

Peptide Research

Automated Peptide-Resin Deprotection/Cleavage by a Robotic Workstation

Ronald N. Zuckermann
and Steven C. Banville
Chiron Corporation

ABSTRACT

A robotic workstation has been constructed that automates the deprotection of peptides with trifluoroacetic acid-labile side-chain protecting groups and cleavage from acid-labile resins. The workstation was constructed around a Zymark robot arm and is integrated with a peptide synthesis workstation. Peptide resin samples are deprotected and cleaved with a trifluoroacetic acid/scavenger cocktail that accommodates all common protecting groups used with Fmoc chemistry. Aqueous-ether continuous extraction is used to remove the scavengers and reaction by-products. The apparatus cleaves a 50–500-mg sample of peptide resin every 2 hours and provides the peptides as an aqueous solution in 10% HOAc. Crude peptides obtained with this apparatus are free from residual scavengers and range in yield from 50%–80%. This method is applicable to all but the most hydrophobic peptides and minimizes human contact with the noxious cleavage reagents.

INTRODUCTION

Automated multiple-peptide synthesis technology has revolutionized peptide research by increasing the rate and ease of peptide synthesis (5,6,8,12, 14). Although the synthesis of peptides has been automated, the subsequent side-chain deprotection and cleavage from the resin still require manual workup and can quickly become the rate-limiting steps in obtaining peptides.

Peptides synthesized by standard Merrifield solid-phase chemistry require side-chain deprotection and concomitant cleavage from the resin to obtain the free peptide (13). For peptides synthesized by Fmoc chemistry, this can be accomplished by treating the peptide-resin with trifluoroacetic acid and a cocktail of scavengers to prevent unwanted side reactions (9). Recovery of the peptide from this mixture is typically accomplished either by ether precipitation or by aqueous-ether extraction (9), both of which are somewhat labor-intensive.

An apparatus of our own design has been constructed which fully automates the side-chain deprotection and resin-cleavage of peptide-resin samples. The apparatus accommodates peptides containing trifluoroacetic acid (TFA)-labile side-chain protecting groups and resin linkages. An established cleavage protocol is used which efficiently removes all side-chain protecting groups used in Fmoc chemistry (9). A continu-

ous aqueous-ether liquid-liquid extraction procedure is used to remove the scavengers and reaction by-products. The workup procedure provides the peptide as an aqueous acidic solution, which can then be lyophilized and/or HPLC purified.

The robotic apparatus consists of two workstations: a cleavage station and an evaporation/extraction station (Figure 1). A robotic arm delivers solvents/reagents and transfers peptide samples to these stations. An external computer is interfaced with the robot and controls the central arm movements, solenoid valves and pneumatic devices (15). Details of the apparatus and chemical methodology are described.

MATERIALS AND METHODS

General

High-purity dichloromethane and dimethylformamide were obtained from Burdick & Jackson and used without further purification. Trifluoroacetic acid (Fluka, Buchs, Switzerland), thioanisole (Aldrich, Milwaukee, WI), 1,2-ethanedithiol (Aldrich), glacial acetic acid (Baker, Phillipsburg, NJ) and phenol (Gibco BRL/Life Technologies, Gaithersburg, MD) were used without further purification.

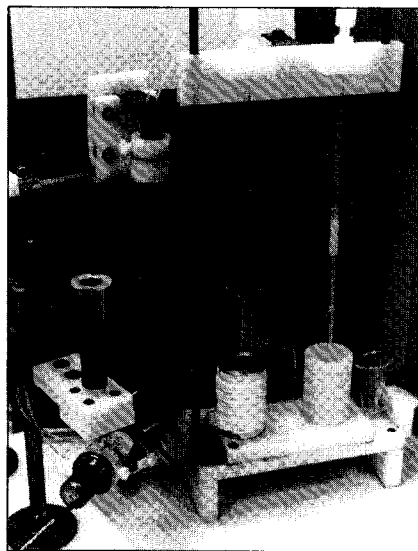


Figure 1. The robotic peptide-resin deprotection/cleavage apparatus, consisting of two substations. The trifluoroacetic acid reactions occur in disposable polypropylene vessels in the cleavage substation (left), and the evaporation and extraction procedures occur in a glass sidearm tube in the evaporation/extraction substation (right).

Fmoc amino acids and polystyrene resins were obtained from Advanced Chemtech (Louisville, KY). Side-chain protecting groups used with the apparatus include: *t*-butyl esters for Asp and Glu, *t*-butyl ethers for Ser, Thr, and Tyr; triphenylmethyl for Cys, His, Asn, and Gln; *t*-butoxycarbonyl for Lys; and 2,2,5,7,8-pentamethylchroman-6-sulfonyl for Arg. 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin (100–200 mesh, 1% crosslinked with divinylbenzene) was used at a 0.40 mmol/g substitution level for the synthesis of C-terminal amides (11).

HPLC analysis of peptides was performed on a Rainin HPX system controller with a C18 reversed-phase HPLC column (Vydac, 25 cm × 4.6 mm; The Separations Group, Hesperia, CA) and a gradient elution (solvent A: H₂O/0.1% TFA and solvent B: CH₃CN/0.1% TFA; 0%–50% B in 4 min).

Amino acid compositions were obtained using the Pico-Tag method of Millipore, Milford, MA) (1). 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).

Robotics

The cleavage/deprotection workstation was designed around a Zymate XP robot (Zymark, Hopkinton, MA), which consists of a central arm that provides a variety of functions via a gripping hand and a modified 30-ml syringe hand (Figure 2). A Remote Computer Interfaces Module Card (Zymark) connects the Zymark controller

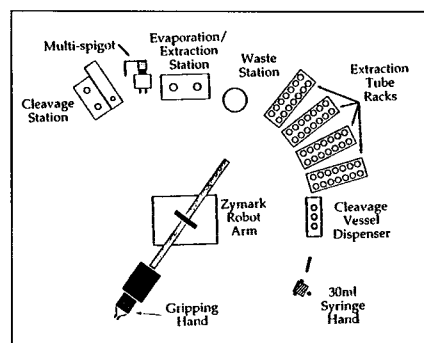


Figure 2. Layout of the deprotection/cleavage apparatus. The Zymark robot arm uses a gripping hand and a syringe hand to process peptide samples through the cleavage and evaporation/extraction substations. Additional racks are provided for extraction tube and cleavage vessel storage.

to a Macintosh II (Apple Computer, Cupertino, CA). The Macintosh is programmed in THINK C language and sends commands through its serial port to the Zymark, which then executes the robotic movements (15). The computer is equipped with a MacADIOS II multifunction analog and digital I/O board and three daughter boards (GW Instruments, Somerville, MA) which allow for the control of over 48 inputs

and outputs. The Macintosh controls the solenoid valves, a pneumatic air cylinder and a diaphragm pump via the multifunction I/O board. The multifunction I/O board is connected to a circuit board containing solid-state relays (5–60 V DC; Newark, Chicago, IL) by a 34-pin ribbon cable. The MacADIOS II board triggers the relays by outputting 0 V and +5 V signals which connect the solenoid valves to a

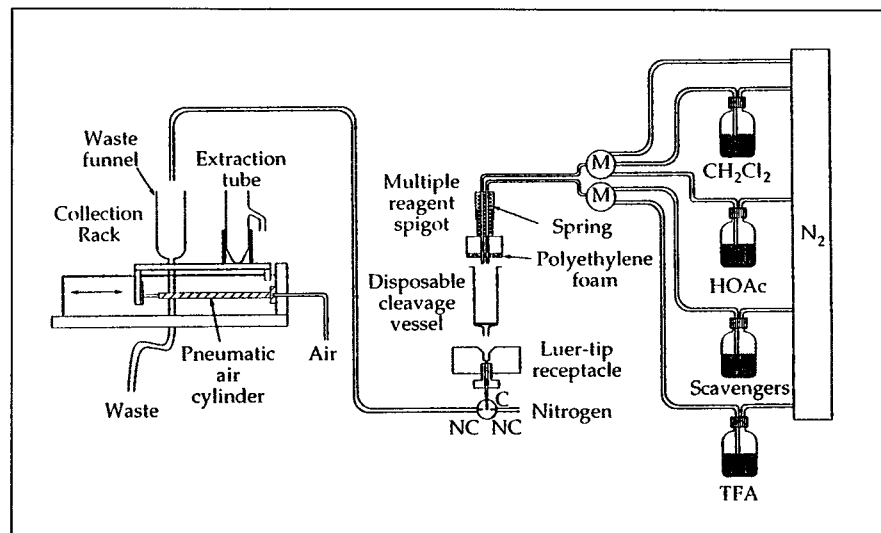


Figure 3. The cleavage station consists of a polypropylene receptacle that forms a leak-tight seal with a disposable cleavage vessel delivered by the robot arm. Reagent mixing and draining are controlled by a solenoid valve that can open the vessel to a nitrogen source or to a drain path (C = common, NC = normally closed). A multiple-reagent spigot is used to deliver a variety of reagents and can form a gas-tight seal when pressed against the top of the cleavage vessel. Contents of the cleavage vessel can be directed to either an extraction tube or to a waste receptacle. M = Multi-port solenoid valve.

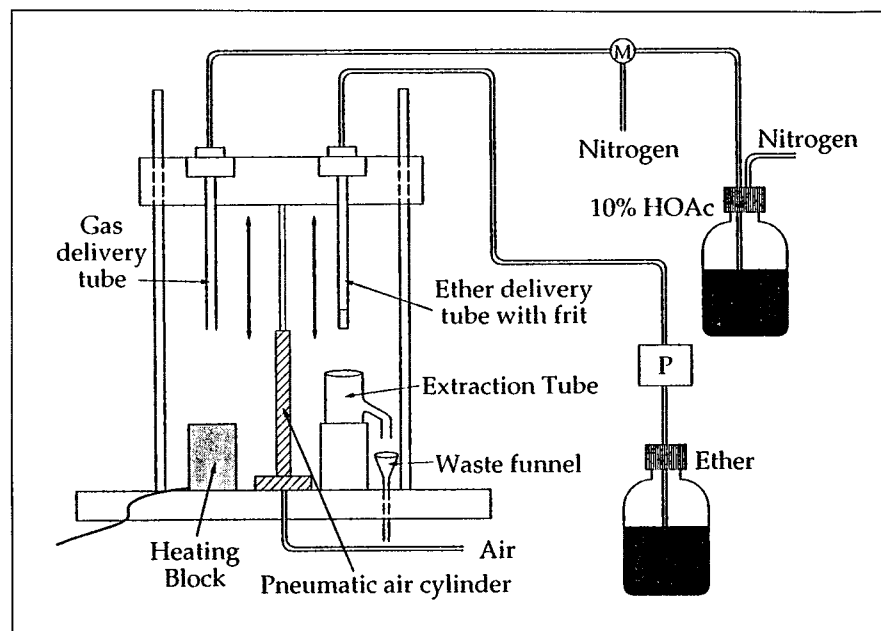


Figure 4. The evaporation/extraction station has two positions that can be vertically accessed by reagent-delivery tubes. Evaporation occurs in the heating block position (left) with the delivery of nitrogen gas. Aqueous acetic acid can also be delivered to this station. Aqueous-ether continuous extraction occurs in the adjacent position. P = Diaphragm pump.

power supply. All solenoid valves (General Valve, Fairfield, NJ) were made from Teflon® and operate at +24 V DC.

Cleavage Workstation

The cleavage workstation (Figure 3) consists of a polypropylene platform with a tapered receptacle (0.166" i.d.) that forms a leak-proof seal with luer-tipped polypropylene vessels. Disposable 15-ml polypropylene vessels are fitted with a 20- μ m polyethylene frit (Varian Sample Preparation Products, Harbor City, CA) and stored in three 4' \times 1" glass dispensers which vertically stack up to 16 reaction vessels each. A 2-solenoid, 3-port (normally closed) valve is located beneath the cleavage platform and can either open to nitrogen pressure to allow reagent mixing or open to an outlet effect draining. Teflon tubing (1/16" o.d.; Cole-Parmer, Chicago, IL) directs fluid drained from the cleavage vessel to either a waste position or an evaporation/extraction tube in the collection rack. The fluid destination is controlled by the activation of a pneumatic air cylinder, resulting in movement of the collection rack (Figure 3).

Dichloromethane and acetic acid are stored in 200-ml pressurized bottles (Rainin Instrument, Woburn, MA) fitted with Teflon spigot lines. The cleavage reagents, 94% TFA/H₂O and the scavenger mixture, are stored separately in 500-ml and 200-ml pressur-

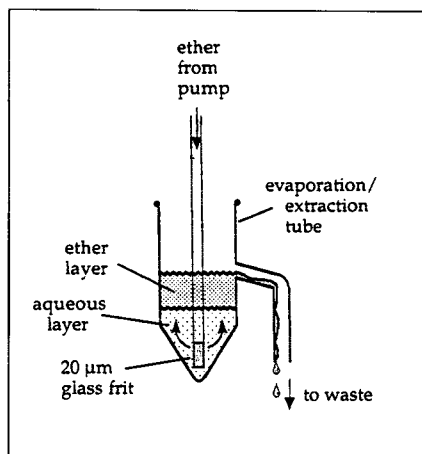


Figure 5. Scavengers and reaction by-products are removed from an acidic aqueous peptide solution by a continuous liquid-liquid extraction procedure. Ether is delivered through a fritted glass tube, flows through the aqueous layer and carries the contaminants away via the sidearm tube.

ized amber bottles, respectively, fitted with Teflon spigot lines. Delivery of all four of these reagents is controlled by 2-way solenoid valves. These reagents are delivered to the cleavage vessel by pressurized Teflon lines (1/16" o.d.) which culminate in a custom-made

multi-channelled spigot (Figure 3). This multi-spigot consists of a polyethylene rod (1.0" o.d.) fitted with an internal spring and a 1/8" layer of polyethylene foam (McMaster-Carr, Los Angeles, CA) on the contacting surface. This design allows an air-tight seal to be made

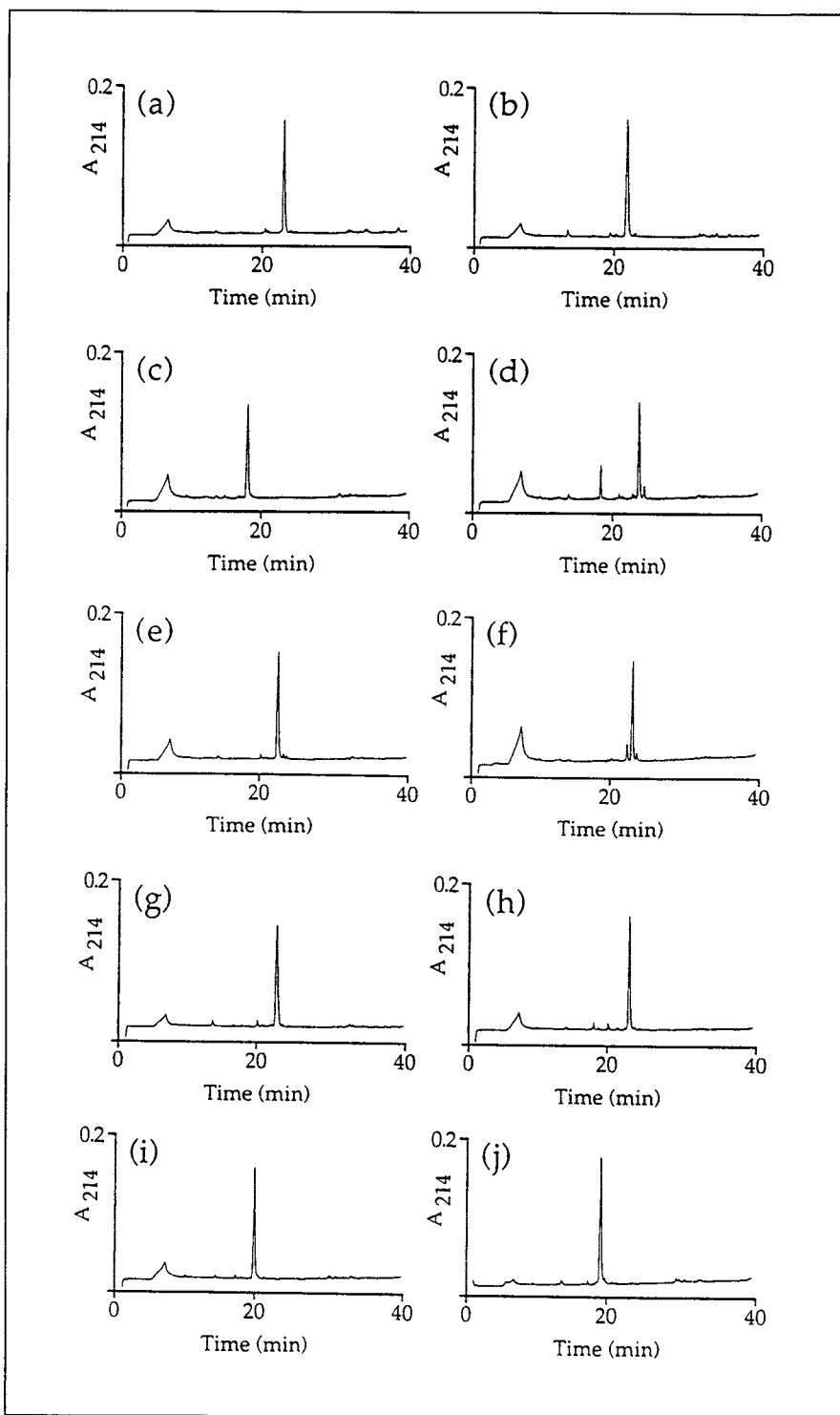


Figure 6. Reversed-phase C18 analytical HPLC analysis of a series of ten crude decapeptides, deprotected/cleaved automatically by the robotic apparatus. All peptides are acetylated and amidated: (a) AAFHTTGRII, (b) RAFHTTGRII, (c) RAAHTTGRII, (d) RAFATTGRII, (e) RAFHATGRII, (f) RAFHTAGRII, (g) RAFHTTARI, (h) RAFHTTGAI, (i) RAFHTTGRAI, (j) RAFHTTGRIA.

with the cleavage vessel to facilitate liquid draining and compensates for any differences in vertical positions. The flow of nitrogen gas through the multi-spigot is controlled by a 2-way solenoid valve and a needle valve (Furon, Anaheim, CA).

Evaporation/Extraction Station

The evaporation station (Figure 4) consists of an aluminum heating block (custom made) wrapped with nichrome heating wire (Ace Glass, Vineland, NJ). A Pyrex® gas-delivery tube (25 cm × 5 mm i.d.) is located directly above the heating unit and is mounted on a movable rack controlled by a pneumatic air cylinder with a 9-inch stroke (Parker Fluidpower, Des Plaines, IL). The rack can be lowered, to submerge the gas-delivery tube into a liquid sample in the evaporation/extraction tube, or raised, to allow removal of the sample tube from the station. The gas-delivery tube is connected to a nitrogen source via a male pipe adapter (1/8" o.d.) and Teflon tubing. Nitrogen gas is delivered through the glass tube by activation of a 2-way solenoid valve, and the flow rate is controlled by a needle valve. Aqueous acetic acid (10%) can also be delivered through the gas-delivery tube and is stored in a 1-liter pressurized bottle.

The extraction station consists of a solid polypropylene block that holds one evaporation/extraction tube (Figure 4). This tube is a 50-ml conical Pyrex centrifuge tube (Ace Glass) modified at the 20-ml level with a downward-sloping sidearm tube (8 mm o.d.). A funnel is located below the outlet of the sidearm and directs liquid to a waste receptacle. A Pyrex gas-dispersion tube (25 cm × 6 mm, Ace Glass) with a fritted end (20 μm) or a tapered end is located directly above the polypropylene block on the same movable rack as the gas-delivery tube. Ether is delivered through the fritted tube by a diaphragm pump (ProMinent Fluid Controls, Pittsburg, PA) operated under computer control.

Cleavage Protocol

The cleavage protocol begins with the delivery of a disposable vessel from the dispenser rack to the cleavage platform by the Zymark gripping hand (Figure 2). The peptide-resin samples

Table 1. Yields of Automatically Deprotected/Cleaved Peptides

Peptide	Weight (mg)	Yield (%)
AAFHTTGRII	30	49
RAFHTTGRII	50	82
RAAHTTGRII	49	80
RAFATTGRII	28	46
RAFHATGRII	48	79
RAFHTAGRII	30	49
RAFHTTARII	46	75
RAFHTTGAI	30	49
RAFHTTGRAI	30	49
RAFHTTGRII	47	77

(ca. 50–500 mg) are transferred from a peptide synthesis station (14), or a suitable storage rack, to the cleavage station with the 30-ml syringe hand. The resin is transferred as a slow-settling slurry in 60% 1,2-dichloroethane/dimethylformamide (14). The transfer process is repeated three times to ensure quantitative transfer of the resin. The transfer solvent is then directed to waste by the application of positive nitrogen pressure by the multi-spigot (Figure 3). The resin is then rinsed with CH₂Cl₂ (1 × 10 ml), dried with nitrogen (1 min), and the cleavage reagents are then delivered. The scavenger cocktail (1.0 ml, ethanedithiol:thioanisole:phenol, 1:2:2) is initially delivered, followed by the 94% TFA/H₂O solution (9 ml). The reaction is allowed to incubate for 2 h with periodic bubbling of argon through the bottom of the reaction vessel. The peptide solution is then filtered into an evaporation/extraction tube by the application of positive nitrogen pressure from the

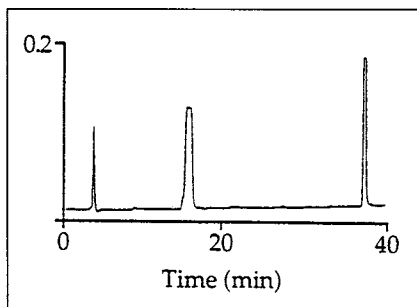


Figure 7. Reversed-phase C18 HPLC analysis of the scavenger cocktail under the same gradient conditions as the peptide chromatograms shown in Figure 6, indicating the effective removal of scavengers by the robotic cleavage/deprotection procedure.

multi-spigot. The resin is washed with TFA (1 × 2 ml), followed by glacial HOAc (1 × 0.5 ml). The spent cleavage vessel is then removed by the gripping hand and placed into a waste receptacle.

The peptide solution is then transferred from the cleavage station to the heating unit of the evaporation workstation by the gripping hand. The TFA solution is evaporated to a volume of approximately 1 ml with a stream of nitrogen and gentle heating (ca. 40°C) for 45 min. The concentrated solution is then diluted with 10 ml of 10% aqueous acetic acid and mixed thoroughly by nitrogen bubbling to form a uniform suspension. The extraction/evaporation tube is then transferred to the extraction station with the gripping hand.

Removal of the scavengers and protecting group by-products is performed by continuous aqueous-ether extraction (Figure 5). The fritted (or tapered) glass tube is positioned at the bottom of the peptide suspension, and diethyl ether or methyl *t*-butyl ether is delivered for 45 min at a flow rate of approximately 2 ml/min. During the continuous-extraction process, excess ether overflows through a sidearm tube and is directed to waste. The extraction tube is then removed by the gripping hand and placed into a storage rack, where the ether is allowed to evaporate. The peptide solution is then ready to be frozen and lyophilized.

The evaporation and extraction workstations are rinsed between samples to avoid cross-contamination. A clean rinse tube is placed into the evaporation station, and 10% HOAc/H₂O is delivered through the gas-delivery tube. The gas-delivery tube is rinsed thoroughly by bubbling this solution (2 × 2 min). This procedure is repeated with a fresh aliquot of solution. The rinse tube is then transferred to the extraction substation, where ether is delivered for 20 min to rinse the fritted ether-dispersion tube.

RESULTS

The cleavage/deprotection apparatus was tested by determining the yield and purity of a set of ten decapeptides. These peptides were synthesized by Fmoc chemistry (3,14) on a TFA-labile Rink amide resin (11). The average peptide yield after the cleavage/

Table 2. Characterization of Automatically Deprotected/Cleaved Peptides

Peptide	[M+H] ⁺ m/z		Amino Acid Analysis observed (theoretical)						
	calc'd.	obs.	Ala	Phe	Gly	His	Ile	Arg	Thr
AAFHTTGRII	1127.5	1127.6	2.01(2)	1.06(1)	1.11(1)	1.06(1)	1.54(2)	1.06(1)	2.00(2)
RAFHTTGRII	1212.7	1212.3	1.00(1)	1.02(1)	1.04(1)	1.04(1)	1.50(2)	1.92(2)	1.93(2)
RAAHTTGRII	1136.6	1136.5	1.92(2)	---	1.05(1)	1.00(1)	1.50(2)	1.82(2)	1.90(2)
RAFATTGRII	1146.7	1146.6	2.00(2)	0.87(1)	1.22(1)	---	1.83(2)	1.92(2)	2.20(2)
RAFHATGRII	1182.7	1182.6	2.00(2)	1.04(1)	1.03(1)	1.04(1)	1.54(2)	1.93(2)	0.98(1)
RAFHTAGRII	1182.7	1182.6	2.01(2)	1.01(1)	1.04(1)	1.02(1)	1.60(2)	1.91(2)	1.00(1)
RAFHTTARII	1226.7	1226.3	2.10(2)	1.08(1)	---	1.07(1)	1.55(2)	2.01(2)	2.00(2)
RAFHTTGAI	1127.5	1127.6	2.17(2)	1.07(1)	1.14(1)	1.14(1)	1.45(2)	0.98(1)	2.13(2)
RAFHTTGRAI	1170.7	1170.6	1.99(2)	1.01(1)	1.00(1)	1.00(1)	1.08(1)	1.92(2)	1.89(2)
RAFHTTGRIA	1170.7	1170.7	2.02(2)	1.01(1)	1.00(1)	1.00(1)	1.09(1)	1.90(2)	1.91(2)

deprotection of 100-mg peptide-resin samples was 65% of theoretical (Table 1). The purity of the peptides was >90% as determined by reversed-phase HPLC (Figure 6). The identity of each major peak (by HPLC) was confirmed by amino acid analysis and mass spectrometry (Table 2). These chromatograms indicate that the automated method quantitatively removes the scavengers; an analysis of the scavenger cocktail under identical HPLC conditions is shown for comparison (Figure 7).

DISCUSSION

A robotic workstation for the deprotection and cleavage of peptides with TFA-labile side chains and resin linkers has been designed and constructed around a Zymark robot. This robotic system has been previously adapted to multiple-peptide and equimolar-peptide mixture (EPM) synthesis (14). The workstation described here interfaces with the EPM synthesizer, allowing for postsynthesis peptide cleavage and deprotection without user intervention.

Special features of the apparatus have been designed which allow the deprotection/cleavage protocol to be efficiently automated:

1. The TFA deprotection/cleavage reactions are performed in fritted, disposable vessels. This allows the acid treatment and subsequent filtration step to be performed in the same vessel, and eliminates cross-contamination.

2. A receptacle for the disposable

vessels was designed to easily form a leak-proof seal with the vessel upon delivery by the robot arm. The ability of the robot to sense vertical pressure is used to establish a successful fit.

3. Reagent mixing in the cleavage vessel can then be achieved by energizing one port of a solenoid valve. This opens the cleavage vessel to nitrogen pressure and thoroughly agitates the resin slurry periodically during the 2-h incubation period. Evaporation of TFA during this period is kept to a minimum by mixing for only 5 out of every 30 seconds.

4. Liquid draining occurs by energizing the other port of the solenoid valve, which directs the fluid to either a waste or collection vessel. The multi-spigot ensures that reagents are drained quickly and reproducibly by forming a seal with the top of the cleavage vessel.

5. In addition to its pressure-sealing capability, the multi-spigot configuration allows for the rapid delivery of several reagents used at the cleavage workstation, thereby minimizing time and the number of robotic movements required. For example, the cleavage/deprotection reagents, which are stored as two separate solutions in order to increase the reagent's stability, are added in rapid succession with the multi-spigot.

6. The TFA-evaporation and ether-extraction steps are performed in the same tube, minimizing losses due to sample transferring. The evaporation time (45 min) is controlled such that the residue does not reach dryness, in order to facilitate peptide dissolution.

Trifluoroacetic acid vapors can be recovered with a condenser if desired. Aqueous acetic acid (10%) is used to dissolve the evaporation residue because of its excellent peptide solubility properties. The only disadvantage with this solvent is its tendency to oxidize methionine.

7. A continuous aqueous-ether extraction process is used which effectively removes all scavengers and reaction by-products from the acidic aqueous peptide solution. The gentle flow of ether in the continuous-extraction process greatly reduces the troubles of emulsion-layer formation and subsequent loss of product often associated with batch liquid-liquid extractions. A fritted tube finely disperses the ether to increase the surface area in contact with the aqueous peptide solutions. In cases where emulsion formation is persistent, the fritted tube can be replaced by a glass taper or Teflon tubing (0.031" i.d.) without significantly compromising extraction efficiency.

The overall processing time is minimized by performing an evaporation and extraction step simultaneously with a cleavage reaction. Since the rate-limiting step is the 2-h TFA cleavage/deprotection reaction, the preceding sample is taken through the evaporation step (45 min) and the extraction step (45 min) during this time. The remaining 30 min are used to rinse the evaporation/extraction station. In this manner, a sample is processed every 2 h. The flexibility of the software can accommodate longer cleavage times as needed.

The productivity of the apparatus could be substantially increased by expanding the workstation's capacity to allow the processing of several samples in parallel. The flexibility of this robotic system can also allow the inclusion of on-line HPLC analysis.

In conclusion, an apparatus has been constructed which automates the cleavage and deprotection of peptides synthesized by Fmoc chemistry. In addition to the automation of an otherwise laborious procedure, the described apparatus minimizes human contact with the noxious cleavage reagents. The workstation processes between 50–500 mg of resin sample every 2 h and provides the peptides in 50%–80% yield as an aqueous acidic solution. More consistent yields are likely to be obtained (especially with hydrophobic peptide sequences) when cleavage/deprotection strategies are used that do not involve extraction or precipitation, such as the incorporation of photolabile (7) or diketopiperazine-forming (2) resin linkers, or the use of affinity-based protecting groups (4,10). This instrument has greatly increased the rate of peptide cleavage/deprotection in the processing of hundreds of peptides in our laboratories to date.

ACKNOWLEDGMENTS

The authors would like to thank Janice Kerr for valuable assistance, Cathy Chu for peptide amino acid analysis and Michael Siani for assistance with software development.

REFERENCES

1. **Bidlingmeyer, B., S. Cohen and T. Tarvin.** 1984. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* 336:93-104.
2. **Bray, A., N. Maeji, R. Valerio, R. Campbell and H. Geysen.** 1991. Direct cleavage of peptides into aqueous buffer. Applications in simultaneous multiple peptide synthesis. *J. Org. Chem.* 56:6659-6666.
3. **Fields, G. and R. Noble.** 1990. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Peptide Protein Res.* 35:161-214.
4. **Funakoshi, S., H. Fukuda and N. Fujii.** 1991. Chemoselective one-step purification method for peptides synthesized by the solid-phase technique. *Proc. Natl. Acad. Sci. USA* 88: 6981-6985.
5. **Gausepohl, H., M. Kraft, C. Boulin and R. Frank.** 1990. Automated multiple peptide synthesis with BOP activation, p. 1003-1004. *In*

- J. Rivier and G. Marshall (Eds.), *Peptides: Chemistry, Structure and Biology* (Proceedings of the 11th American Peptide Symposium) 1990. ESCOM, Leiden.
6. **Geysen, H., R. Meloen and S. Barteling.** 1984. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. USA* 81: 3998-4002.
7. **Hammer, R., F. Albericio, L. Gera and G. Barany.** 1990. Practical approach to solid-phase synthesis of C-terminal peptide amides under mild conditions based on a photolysable anchoring linkage. *Int. J. Peptide Protein Res.* 36:31-45.
8. **Houghten, R.** 1985. General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* 82:5131-5135.
9. **King, D., C. Fields and G. Fields.** 1990. A cleavage method which minimizes side reactions following Fmoc solid-phase peptide synthesis. *Int. J. Peptide Protein Res.* 36:255-266.
10. **Ramage, R. and G. Raphy.** 1992. Design of an affinity-based N^α-amino protecting group for peptide synthesis: Tetrabenzo[a,c,g,i]fluorenyl-17-methyl urethanes (Tbfmoc). *Tetrahedron Lett.* 33:385-388.
11. **Rink, H.** 1987. Solid-phase synthesis of protected peptide fragments using a trialkoxydiphenyl-methyl ester resin. *Tetrahedron Lett.* 28:3787-3790.
12. **Schnorrenberg, G. and H. Gerhardt.** 1989. Fully automatic simultaneous multiple peptide synthesis in micromolar scale - rapid synthesis of series of peptides for screening in biological assays. *Tetrahedron* 45:7759-7764.
13. **Tam, J. and R. Merrifield.** 1987. Strong acid cleavage of synthetic peptides: mechanisms and methods, p. 185-248. *In The Peptides*, Vol. 9. Academic Press, New York.
14. **Zuckermann, R., J. Kerr, M. Siani and S. Banville.** 1992. Design, construction and application of a fully automated equimolar peptide mixture synthesizer. *Int. J. Pept. Protein Res.* (In press).
15. **Zuckermann, R., M. Siani and S. Banville.** 1992. Control of the Zymate robot with an external computer: construction of a multiple peptide synthesizer. *Laboratory Robotics Automation* (In press).

Address correspondence to:

Ronald N. Zuckermann
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608