acids to the solid support with HBTU proceeded smoothly and quantitatively, according to FMOC cleavage measurements taken after each coupling. The quantitativeness of the coupling is advantageous since we can assume that the loading capacity (and therefore the number of moles per gram of resin) is approximately constant, no matter at what stage we are at in the synthesis or deconvolution of the library.

The binding assays against the β-endorphin antibody were performed with the peptide libraries remaining attached to the Tentagel resin. The deconvolution sequence can be followed pictorially in Figure 3 showing Scheme 3 and the corrected absorbances for each round are depicted in Figure 4. In the 4 pools of the pentapeptide libraries, where only the N-terminal amino acid was defined, TG-Xaa-Xaa-Xaa-Xaa-Tyr clearly gave the strongest absorbance. From this point, the 4 tetrapeptide libraries, TG-Xaa-Xaa-Xaa-Gly-FMOC, TG-Xaa-Xaa-Xaa-Leu-FMOC, TG-Xaa-Xaa-Xaa-Phe-FMOC and TG-Xaa-Xaa-Xaa-Tyr-FMOC, saved and labeled during the formation of the pentapeptide combinatorial library, were all coupled with Tyr-FMOC. Of the pools TG-Xaa-Xaa-Xaa-Gly-Tyr, TG-Xaa-Xaa-Xaa-Leu-Tyr, TG-Xaa-Xaa-Xaa-Phe-Tyr, and TG-Xaa-Xaa-Xaa-Tyr-Tyr, TG-Xaa-Xaa-Xaa-Gly-Tyr, obviously gave the strongest binding. This method of deconvolution was repeated to deduce that TG-Xaa-Xaa-Gly-Gly-Tyr was the best binder to the β-endorphin antibody. Solving for the fourth amino acid did not give a clear winner. TG-Xaa-Phe-Gly-Gly-Tyr, the sequence leading to the native epitope to the antibody, was the strongest binder. However, TG-Xaa-Leu-Gly-Gly-Tyr also showed significant binding to the antibody. Significant enough, in fact, to warrant the coupling of Leu-Gly-Gly-Tyr as well as Phe-

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Gly-Gly-Tyr to the monocoupled Tentagel libraries saved from the first step of the combinatorial synthesis.

In the final analysis, TG-Leu-Phe-Gly-Gly-Tyr, the native epitope, was the most extensive binder. Other weaker binders were also deduced, TG-Phe-Phe-Gly-Gly-Tyr and TG-Leu-Leu-Gly-Gly-Tyr also showed significant binding to the β-endorphin antibody.

Iterative methods of deconvolution have a number of advantages. A variety of chemical libraries, not just peptide or oligonucleotide libraries, can be solved iteratively. An advantage of an iterative deconvolution over encoded libraries is that the use of a decoding tag is avoided. This averts the concern of whether the decoding tag is involved in the binding of a library member to a receptor. Also, an iterative strategy avoids the sensitivity concerns of solving for the active member of a library because iterative methods are not dependent upon sequencing or decoding for the library members bound to a receptor.

Making combinatorial libraries by the split pool method is a cumbersome process. By cataloguing sublibraries along each step of a combinatorial synthesis, only one library needs to be made by the split pool method. While this may not be a tremendous advantage in making libraries that are synthesized in a highly automated manner, like oligonucleotide libraries, in the synthesis of other libraries not yet amenable to automated processes, like small molecule libraries, this iterative method avoids much of the time and labor involved in making split pool libraries for the previous iterative approaches.

Other embodiments are within the following claims.

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Claims

1. A kit useful for indentification of a desired compound comprising:

a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said first plurality of vessels comprising polysubunits having a different known subunit at said terminus, and

a second plurality of vessels comprising different polysubunits each constructed from said known number minus one subunit joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each one of said second plurality of vessels comprising polysubunits having a different known subunit at said terminus.

2. The kit of claim 1, wherein said kit further comprises a third plurality of vessels comprising different polysubunits constructed from said known number minus two subunits.

3. The kit of claim 1, wherein said kit further comprises a fourth plurality of vessels comprising different polysubunits constructed from said known number minus three subunits.

4. The kit of claim 1, wherein said kit comprises further pluralities of vessels comprising different polysubunits, each said plurality of vessels differing from said other plurality of vessels in the number of subunits present in a said polysubunit.

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5. The kit of claim 4, wherein said further pluralities of vessels include polysubunits differing from other pluralities of vessels by having from said known number minus two subunits to one subunit.

6. Method for construction of a kit useful for identification of a desired compound, comprising the steps of:

(a) providing a first plurality of vessels comprising different polysubunits each constructed from a known number of subunits joined together by one or more bonds, wherein each said subunit and bond can be the same or different, each said different polysubunit comprising a different sequence of subunits but having the same known subunit at one terminus of said polysubunit, each said vessel comprising polysubunits having a different known subunit at said terminus,

(b) combining aliquots of said different polysubunits from each said vessel to form a mixture, wherein said aliquots are less than all of the contents of each said vessel,

(c) dividing said mixture into a second plurality of vessels, and

(d) bonding a further subunit to each said polysubunit in said second plurality of vessels; wherein said subunit is different for each said second plurality of vessels.

7. The method of claim 6, wherein said method further comprises repeating said steps (a) through (d) for said second plurality of vessels.

8. The method of claim 7, comprising repeating said steps (a) through (d) a plurality of times.

9. Method for identification of a desired compound, comprising the steps of:

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(a) providing a kit of claim 1 and screening said first plurality of vessels to identify a said vessel having a desired activity in an assay, and thereby identifying a first known subunit; and

(b) coupling said first known subunit to a terminus of an aliquot of the contents of each of said different polysubunits in said second plurality of vessels.

10. The method of claim 9, wherein said method further comprises screening said different polysubunits comprising said first known subunit for said desired activity in said assay, and thereby identifying a second known subunit.

11. The method of claim 9, wherein said steps (a) and (b) are repeated a plurality of times.

12. The method of claim 10, wherein said steps (a) and (b) are repeated a plurality of times.

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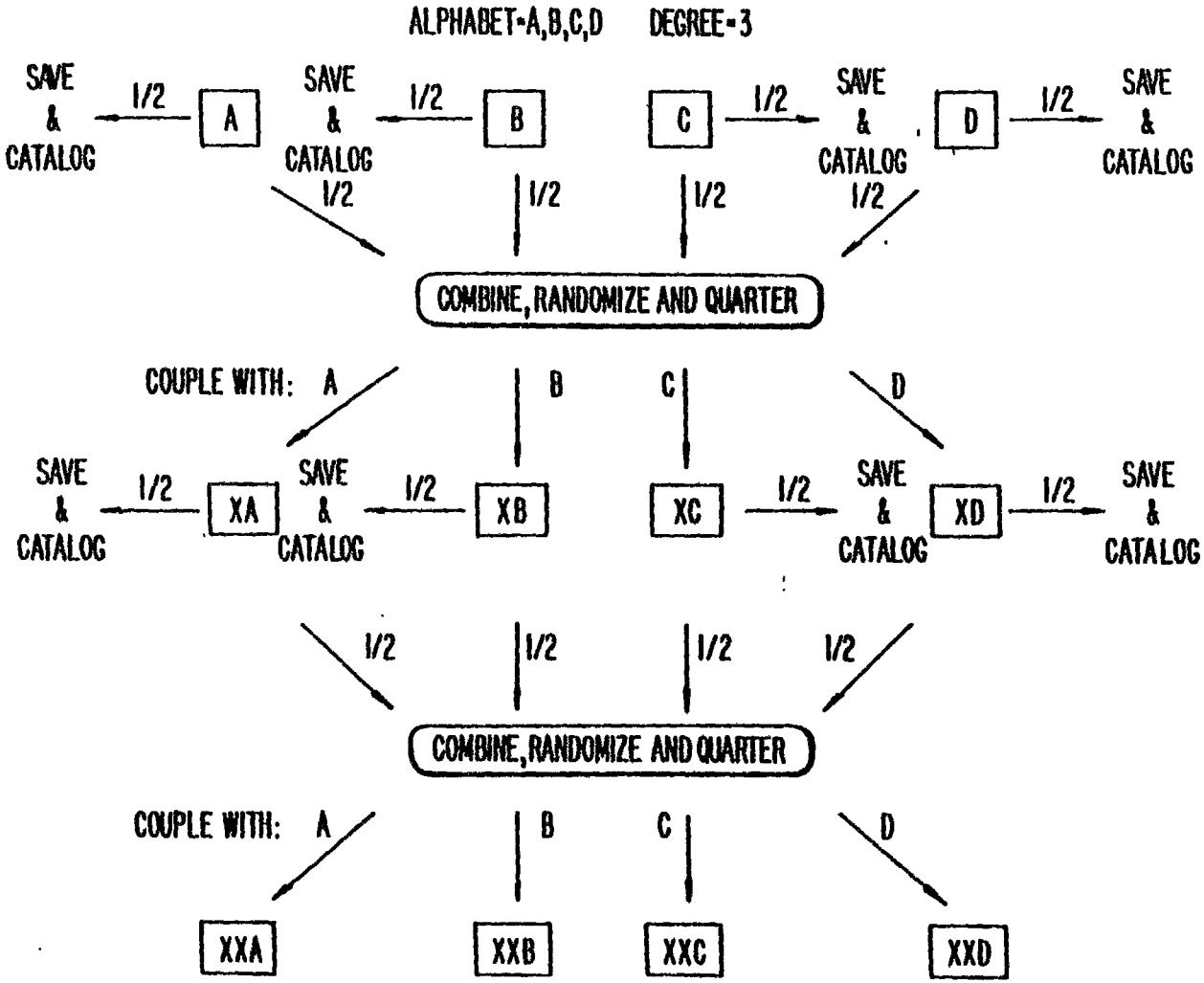
COMBINATORIAL LIBRARIES AND METHODS FOR THEIR USE

Abstract

Kit, and method for its use and construction, which includes a first plurality of vessels containing different polysubunits, each constructed from a known number of subunits. Each subunit is joined by one or more bonds and each subunit and bond can be the same or different. Each of the different polysubunits has a different sequence of subunits but has the same known subunit at one terminus. Each vessel contains polysubunits which have a different known subunit at one terminus. The kit further includes a second plurality of vessels which includes different polysubunits, each constructed from the known number minus one subunit, as described above.

FIGURE 1.

FIG. 1.

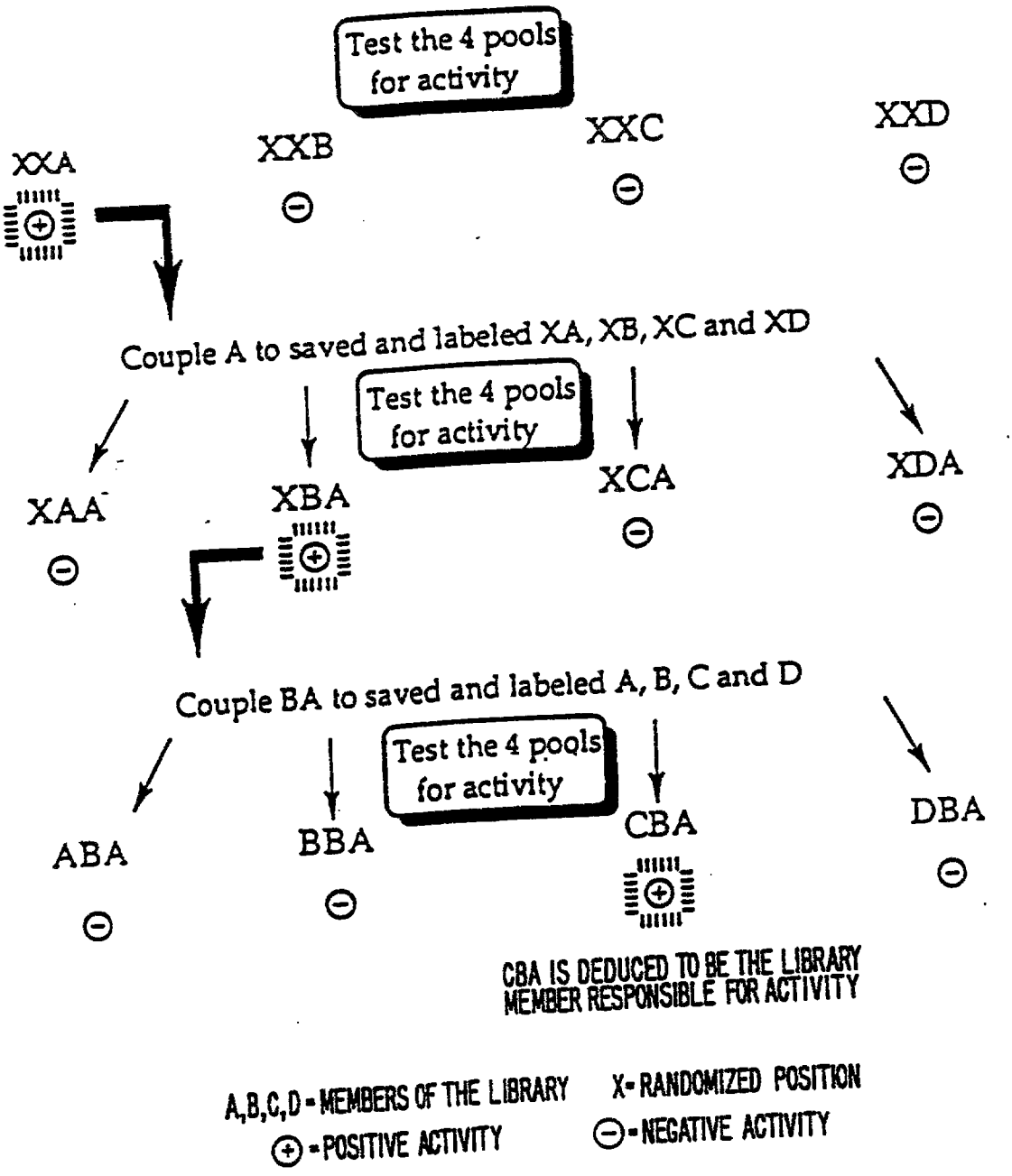


EACH POOL CONTAINS 16 MOLECULES. THERE ARE A TOTAL OF 64 UNIQUE MOLECULES.

~~SCHEME 1. A DEPICTION OF THE SPLIT POOL SYNTHESIS OF A COMBINATORIAL LIBRARY WITH AN ALPHABET OF 4 AND A DEGREE OF 3. AFTER EACH STEP OF THE SYNTHESIS, A PORTION OF EACH SUBLIBRARY IS SAVED AND CATALOGUED BEFORE THE RANDOMIZATION STEP.~~

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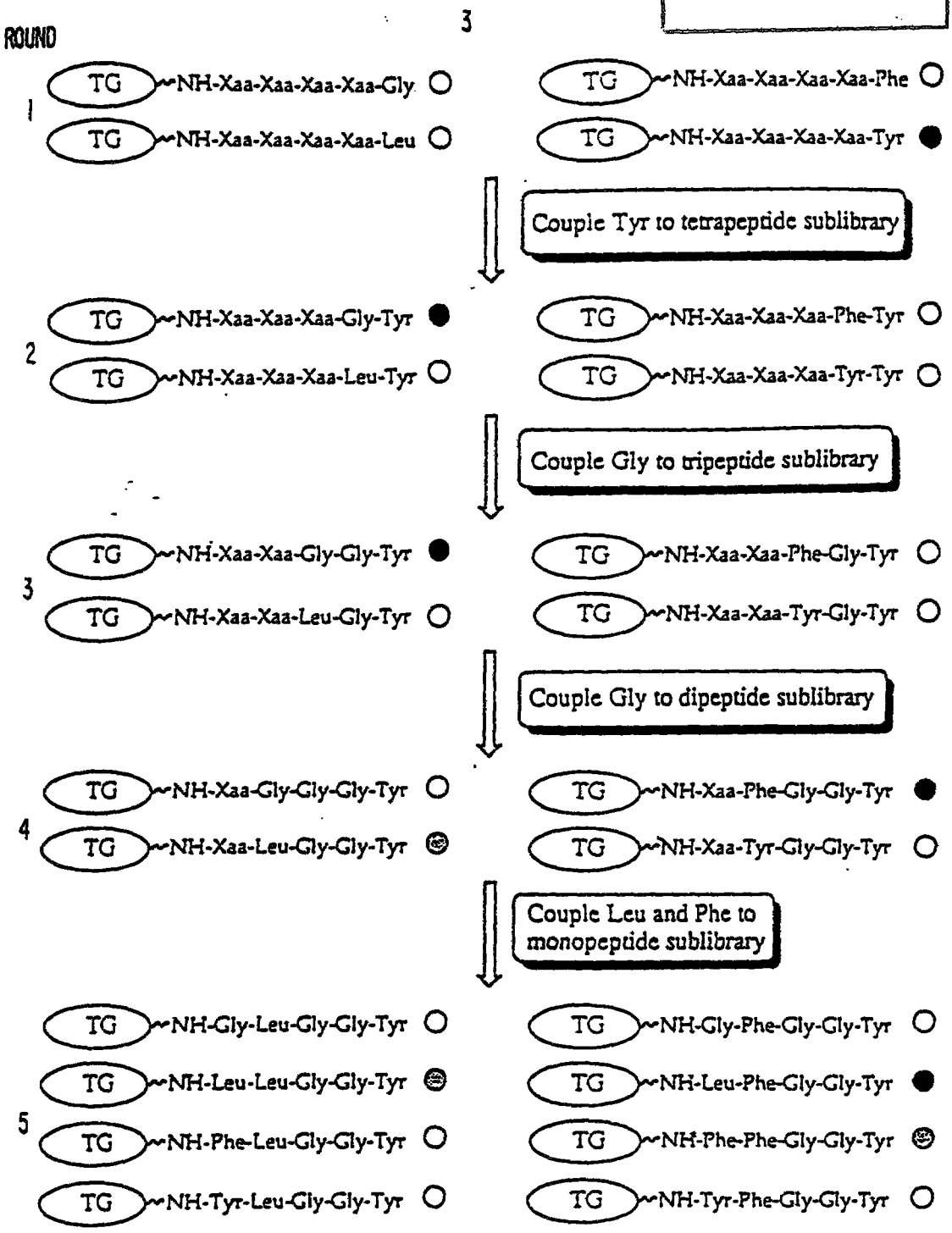
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~~SCHEME 2. A DECONVOLUTORY PATHWAY FOR SOLVING THE ACTIVE MEMBER OF A COMBINATORIAL LIBRARY.~~

FIG. 2.

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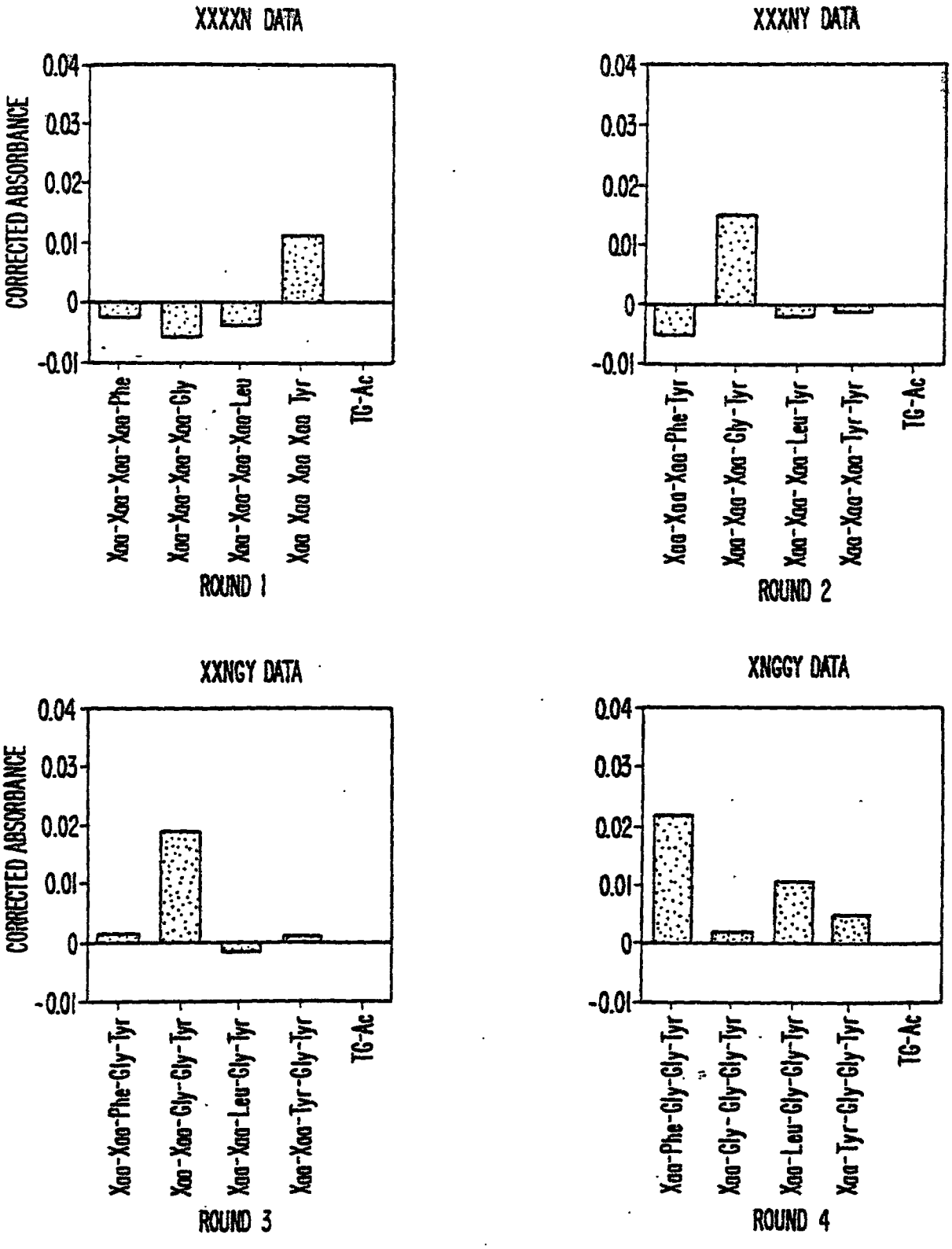


~~SCHEME 3. ROUTE OF DECONVOLUTION OF THE TENTAGEL BOUND COMBINATORIAL PENTAPEPTIDE LIBRARY. EACH PEPTIDE POOL OF A ROUND WAS TESTED AGAINST THE ANTI-β-ENDORPHIN ANTIBODY. THE DEFINED POSITIONS OF THE POOL WITH THE STRONGEST AND SIGNIFICANT BINDING WERE NOTED AND COUPLED TO THE APPROPRIATE SUBLIBRARY FOR THE NEXT ROUND.~~

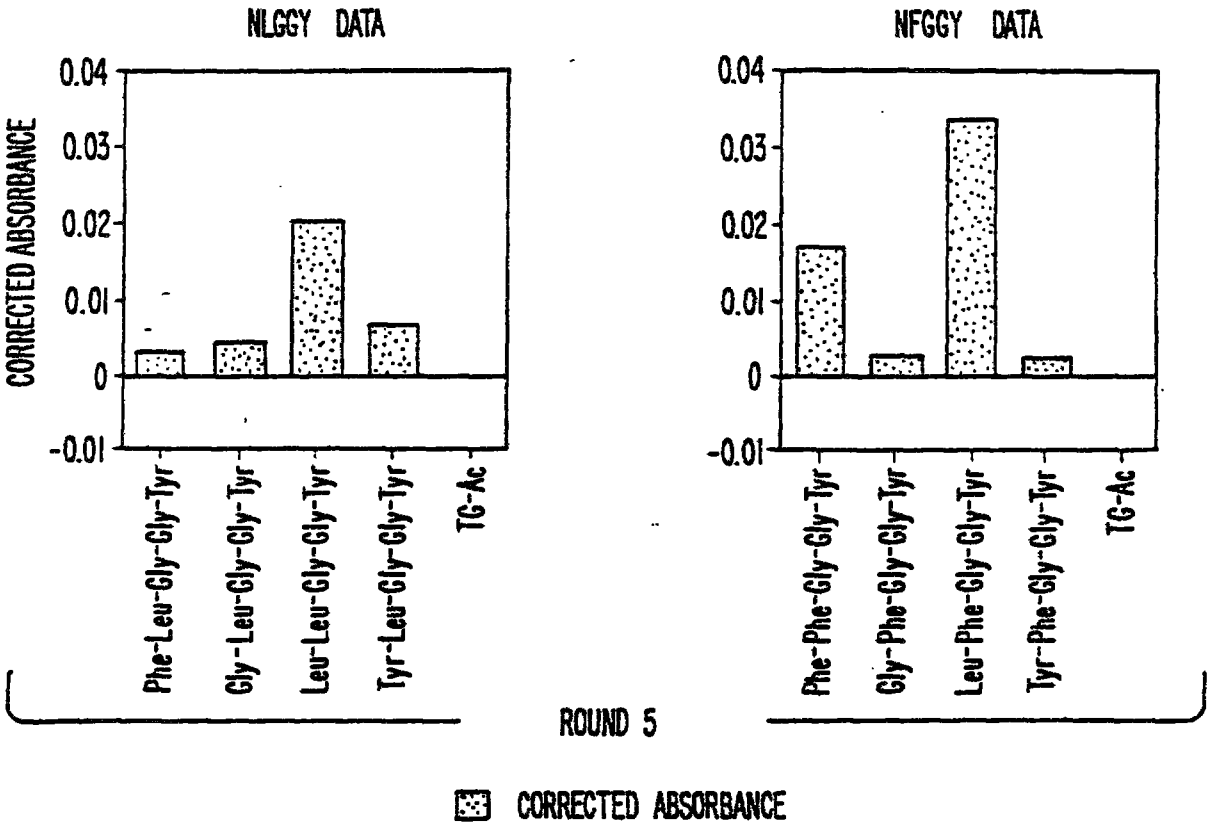
FIG. 3.

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FIG. 4.



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~~CORRECTED ABSORBANCES OF EACH POOL IN COMPARISON TO THE ACETYLATED TentoGel CONTROL POOL.~~
~~N DENOTES THE AMINO ACID TO BE DEFINED IN THAT ROUND. Xaa IS A RANDOMIZED POSITION.~~

FIG. 4 cont.