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# 14
# MARS - MULTIPLE AUTOMATIC ROBOT SYNTHESIZER FEATURING A NOVEL TIMING PROCEDURE AND RANDOM ACCESS TO REAGENTS AND REACTION VESSELS.

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*An automated system for organic solid-phase synthesis was designed and constructed. Its main features are a flexible, modular, and open access design, and a novel timing procedure for handling multiple synthetic tasks that eliminates any unnecessary time delay during ongoing chemical reactions keeping the robot arm continuously in operation. Sequential rather than batchwise processing enables one to modify the queue for execution at any time during operation. When the synthesis of a compound has finished, the initiation of the next one from the synthetic queue automatically begins. The hardware design permits access to any number of reagents, reaction vessels, and accessories. Polypropylene syringes equipped with a polypropylene frit at the bottom serve as reaction vessels and all operations are performed by a robot arm with a specially designed gripper to hold the syringe and to aspirate or dispense liquids.*

## INTRODUCTION

Since its inception, synthesis on an insoluble solid support which was introduced by Bruce Merrifield [1] has been automated countless number of times. The first instruments obviously synthesized single peptides, an achievement without precedence at that time. Today synthesizers are very sophisticated instruments enabling unattended concurrent synthesis of many peptides [2]. Recently, the first instrument for the automation of organic reactions on solid phase has been launched by Advanced ChemTech.

Despite the number of synthesizers on the market produced by Advanced ChemTech/Zinsser [3], Abimed [4], Rainin, Spyder [5] and Shimadzu [6], we believe that there is still room for improvement. The routine work of a chemist has taught us that the demand for synthetic peptides comes steadily rather than in quantum (batchwise) fashion. Current synthesizers work in quantum fashion where from time to time (e.g. once a week) a synthesizer is loaded with reagents and solvents initiating the synthesis of a set (quantum) of peptides. Before this batch is finished, there is no way (except manual synthesis) to start a synthesis of even a single peptide.

Probably the most simple reaction vessel for solid-phase synthesis is a polypropylene plastic syringe [7]. Equipped with a frit that keeps the insoluble solid support inside, the syringe enables aspirating and dispensing liquid by simply moving the syringe plunger. Further, the material is compatible with all solvents used in synthesis of peptides, including TFA. We have been using syringes for the manual synthesis of peptides for quite a while with very good results, the only complaint being the boring repetition of plunger moves.

The obvious solution to eliminate this drawback was automation of the entire process. The result is MARS (multiple automatic robot synthesizer), an instrument featuring sequential processing of synthesized compounds using plastic syringes as reaction vessels.

## MATERIALS AND METHODS

### Instrument Description

The robot table layout is shown in Fig. 1. The synthesizer was built using the Small Industrial Robot System, model A251 (CRC Plus Inc., Harrington, Canada) interfaced with an IBM PC and operated by TCW system (Hudson Total Control for Windows, Hudson Control Group Inc., Springfield, New Jersey). The robot arm is equipped with a gripper that can take any syringe and transport it to any defined destination on the table and it can aspirate and dispense liquid by moving the syringe plunger. This gripper was designed to handle both 2.5 and 10 ml syringes. Syringes loaded with resin are placed in the incoming rack that can hold thirty 10 ml and thirty 2.5 ml syringes. Amino acid solutions are also stored in syringes where they are placed in two racks holding one hundred 2.5 ml and one hundred 10 ml syringes each. Solvent (DMF) and reagents (piperidine/DMF, DIPCDI/DMF) are delivered from solvent reservoirs to delivery cups from which the liquid is aspirated by the syringe. While the first cup is used for all DMF washes and for the delivery of protected amino acid and coupling reagent (DIPCDI), the second cup serves the delivery of the deprotection mixture (piperidine/DMF). The system is equipped with four 10 ml piston pumps (Hamilton MicroLab 900, Reno, Nevada), three pumps deliver liquid from reservoirs to the cups (DMF, piperidine/DMF, and DIPCDI solution), the last one removes washings from the cup, where the amino acid and DIPCDI are mixed. During the reactions (deprotection and coupling) the syringes are placed on a 2.5 ml or 10 ml tumbling rack, each holding up to 18 syringes. Whenever the robot arm picks a new syringe it passes the optical sensor to confirm that the syringe has been gripped. Syringes with finished peptides are placed into the basket.

### Chemistry

Standard Fmoc/tBu chemistry has been applied, where polystyrene (Rink resin) or polyethyleneglycol grafted polystyrene (TentaGel) resins have been used as solid supports. One synthetic cycle consists of the following steps:

(i) washing, DMF, 3 times for 30 seconds;

(ii) deprotection, 50% piperidine/DMF (v/v), 10 min.;

(iii) washing, DMF, 5 times for 30 seconds;

(iv) coupling, 3 molar excess of DIPCDI/HOBT activated Fmoc protected amino acids, 2 hours.

After finishing the last cycle, the synthesis is finished by washing (step (i)) or by washing, deprotection, and washing (steps (i) - (iii)).

## RESULTS AND DISCUSSION

The simple chemical protocol for one synthetic cycle of a peptide synthesis consists of four steps: (1) washing, (2) deprotection, (3) washing, and (4) coupling. When automated, all steps involving liquid handling require robot operation, whereas during the time necessary for a chemical reaction to be completed the robot is not in use (the syringe rests on tumbler). Timing of the synthesis of individual peptides can be arranged as shown in Fig. 2. For example, we use a protocol consisting of 5 min. washes, a 10 min. deprotection and a 2 hours coupling time. After finishing the initial wash and starting the deprotection of the first peptide, there is 10 min. left for the robot to start a synthesis of the second and then the third peptide. The only condition that has to be fulfilled is that the robot has to be able to finish all consequent operations once the synthesis has started. Before the first coupling of the first peptide is finished, the synthesis of 11 peptides can be initiated.

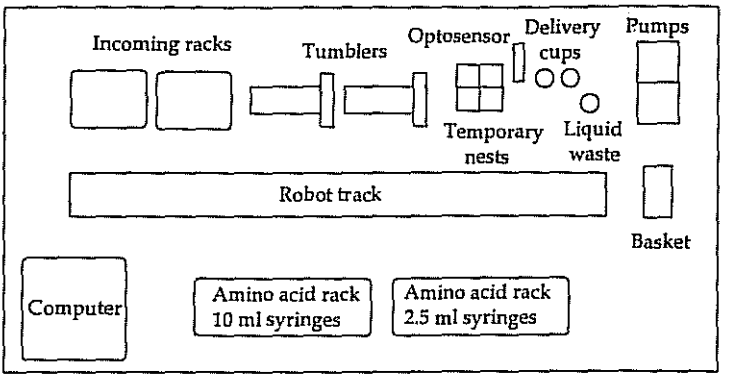


**Figure 1.** Robot table layout

The MARS performs a synthesis exactly as it is done manually, using the gripper to hold the syringe and to move the plunger instead of a human hand used in a manual synthesis. We consider the main advantage of this concept to be operational flexibility. Depending on the chemical protocol, the MARS is synthesizing a certain number of peptides all of the time. Once the synthesis of one peptide is finished, it automatically takes the next peptide from the waiting list for synthesis. A practical consequence of this feature is that the operator can update the synthetic waiting list at any time which eliminates an instrument start or stop period. Taking an example of decapeptides and the protocol described in the Material and Methods section, up to 12 peptides can be synthesized simultaneously and the daily throughput averages 10 completed peptides.

Approximately every two hours a synthesis of a new peptide begins. This means, that in the case of a very urgent peptide synthesis, the longest waiting time for the initiation of this synthesis would be 2 hours.

An additional advantage of the MARS is the independent handling of reaction vessels and random access to reagents. Peptides with any length can be synthesized concurrently without

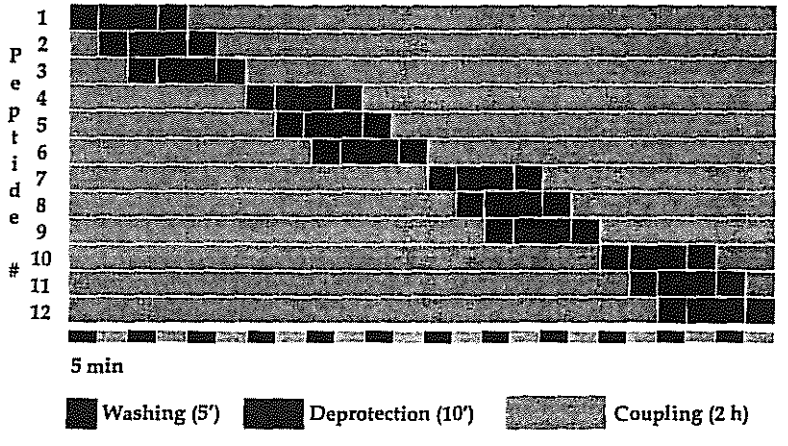


**Figure 2.** Timing of individual steps during multiple synthesis of 12 peptides

compromising the total throughput. Further, any peptide in the synthesis can be removed any time without stopping the run and different sizes of reaction vessels (plastic syringes) can be used together. Because of its modular design, the synthetic flexibility can be increased by the addition of reaction accessories where chemistries other than traditional amide bond formations can be accommodated.

This concept and completed system was validated by the multiple synthesis of peptides. The current version of MARS is capable of synthesizing 5 to 15 peptides of different length simultaneously. While current quality of crude peptides is consistently high, prior unexpected results were always connected with hardware or software problems during the relatively long period of debugging the instrument.

## CONCLUDING REMARKS

We have designed and constructed MARS, a multiple automatic robot synthesizer, the most important feature of which is a novel timing procedure and random access to reagents and reaction vessels. The advantageous timing was made possible by independent handling of each peptide in independent reaction vessel. As a result of this protocol MARS can run continuously; after finishing any of peptides that are currently being synthesized it automatically starts the synthesis of the first peptide on the waiting list without interrupting the synthesis of peptides that are in progress.

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