Design, construction and application of a fully automated equimolar peptide mixture synthesizer

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A fully automated peptide synthesizer has been constructed that is capable of the synthesis of equimolar peptide mixtures and the simultaneous synthesis of 36 individual peptides. The synthesizer was constructed from a workstation of our own design utilizing a Zymark robot arm. A Macintosh II computer coordinates the movements of the robotic arm, the switching of over 40 solenoid valves and the monitoring of sensors in the workstation. The robot hands are used to deliver solvents from pressurized spigot lines and to pipet amino acid solutions from reservoirs to an array of reaction vessels. Liquid dispensing, reagent mixing and solvent removal are controlled from a multifunction I/O board in the computer. The design features of the synthesizer are presented, as well as the characterization of multiple individual peptides, a simple mixture of 19 components, and a complex mixture of 15625 components.

Key words: combinatorial synthesis; molecular diversity; multiple peptide synthesizer; peptide library; robotic synthesis

The extensive use of peptides in drug discovery programs and research involving monoclonal antibodies, vaccines, therapeutics and diagnostics has resulted in the need for rapid peptide synthesis methodologies. Simultaneous multiple peptide synthesis technologies have recently met this need and serve as a source of peptide diversity. Geyser's multi-pin technology (1) and Houghten's "tea bag" method (2) were among the first innovations that allowed for the simultaneous synthesis of hundreds of peptides. These semi-automated methods were followed by more fully-automated robotic synthesizers which include a 96 mini-tube format (3) and a 48 flow-through reactor system (4). Solid-phase chemistry using photolabile protecting groups and photolithography has recently been used to synthesize $\sim 10^4$ peptides bound to a silica solid support (5).

An alternative strategy for generating peptide diversity is to synthesize mixtures of peptides by solid-phase synthetic procedures (6–12). In one method, mixtures are synthesized by coupling a mixture of amino acids to a single batch of resin (6–8). In order to synthesize mixtures of known composition, however, knowledge of individual coupling rate constants is required since the amino acids are competing for a single batch of resin (8). In another method (Fig. 1), mixtures of known composition can be readily prepared by physically separating the solid support into equal aliquots, coupling a unique amino acid to each aliquot, and then mixing all the resin aliquots (9–12). Recently, this resin-splitting method was used by two different groups to synthesize peptide libraries containing $\sim 10^6$ components (10, 11). These libraries led to the discovery of novel protein-binding ligands (10) and biologically active peptides (11). The latter method has also been used to generate mixtures of lower complexity to determine quantitative structure-activity relationships in receptor-binding (12) and enzyme reactivity (13) studies. Affinity selection experiments have also been performed with solution-phase peptide mixtures in which the highest affinity components of a mixture were directly identified (12,23). Although the screening of peptide mixtures generated by the resin-splitting method increases the throughput of compounds assayed in drug discovery programs, the (manual) synthetic procedure is quite labor intensive.

We report here the design and construction of a fully automated peptide synthesizer that is capable of both the synthesis of equimolar peptide mixtures by the resin-splitting method (9–12) and the simultaneous synthesis of up to 36 individual peptides. The instrument consists of a workstation of our own design and a Zymate XP robot interfaced with a Macintosh II computer. The computer provides calculation power and event control capabilities that are not available in the
HPLC characterization of peptides was performed on a Rainin HPX system controller with a C18 reversed-phase HPLC column (Vydac, 25 cm x 4.6 mm) and a gradient elution (solvent A: H2O/0.1% TFA and solvent B: CH3CN/0.1% TFA; 0%-50% B in 40 min).

Deprotection of the amino acid side-chains and cleavage of the peptides from the resin were accomplished automatically by a robotic workstation as described (16). The peptide-resin was treated with 82.5% trifluoroacetic acid/5% phenol/5% water/thioanisole/2.5% ethanediol (10 mL per 500 mg resin) (17). The deprotection mixture was incubated at room temperature for 2 h, and concentrated to a 1 mL volume by gentle heating under a stream of nitrogen. The peptide residue was then diluted with 10% HOAc/H2O (10 mL), extracted with diethyl ether, and lyophilized.

Amino acid compositions were obtained using the Pico-Tag method of Waters Associates (Milford, MA) (18). Mass spectra were analyzed in a glycerol matrix by Liquid Matrix Secondary Ion Mass Spectrometry on a VG Analytical ZAB 2SE mass spectrometer at Mass Search (Modesto, CA).

**Robotic apparatus**

The robotic equimolar peptide mixture (EPM) synthesizer was designed around a Zymate XP robot (Zymark Corp., Hopkinton, MA) which consists of a central robotic arm that provides a variety of functions via a 5 mL pipetting hand, a gripping hand, and a modified 30 mL syringe hand (Fig. 2). A Macintosh II computer (Apple Computer, Cupertino, CA) is interfaced with the Zymark controller as described (14). A Remote Computer Interface Module Card (Zymark Corp.) connects the Zymark controller to the computer. The computer is programmed in THINK C language version 5.0 (Symantec Corp., Cupertino, CA) and sends commands through its serial port to the Zymark which then executes the robotic movements. The computer is equipped with a MacADIOS II multifunction analog and digital I/O board and three daughter boards (GW Instruments, Somerville, MA) which can control over 48 inputs and outputs. These boards are used to control solenoid valves and to monitor sensors. The multifunction I/O board is connected to a circuit board containing solid-state relays (5–60 V DC, Newark) by a 34 pin ribbon cable. The board triggers the relays, which connect the solenoid valves to a ±24 V DC power supply, by outputting 0 V or +5 V DC signals. The relays are enclosed in an aluminum chassis equipped with LED indicator lights.

A custom-designed 12" x 22" reaction vessel rack was constructed from high density polyethylene sheets and rods (Interstate Plastics, Oakland, CA). The rack is equipped with one fritted glass resin-mixing chamber (150 mL capacity), thirty-six 1.5 x 10 cm and twelve 2.5 x 10 cm glass reaction vessels, each fitted with a 20 µm polyethylene frit (Kontes, Vineland, NJ). The smaller reaction vessels are modified to contain a slight
bulge in the wall, 3 cm from the bottom, to optimize resin mixing and prevent resin from rising up the wall (Fig. 3). Reaction vessels are attached to the rack with polypropylene bulkhead luer fittings (Cole-Parmer, Chicago, IL). The solenoid valves, plumbing, reaction vessels and vacuum/pressure manifolds are constructed from solvent-inert materials (Teflon, polypropylene, polyethylene, glass) and are configured as shown in Fig. 4. Liquid delivery and removal from the reaction vessels is controlled by Teflon 24 V DC, 1/16” orifice solenoid valves (General Valve Corp., Fairfield, NJ). The reaction vessels are grouped in rows of six such that there are two rows of large vessels and six rows of small vessels. Teflon tubing (1/16” i.d.) connects each row of reaction vessels to both the pressure and vacuum manifolds via a custom-designed 6-to-1 Teflon connector and a 2-solenoid, 3-port (normally closed) valve. The Teflon lines are positioned at a level higher than the reaction vessels to prevent cross-contamination during the filling of reaction vessels. In addition to the 2-solenoid, 3-port valve, one row of both the small and large vessels have reaction vessels that are individually controlled by 2-way (normally closed) valves. The pressure and vacuum manifolds are constructed from polyethylene hollow rods (3/4” i.d., McMaster-Carr, Los Angeles, CA) fitted with 9 Teflon male pipe adapters (1/8” NPT x 1/8” o.d., Cole-Parmer). The vacuum system is controlled by two 3-way valves (24 V DC, 1/8” i.d., Furon, Anaheim, CA) and consists of a 4 L polypropylene vacuum bottle (Naige Co., Rochester, NY), a 25 L carboy, a vacuum pump, and a refrigerated (-90°) condensation trap (Savant Instruments, Farmingdale, NY) as shown in Fig. 5. Solvents (DMF, 20% piperidine/DMF, and 65% 1,2-dichloroethane/DMF) are stored in 2000 mL or 5000 mL pressurized bottles (Rainin Instrument Co., Woburn, MA) fitted with Teflon spigot lines that are accessed by the robot arm. Small volumes (µL amounts) are accurately delivered with a multichannel syringe pump (Master Laboratory Station, Zymark Corp.) and corresponding spigot.

Individual peptide synthesis

Peptides are synthesized on polystyrene resin with standard Fmoc chemistry (19). There are three major steps in each cycle of the synthesis: (a) deprotection of the N-terminal Fmoc group on the peptide-resin, (b) acti-
Active mixing of reagents and solvents with the resin is achieved by the periodic bubbling of argon gas through the fritted bottom of the reaction vessel. This also blanks the reaction mixture with a layer of argon gas.

![Image of apparatus](image)

**FIGURE 3**

Plumbing configuration for the 36 small reaction vessels. The 12 large reaction vessels are configured in a similar manner (not shown). This configuration allows for the synthesis of any number of peptides between 1 and 36 with a minimum number of solenoid valves. All valves are shown de-energized.

FIGURE 4

Automation & coupling of amino acids, followed by (c) acetylation of unreacted amino groups (optional step). The reagent concentrations, volumes, reaction times, and number of repetitions performed in the robotic synthesis protocol are shown in Table 1. Reaction conditions (aspirate times, reagent volumes and wash volumes) are based on the reaction scale which can range from 25 μmol to 125 μmol in the small reaction vessels. The time required to drain a given volume of solvent was determined empirically for a range of reaction scales and depends on both the solvent volume and the number of reaction vessels in use.

Operation of the synthesizer requires the user to input the peptide sequences, the molecular weights of the

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Rxn Time</th>
<th>Volume</th>
<th>Repetition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20% Piperidine/DMF</td>
<td>1 min</td>
<td>2.0 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>2</td>
<td>DMF</td>
<td>20 min</td>
<td>2.0 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>3</td>
<td>0.3 M AA/0.3 M HOBT</td>
<td>1–30 min</td>
<td>0.83 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>4</td>
<td>3.2 M DIC</td>
<td>1–5 min</td>
<td>0.20 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>5</td>
<td>DMF</td>
<td>30 min</td>
<td>2.0 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>6</td>
<td>DMF</td>
<td>3 min</td>
<td>2.0 mL</td>
<td>3 x</td>
</tr>
<tr>
<td>7</td>
<td>0.25 mM Ac₂O/DIEA</td>
<td>20 min</td>
<td>2.0 mL</td>
<td>1 x</td>
</tr>
<tr>
<td>8</td>
<td>DMF</td>
<td>5 min</td>
<td>2.0 mL</td>
<td>1 x</td>
</tr>
<tr>
<td></td>
<td>Total cycle time:</td>
<td>126–192 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Solvent and reagent volumes are a function of the reaction scale, which can range from 25 to 125 μmol peptide per reaction vessel.

* The addition of each amino acid requires ~45 seconds if a tip change is required.
Figure 5
Pressure and vacuum systems for the robotic peptide synthesizer. The pressure system is maintained at 7–8 psi of argon gas, and is used for the delivery of solvents through spigot lines, and the mixing of resins in the reaction vessels. The vacuum system is maintained at 28–29" Hg and collects solvent waste in the 4 L vacuum trap. The 4 L trap can be backflushed with argon pressure into a 25 L carboy to prevent overfilling. (C = common; NC = normally closed).

Amino acids, and the reaction scale. A calculation protocol then outputs the amount of amino acids and activator needed. The user then adds resin to the appropriate reaction vessels, prepares the amino acid and activator solutions, and fills the solvent reservoirs. The automated synthesis then begins by first swelling the resin in DMF. The gripping hand accesses the DMF spigot and adds DMF to each vessel through the argon pressurized line (7–8 psi). The DMF spigot is sealed at the end and contains eight radial holes that rinse the walls of the reaction vessels. The resin slurry is thoroughly mixed by periodic bubbling. The DMF is then removed by the application of vacuum (27–29 in Hg) which draws solvents through the fritted bottom of the reaction vessels into the 4 L Nalgene bottle (Fig. 5). A purge function periodically transfers this solvent waste to a vented 25 L waste bottle with argon pressure. The N-terminal Fmoc group on the peptide-resin is then removed by the addition of 20% piperidine/DMF (1 x 1 min, followed by 1 x 20 min) with periodic mixing. The resin is then washed thoroughly with DMF (7 x). The coupling cycle begins with the addition of amino acid by the 5 mL pipetting hand. Amino acids are stored in an open 5 x 5 array of 250 mL polypropylene bottles and are periodically blanketed with argon to prevent the absorption of water. Fmoc amino acids are added to the peptide-resin in a 5-fold molar excess to amino groups as a 0.3 M solution in DMF containing 0.3 M 1-hydroxybenzotriazole (HOBr). The delivery of each amino acid aliquot to a reaction vessel (including changing pipet tips) takes ~45 seconds. The amino acids are then activated in situ by the addition of N,N'-disopropylcarbodiimide (DIC) to each reaction vessel via a multichannel syringe pump and corresponding spigot. Activation chemistries compatible with this format also include the HBTU (20) and PyBOP (21) reagents which require the addition of small volumes of base. The distribution of a common activator solution to all reaction vessels requires ~5 min. Coupling reactions are then typically allowed to proceed for 30 min, although the time can be specified by the user. After the initial coupling, the reaction solutions are removed, the resins are washed, and fresh aliquots of amino acid and activator are added. Following the second coupling, any unreacted amino groups are acetylated (optional step) by the addition of 0.25 mM acetic anhydride, 0.25 mM disopropylethylamine (DIEA) in DMF for 20 min.

Equimolar peptide mixture synthesis
Synthesis of an equimolar peptide mixture uses the same synthesis protocol as described for individual peptides, but requires two additional steps that involve the transfer of resin particles (Fig. 1). Distribution and recombination of the resin, as required by the resin-splitting method, is performed with a modified 30 mL syringe hand as shown in Fig. 6. The first step in the resin splitting algorithm involves the distribution of resin into a variable number of reaction vessels between 2 and 36. The resin is suspended as a 3% w/v free flowing slurry in 65% 1,2-dichloroethane/DMF, and the resin slurry is divided into equal portions by volume. Since the
Peptide-resin particles are robotically transferred in and out of reaction vessels as an isopercnic slurry via a hand fitted with a 30 mL disposable syringe.

FIGURE 6

A level sensor is used to obtain a pre-determined volume of resin slurry despite changes in the resin volume throughout the synthesis. The resin-mixing chamber is initially filled with a slight excess of solvent, and the solvent is then drained to the sensor level by opening the bottom valve to vacuum. The slurry is mixed by opening the valve to argon pressure. Solvent is dispensed from a pressurized line containing eight radial holes which spray the walls.

FIGURE 7

A level sensor is used to obtain a pre-determined volume of resin slurry despite changes in the resin volume throughout the synthesis. The resin-mixing chamber is initially filled with a slight excess of solvent, and the solvent is then drained to the sensor level by opening the bottom valve to vacuum. The slurry is mixed by opening the valve to argon pressure. Solvent is dispensed from a pressurized line containing eight radial holes which spray the walls.

RESULTS AND DISCUSSION

Individual peptide synthesis

A robotic peptide synthesizer has been constructed that can function in a multiple-peptide synthesis mode. Any
number of individual peptides from 1 to 36 can be synthesized on a scale from 25 to 125 μmol per peptide, which provides enough peptide for complete chemical characterization, structural studies and numerous biological assays. The robotic design and software flexibility enable any amino acid to be added to any reaction vessel, thus allowing for the simultaneous synthesis of unrelated sequences of variable length. Standard Fmoc amino acids and polystyrene resins are used with carbodiimide, PyBOP or HBTU activation chemistries (19). One complete cycle of monomer addition takes from 1.5 to 3 h per residue (without capping), depending on the number of peptides synthesized (Table 1). Presently, up to 25 different amino acid monomers can be added, but this number is limited only by available workspace.

The ability of the EPM synthesizer to synthesize individual peptides of high purity was demonstrated by the simultaneous synthesis of eight decapeptides. These peptides comprise a set of decapeptide sequences obtained from the affinity selection of a bacteriophage library with a monoclonal antibody (24). The majority of these peptides were synthesized in >95% purity as determined by analytical reversed-phase HPLC (Fig. 8). The identity of each peptide was confirmed by mass spectrometry and amino acid analysis (data not shown).

**Equimolar peptide synthesis**

The EPM synthesizer has been designed to synthesize peptide libraries of known composition, that contain any combination of constant and mixed residues. For example, a specific library designed to probe structure-activity relationships between peptide analogs can be synthesized where a mixture is introduced at specific positions in the lead compound. Alternatively, a generic library, intended for the random screening of any receptor, can be synthesized by placing a mixture of amino acids at every position of a peptide. Either type of library can be used for screening receptors or enzymes as resin-bound peptides (10), or cleaved into solution and used in competition binding assays (11-12, 23), enzyme assays (13), or affinity selection experiments (12, 23). The EPM synthesizer was designed to accommodate all of the above mixture strategies. The synthesizer can generate up to 36 related peptide mixtures on a scale of 1.8-3.5 mmol total (which can yield 2-4 g of total decapeptide).

In addition to quantitative amino acid couplings, the synthesis of equimolar peptide mixtures requires the quantitative transfer of resin particles in and out of reaction vessels. Quantitative resin transfer is achieved with a robotic pipet hand modified with a 30 mL disposable syringe (B-D, VWR Scientific, Plainfield, NJ) as shown in Fig. 6. This hand can accurately transfer volumes between 4 and 20 mL to within ± 0.1 mL. The resin is suspended as a 3% w/v free-flowing slurry in a solvent system that has a similar density to polystyrene, causing the resin particles to settle very slowly.

**FIGURE 8**

Reversed-phase C18 analytical HPLC analysis of a series of eight crude decapeptides synthesized individually on the robotic synthesizer. All peptides are acetylated and amidated: (a) SSRPMRPTKT, (b) ESTRPMAGDA, (c) PSTPMPQSRG, (d) RALESTRAME, (e) SSDRAWVCCS, (f) SIVSVREVFR, (g) GRAWGVADKR, (h) LARKDGFGGW.

This allows the resin slurry to be divided into equal portions by volume. The accuracy with which the synthesizer distributes resin by this isophasic slurry method was determined by partitioning 2.5 g of resin (1.25 mmol) from the mixing chamber into 19 reaction vessels. The dry weight of each aliquot was determined to be within a ± 3% standard deviation of the mean weight (data not shown).

In addition to the accuracy of resin transfer, the equimolarity of a mixture depends on amino acid coupling yields and efficiency of resin mixing. A nineteen component decapeptide mixture: H2N-DKLYDLXPE-NH2 was synthesized containing nine constant positions and one mixture position (X) at which all the natural amino acids (except Cys) were substituted. The equimolarity of this mixture was determined by amino acid analysis (Fig. 9). The expected
composition of the constant residues varies from 10.53% to 31.06%, whereas the composition of these residues found only at the mixture position (constant residues excluded) is expected to be 0.53%. The variation in composition of these 11 residues (0.55% ± 0.07) indicates that the mixture components were present within a ± 12% standard deviation of the expected value. Although the observed composition of the constant residues closely matched the predicted values, the magnitude of these values are much greater than that of a single mixture residue, and therefore cannot be used to assess the mixture equimolarity. The presence of every component was confirmed by LSIMS mass spectrometry of the mixture (data not shown). These results demonstrate the ability of the EPM synthesizer to automatically and accurately perform equimolar peptide mixture syntheses.

The EPM synthesizer was then used to generate a 15625-component generic library intended for screening against a variety of receptors: \( H_2N-GGB_{B_1}B_2XXXG-NH_2 \) where \( B_1, B_2 \) and \( X \) are equimolar mixtures of Tyr, Glu, Ser, Ile, and Arg. This subset of amino acids was chosen because it comprises a chemically diverse set of functionalities. Since peptides are synthesized from their \( C \) to \( N \)-termini, the synthesis of related subpools that differ in the identity of amino terminal residue(s) was accomplished by omitting a resin recombination step as shown in Fig. 1. This library was synthesized as 25 separate pools, each containing 625 peptides and a unique combination of \( N \)-terminal mixture positions \( B_1 \) and \( B_2 \). Specifically, the synthesis of the four \( C \)-terminal mixture positions was followed by the distribution of the resin from the mixing chamber into five large reaction vessels. A different amino acid, corresponding to each \( B_2 \) residue, was then added to each of the five resin samples. These five pentapeptide mixture were each subsequently distributed into five small reaction vessels, and the \( B_1 \) residue was added to all 25 vessels.

The twenty-five 625-component mixtures were characterized by amino acid analysis. Unfortunately, amino acid analyses of mixtures this complex are only capable of highlighting gross variations in composition, and is not capable of indicating the absence of a particular sequence. The composition of 23 out of the 25 pools were within ± 15% of the expected values after cleavage from the resin (data not shown). The two pools that were outside of this composition range were obtained in very low yields after side-chain deprotection and cleavage from the resin; the \( B_1B_2 = YY \) and \( YI \) pools were obtained in 12% and 10%, respectively. These two are among the most hydrophobic pools synthesized, and it is likely that a significant portion of these pools were lost during the post-synthesis workup which involved extraction with ether. Yields for the remaining pools ranged from 40–60%. Two representative amino acid analyses are shown (Fig. 10), one for a high-yielding pool (\( B_1B_2 = SI \)) and a low-yielding pool (\( B_1B_2 = YY \)).

Since each mixture was generated from a diverse set of functionalities, a mixture population is expected to be composed of peptides with a wide range of chemi-

![Amino Acid Analysis](image-url)

**FIGURE 9**
Amino acid analysis of a 19-component decapeptide mixture: \( H_2N-DKNLYDLXP6-NH_2 \) where \( X = \) all natural amino acids (except cysteine). The composition of 11 of the 19 mixture residues indicate that the mixture is approximately equimolar. (■ = predicted values, □ = observed values).

![Amino Acid Analysis](image-url)

**FIGURE 10**
Amino acid analysis of two of the 25 sub-pools of the 15625-component mixture synthesized on the EPM synthesizer. Each pool theoretically contains 625 components. Analyses are shown for: (a) a high (50%) yielding pool \( B_1B_2 = SI \), and (b) a low (12%) yielding pool \( B_1B_2 = YY \). (■ = predicted values, □ = observed values).
cal and binding properties. Although this may be desirable from a random screening point of view, selective losses during the post-synthesis workup may pose a significant problem. For example, the observed low value of isoleucine (−34%) in the YY pool suggests that sequences containing isoleucine were preferentially lost during ether extraction. The use of cleavage/deprotection strategies that do not involve extraction or precipitation would be desirable when working with hydrophobic sequences. Potential solutions to this problem include the use of volatile scavengers (data not shown), or the incorporation of a photo-labile linker to the solid-support.

**Synthesizer design features**

The EPM synthesizer was designed around a Zymark robot because of its ability to perform a variety of functions with its interchangeable hands. A Macintosh II computer, interfaced with the Zymark robot, allows for the control of both robotic movements and solenoid valves (14). The robot hands are used to deliver solvents from pressurized spigot lines, pipet amino acid solutions to the reaction vessels, and transfer peptide-resin particles. Liquid dispensing, resin mixing, solvent removal, and the monitoring of sensors is controlled by the Macintosh computer via a multifunction analog and digital I/O board. The synthesizer has been expanded to include an automated peptide resin cleavage/deprotection station (16) and additional pressurized reagent lines which accommodate cyclization chemistries (25). The incorporation of an on-line HPLC analysis station is in progress.

The solenoid valve configuration is designed to allow for the synthesis of any number of peptides between 1 and 36 with a minimum number of solenoid valves (Fig. 4). The reaction vessels are arranged in six rows of six. One row has a separate solenoid valve under each reaction vessel that allows for independent mixing and draining of each vessel. Each of the other five rows is controlled by a single solenoid valve. Up to 36 reaction vessels can thus be independently controlled with only 11 valves. This capability is especially important for the synthesis of peptide mixtures, since each position of a peptide may have a different number of mixture components and therefore requires a variable number of reaction vessels.

The two rows of large reaction vessels are similarly configured to allow for the independent control of up to twelve vessels. The large reaction vessels allow peptides to be synthesized on an intermediate scale (125–900 μmol) between the small reaction vessels (25–125 μmol) and the mixing chamber (1.0–3.5 mmol). The ability to synthesize peptides on scales that range over two orders of magnitude is necessary for the synthesis of peptide mixtures by the resin-splitting method, since the reaction scale is inversely proportional to the number of divided resin aliquots.

Each row of large and small reaction vessels can access either the argon pressure manifold or the vacuum manifold via a 3-port valve with two normally closed, independently-controlled ports (Fig. 4). Reagent mixing in the reaction vessels is achieved by energizing one port of the solenoid valve which opens a row of reaction vessels to argon pressure. This causes gas to bubble through the fritted bottom of each reaction vessel, thoroughly agitation the resin slurry (Fig. 3). Active mixing is a unique feature of the synthesizer and is performed at each step of the peptide synthesis. Deprotection, coupling, capping and all washing steps are periodically mixed (1 out of every 10 seconds). Active mixing is particularly important during the synthesis of peptide mixtures. As many as 36 different resin samples can be transferred into the_mixing chamber and must be thoroughly mixed prior to any redistribution steps to ensure equimolarity. The argon gas mixing method facilitates the robotic delivery of reagents because there is no need to physically move the reaction vessels. In addition, the mixing also prevents reactants from absorbing water by blanketing them with argon.

Reagents are removed from a row of reaction vessels by energizing one port of the solenoid valve opening it to the vacuum manifold, which causes the liquid to drain through the bottom of each vessel. The flow-through vessels and vacuum manifold configuration are designed to completely remove reagents and wash solvents from the bottom of the reaction vessel in a minimal amount of time. Application of vacuum is performed on the vessels at a time with a short vacuum recovery time between rows for highest reproducibility. Waste solvents collects in a 4 L vacuum trap and is periodically transferred with argon pressure to a larger storage container.

In conclusion, a fully automated peptide synthesizer has been constructed that is capable of generating up to 36 individual peptides or equimolar peptide mixtures. In addition to performing repetitive amino acid couplings in high yield, we have demonstrated automation of the quantitative transfer of peptide-resin particles. The open vessel design, active resin mixing, and resin transfer by the isopycnic slurry method, allows resin to be divided accurately into equal portions by volume. The equimolar peptide mixture synthesizer automates an otherwise laborious method for the generation of synthetic polymer libraries. This technology holds great promise for the rapid discovery of new diagnostic and therapeutic agents.

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