



ABI 433A Peptide Synthesizer

User Guide

Volume 1 of 2

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1 About This Guide

This guide describes how to operate the ABI 433A Peptide Synthesizer, with conductivity monitoring, for the novice and experienced user.

In this introductory section, you can find explanations of the User Attention Words and Abbreviations found throughout the text of this guide. This section also contains summaries of each chapter and appendix of the User Guide to help you locate information.

This guide assumes that an Applied Biosystems technical representative has installed your ABI 433A Peptide Synthesizer.

This guide also assumes that you have a working knowledge of the Windows® 2000 operating system.

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Related Documentation

The following related documents are shipped with the system:

- *SynthAssist[®] Software Version 3.0 User Guide* (or higher versions) can be printed from the SynthAssist[®] 3.0 (or higher version) pdf file found on the SynthAssist Software CD. The software user guide describes the PC-compatible peptide synthesis software system designed for use with the ABI 433A Peptide Synthesizer.
- *ABI 433A Peptide Synthesizer Site Preparation Guide* (P/N 902475) describes the site preparation and requirements to install an ABI 433A Peptide Synthesizer system.

Common Abbreviations and Units

#	=	number
°C	=	degrees Celsius
°F	=	degrees Fahrenheit
µL	=	microliter
µm	=	micron
AB	=	Applied Biosystems
AUFS	=	absorbance units full-scale
ft.	=	foot
i.d.	=	inside diameter
in.	=	inch
L	=	liter
m	=	meter
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
o.d.	=	outside diameter
psi	=	pounds per square inch
sec	=	second
V	=	volt

Chemical Abbreviations

Abbreviation	Definition
Ac	acetyl
Ac ₂ O	acetic anhydride
AcI	acetylimidazole
Acm	acetamidomethyl
ACT	activator vessel
BHA resin	benzhydrylamine resin
<i>t</i> -Boc	<i>tert</i> -butoxycarbonyl
Bzl	benzyl
Br-Z	2-bromobenzyloxycarbonyl
<i>t</i> -Bu	<i>tert</i> -butyl
CHO	formyl
CH ₃ Bzl	4-methylbenzyl
CH ₃ OBzl	4-methoxybenzyl
Cl-Z	2-chlorobenzyloxycarbonyl
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Dnp	2,4-dinitrophenyl
Et	ethyl
EtOH	ethanol
Fmoc	9-fluorenylmethyloxycarbonyl
HATU	N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-yl-methylene]N-methylmethan-aminium hexafluorophosphate N-oxide
HBTU	2-(1 H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
HMP resin	p-hydroxymethylphenoxymethylpolystyrene resin
HOAc	acetic acid
HOBt	1-hydroxybenzotriazole

MBHA resin	4-methylbenzhydramine resin
MeOH	methanol
Mob	4-methoxybenzyl
Mtr	4-methoxy-2,3,6-trimethylbenzene sulfonyl
Mts	Mesitylene-2-sulfonyl
NMP	N-Methylpyrrolidone, N-methyl-2-pyrrolidone
OBt	1-benzotriazolyl ester
OBzl	benzyl ester
OEt	ethyl ester
OMe	methyl ester
PAM resin	phenylacetamidomethyl resin
Pbf	2,2,4,6,7-Pentamethyldihydro- benzofuran-5-sulfonyl
Pmc	2,2,5,7,8-Pentamethylchroman-6-sulfonyl
RV	reaction vessel
SPPS	solid-phase peptide synthesis
TFA	trifluoroacetic acid
TFMSA	trifluoromethane sulfonic acid
Tos	4-toluenesulfonyl (tosyl)
Trt	trityl
Z	benzyloxycarbonyl

Contents of Volume 1 User Guide

Laminated reference sheet - A quick reference guide to the ABI 433A Peptide Synthesizer. The quick reference includes a plumbing schematic, a list of software functions, a Synthesis Preparation Checklist, and a brief list of the flow tests most often used.

Quick Start Card - Designed primarily for new ABI 433A Peptide Synthesizer users, this colorful aid uses a flowchart format to show the necessary steps to get the ABI 433A instrument up and running the first synthesis.

Chapter 1 Introduction - Briefly describes manual conventions, abbreviations, and each chapter of the User Guide. Describes safety information, safety symbols and labels, and electromagnetic compatibility standards.

Chapter 2 ABI 433A Peptide Synthesizer Operation - Gives step-by-step procedure for preparing the instrument for a routine synthesis. Briefly describes how to use the Cycle Monitor menu to monitor and control synthesis operations. Describes how to change reagent bottles on the ABI 433A instrument when converting from one chemistry option to another.

Chapter 3 Chemistry - Gives chemistry background on the stages of automated solid-phase peptide synthesis and each of the chemistry options available on the ABI 433A instrument, with references.

Chapter 4 Chemistry Options - Describes the protocols, reagents, and modules that characterize the three chemistry options available for peptide synthesis on the ABI 433A instrument: *FastMoc*[™], Fmoc/HOBt/DCC, and Boc/HOBt/DCC chemistry.

Chapter 5 Monitoring - Explains how to use pre-defined *FastMoc* Chemistry files in SynthAssist Software for conductivity monitoring of deprotection with feedback and the conditional modules for extended deprotection and coupling with capping.

Chapter 6 Maintenance and Troubleshooting - Describes procedures and flow tests used to maintain optimum ABI 433A instrument performance.

Chapter 7 Advanced Operations - Describes modifications that may be made to SynthAssist Chemistry and Run files to customize ABI 433A instrument operation.

Chapter 8 System Description - Describes user-accessible hardware components of the ABI 433A instrument and explains embedded software functions.

Chapter 9 Software - Describes the ABI 433A Peptide Synthesizer LCD (liquid crystal display) and the keys available for interacting with the ABI 433A instrument software. Explains each of the software menus that can be viewed on the LCD.

Index - Does not include Appendices.

Contents of Volume 2 User Guide

Appendix A - The Applied Biosystems Limited Warranty for the ABI 433A Peptide Synthesizer.

Appendix B - The quantitative ninhydrin procedure for measuring coupling efficiency, along with resin drying techniques and instructions on the correct use of a repeater pipet. Also included are procedures for performing the post-synthesis calculations that SynthAssist[®] 3.0 software can perform for you automatically.

Appendix C - Chemicals and Reagents used on the ABI 433A Peptide Synthesizer, with their Applied Biosystems part numbers.

Appendix D - Chemical structures of the amino acid derivatives that may be used in peptide synthesis, and a table of molecular weights of amino acids and protected amino acids.

Appendix E - Annotated lists of all the steps in each of the pre-defined modules available in SynthAssist with a list of all the function names as they appear on the ABI 433A Peptide Synthesizer LCD and in SynthAssist software.

Appendix F - Illustrated list of ABI 433A Peptide Synthesizer parts and part numbers.

How to Obtain Support

For the latest services and support information for all locations, go to **<http://www.appliedbiosystems.com>**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to: **techpubs@appliedbiosystems.com**

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT	<i>Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.</i>

Caution	<i>Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.</i>

WARNING	<i>Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.</i>

DANGER!	<i>Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.</i>

Examples

The following examples show the use of safety alert words:

IMPORTANT	<i>You must create a separate a Sample Entry Spreadsheet for each 96-well plate.</i>

Caution	<i>The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.</i>

WARNING	<i>CHEMICAL HAZARD. Formamide. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

DANGER!	<i>ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.</i>

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the On position of the main power switch.
	Indicates the Off position of the main power switch.
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see *Safety Labels on Instruments* on page 1-12. These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.

<i>Symbol</i>	<i>Description</i>
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

<i>English</i>	<i>Francais</i>
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	ATTENTION Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	ATTENTION Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	ATTENTION Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la réglementation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	AVERTISSEMENT Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	AVERTISSEMENT Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	ATTENTION Surface brûlante.
DANGER High voltage.	DANGER Haute tension.

<i>English</i>	<i>Francais</i>
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	AVERTISSEMENT Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
DANGER Class 3B laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	DANGER Class 3B rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.
DANGER Class 3B laser radiation when open. Avoid direct exposure to laser beam.	DANGER Class 3B rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 laser radiation present when open and interlock defeated. Do not stare directly into the beam	DANGER de Class 2 rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 laser radiation present when open. Do not stare directly into the beam.	DANGER de Class 2 rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 LED when open and interlock defeated. Do not stare directly into the beam.	DANGER de Class 2 LED en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 LED when open. Do not stare directly into the beam.	DANGER de Class 2 LED en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	ATTENTION Parties mobiles.

General Instrument Safety

WARNING ***PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.***

Moving and Lifting the Instrument

Caution ***PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.***

Moving and Lifting Stand-Alone Computers and Monitors

WARNING ***Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.***

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See “About MSDSs” on page 1-17.

WARNING ***PHYSICAL INJURY HAZARD. Use this instrument as specified by Applied Biosystems. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.***

Chemical Safety

Chemical Hazard Warning

WARNING	CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING	CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING	CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING	CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **<https://docs.appliedbiosystems.com/msdssearch.html>**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
 - **Open** – To view the document
 - **Print Target** – To print the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See <z-x-ref>“About MSDSs” on <z-x-ref>page 17.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

Caution	<i>HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.</i>

WARNING	<i>CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.</i>

WARNING	<i>CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.</i>

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.

-
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT *Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.*

Electrical Safety



DANGER ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the ABI 433A Peptide Synthesizer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power



DANGER ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.



DANGER ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.



DANGER ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The ABI 433A Peptide Synthesizer system has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Compressed Gases

WARNING **PHYSICAL HAZARD.** Nonflammable compressed gas (Nitrogen). Contents are under pressure. Receive proper training on the handling of compressed gases before use. Exposure to rapidly expanding gas may cause frostbite. High concentrations of vapors in the immediate area can displace oxygen and cause asphyxiation. Use only in areas with adequate ventilation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **EXPLOSION HAZARD.** Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Moving Parts

WARNING **PHYSICAL INJURY HAZARD.** Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Solvents and Pressurized Fluids

WARNING ***PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.***

WARNING ***PHYSICAL INJURY HAZARD. To avoid hazards associated with high-pressure fluids in polymeric tubing see the bulleted list below.***

- Be aware that PEEK[™] tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.
- Always wear eye protection when in proximity to pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use PEEK tubing that has been severely stressed or kinked.
- Do not use PEEK tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause PEEK tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

Caution	<i>MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.</i> <i>These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.</i>

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- *U.S. and Canadian Safety Standards*
- *Canadian EMC Standard*
- *European Safety and EMC Standards*
- *Australian EMC Standards*



U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 3101-1, “Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements.”

This instrument has been tested to and complies with standard CSA 1010.1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.



European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standard EN 61010-1:2001, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements”.

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”



Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”



2 ABI 433A Peptide Synthesizer Operation

This chapter briefly describes the:

- ABI 433A Peptide Synthesizer with conductivity monitoring
- Available chemistry options
- Operating software
- Procedures for preparing the instrument for synthesis
- Running a synthesis
- Procedures for converting from one chemistry option to another

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Introduction to the ABI 433A Peptide Synthesizer

Instrument Description

The ABI 433A Peptide Synthesizer is a fully automated, programmable instrument that performs the chain assembly steps in solid-phase peptide synthesis. You can program the real-time, feedback monitoring feature of the ABI 433A instrument for use in *FastMoc*[™] chemistry. Feedback monitoring control is based on the measurement of either the conductance or the ultraviolet (UV) absorbance of the reagent, solutions, and solvents used in a synthesis cycle. A built-in conductivity cell measures conductivity; the UV deprotection monitoring is available with the U.V. monitoring Accessory Kit (P/N 4335867). Results of the monitoring are returned in real-time to specific on-going steps, as well as to future steps, in a synthesis cycle.

The user sets the parameters for feedback monitoring by defining and applying monitoring functions at steps in the synthesis process.

Figure 2-1 and Figure 2-2 show front and rear views of the ABI 433A instrument and the locations of its controls.

Front and Rear Views of the ABI 433A Instrument

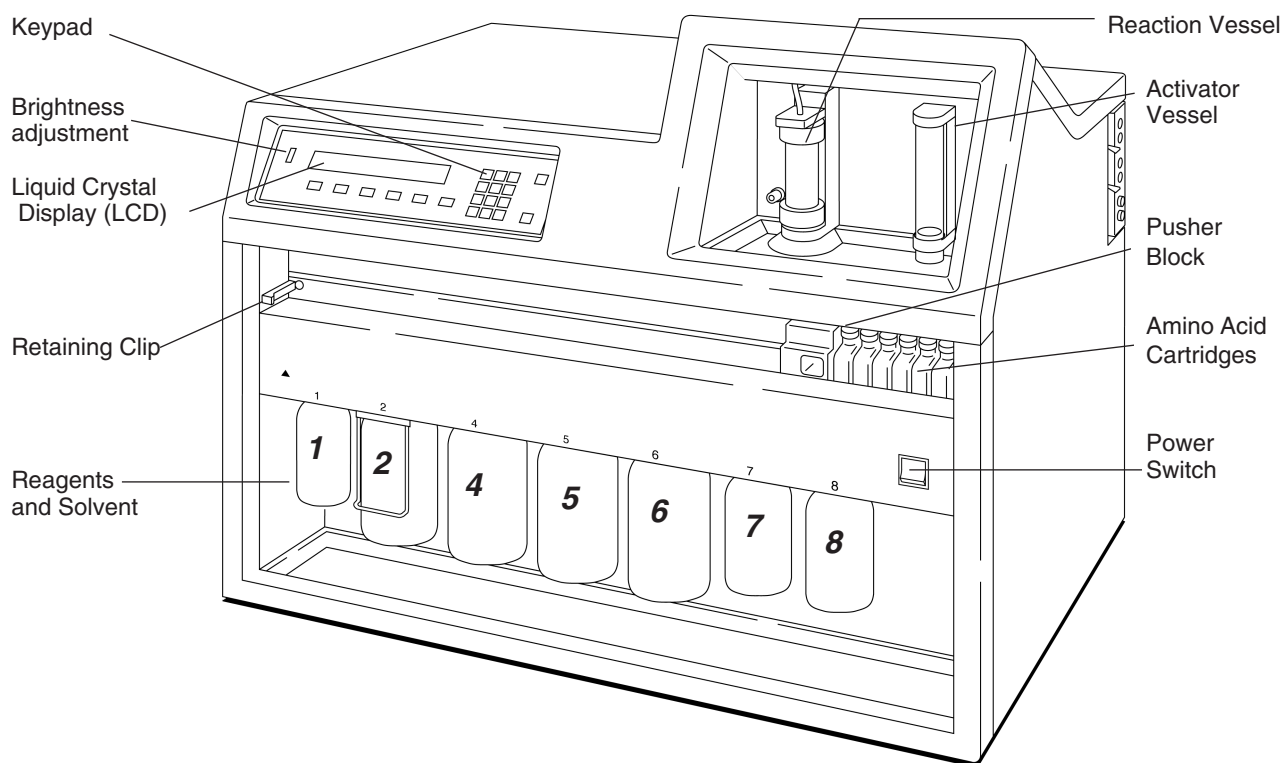


Figure 2-1. ABI 433A Peptide Synthesizer—front

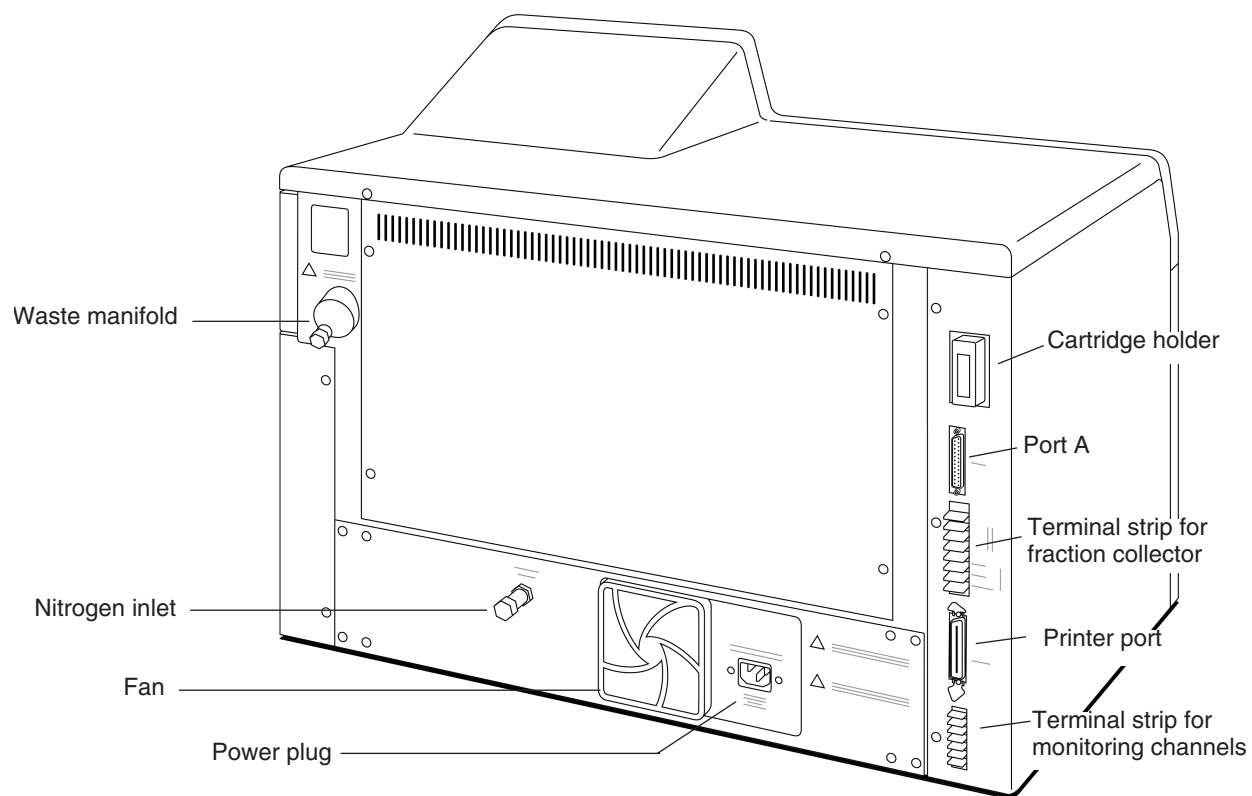


Figure 2-2. ABI 433A Peptide Synthesizer—rear

Chemistry Description

ABI 433A Peptide Synthesizer chemistry options supported by Applied Biosystems include:

- *FastMoc* 1.0 mmol
- *FastMoc* 0.25 mmol
- *FastMoc* 0.10 mmol
- Fmoc/HOBt/DCC 0.25 mmol
- Fmoc/HOBt/DCC 0.10 mmol
- Boc/HOBt/DCC 0.50 mmol
- Boc/HOBt/DCC 0.10 mmol

Table 2-1 outlines the differences among the seven chemistry options with respect to the amount of resin and amino acid used, waste generated, and time per cycle. To change from one option to another, refer to *Conversion of FastMoc, Fmoc/HOBt/DCC, and Boc/HOBt/DCC Chemistries* on page 2-48.

Table 2-1. Comparison of Chemistry Options

ABI 433A Instrument Cycles	Chemistry	Resin (mmol)	Amino Acid (mmol)	Cycle Time (min)	Waste ^a per Cycle (mL)	Ratio AA: Resin
<i>FastMoc</i> 1.0 mmol	Fmoc/HBTU	1.00	3.00	70 ^b	270 ^b	3:1
<i>FastMoc</i> 0.25 mmol	Fmoc/HBTU	0.25	1.00	45 ^b	100 ^b	4:1
<i>FastMoc</i> 0.10 mmol	Fmoc/HBTU	0.10	1.00	24 ^b	50 ^b	10:1
Fmoc/HOBt/DCC 0.25 mmol	Fmoc/HOBt/DCC	0.25	1.00	108	273	4:1
Fmoc/HOBt/DCC 0.10 mmol	Fmoc/HOBt/DCC	0.10	1.00	60	95	10:1
Boc/HOBt/DCC 0.50 mmol	Boc/HOBt/DCC	0.50	2.00	104	370	4:1
Boc/HOBt/DCC 0.10 mmol	Boc/HOBt/DCC	0.10	2.00	65	120	20:1

^a Values in "Waste per Cycle" column are approximate

^b These values increase when the monitoring feature is used.

Software Introduction

The LCD

The LCD (liquid crystal display), shown in Figure 2-3, shows available operations. The “soft keys” under the LCD correspond to selections. To operate the instrument, press the key directly under your choice.

The Keyboard

Use the alphanumeric keyboard to the right of the screen to make numeric and letter entries. Repeatedly press a key on the keyboard to select that key continuously.

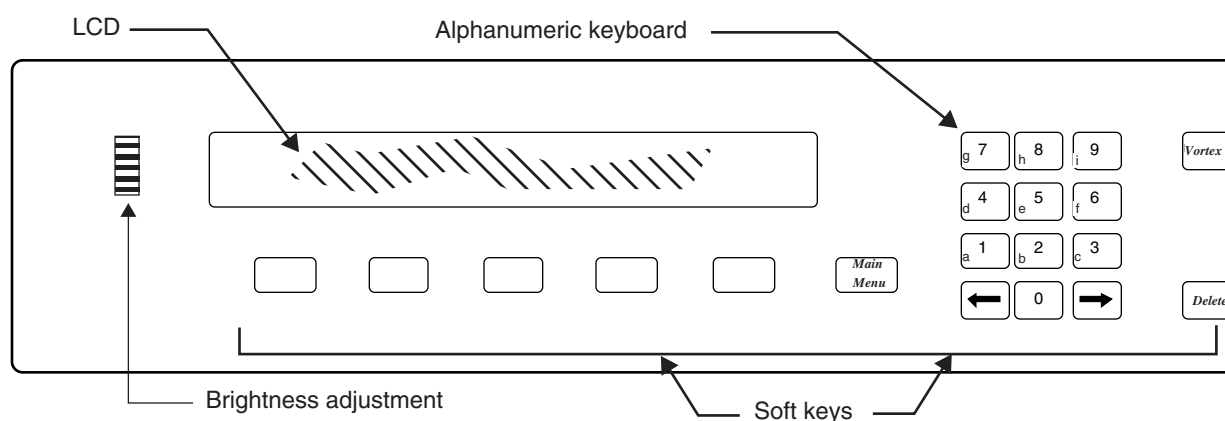


Figure 2-3. LCD and keyboard

Use the number keys (0 – 9) and the letter keys (a – i) to respond to prompts on the screen. The position of the cursor, which appears as a bar (|), indicates where the selected numbers and letters appear on the LCD.

- Use the number keys to indicate quantity.
- Use the letter keys to indicate modules.

→ ← Use the arrow keys to move the cursor across the screen in the direction of the arrows.

Delete Press the **delete** key to erase an entry at the cursor position. When the cursor is not under an entry, the deletion is made to the left of the cursor.

Main Menu Use this key at any time to return to the Main Menu choices.

Vortex This key is like a toggle switch that controls RV mixing. When the RV is mixing, press this key to mix the contents of the RV. Mixing will stop when this key is released. When the RV is mixing, press this key to suspend RV mixing. Mixing continues when this key is released.

Software Menus

When the ABI 433A Peptide Synthesizer is first turned on, the screen shows the display below.

ABI 433A Peptide Synthesizer				
Chemistry: NOT DECLARED!!!			Main Menu->	

Main Menu

Main Menu displays the available options. There are three Main Menu 'pages' (see Figure 2-4). Press **more** from any Main Menu page to view the next page. Any selection you choose from the Main Menu brings up a new 'menu' on the screen. For example, move to the 433A Editors Menu by pressing **433A editors**. From the 433A Editors Menu, press the **main menu** key to return to the Main Menu.

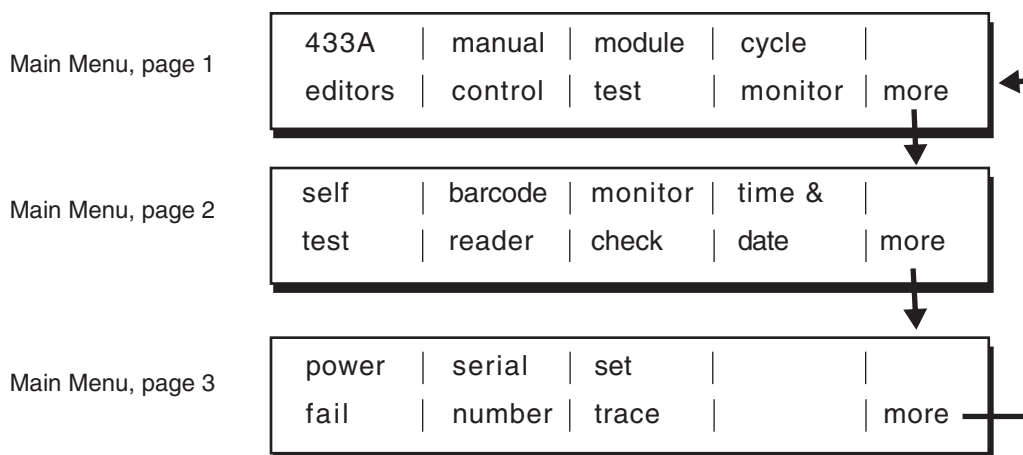


Figure 2-4. Three pages of the main menu

Description of Main Menu Options

433A Editors Choose this menu to edit the Run Editor, the Module Editor, or user-defined functions.

Manual Control Use this menu to manually control individual valves or functions and facilitate testing or manipulation of fluid flows through the instrument. Manually control the vortexer and the autosampler to test proper operation. If a synthesis is underway, you must first press the pause soft key before using the Manual Control Menu.

Module Test Use this menu to select and run any module, especially when running Flow Tests or modules that check instrument performance.

Cycle Monitor Start synthesis from this menu. After synthesis is started, you can view the current step of the synthesis including the function number, countdown, and the add time. From this menu, you can terminate a synthesis, make the synthesizer pause at the current step, use the **set interrupt** key to make the synthesizer pause at some future step, jump to a different step in the cycle, or hold (prolong) a step.

Self Test Use Self Test to verify proper operation of the instrument's electrical and mechanical components.

Barcode Reader Use this menu to calibrate the barcode reader and check the barcode labels on the amino acid cartridges against the peptide sequence entered in SynthAssist® Software and sent to the ABI 433A instrument.

Monitor Check Use this menu to check ground, voltage reference, conductivity voltages, channel 2 and channel 3.

Time & Date Set the hour, minute, month, day, and year in this menu.

Powerfail Use this menu to designate the amount of time (1 to 99 minutes) that an interruption in power must last to pause a synthesis. If the duration of a power failure is greater than the time entered in the powerfail menu, synthesis is interrupted at the start of an activation.

Serial number Open this menu to see the instrument's serial number. This number was determined during manufacturing.

Set Trace Use this menu to select the level of data detail to be recorded in the SynthAssist log.

Synthesis Preparation Checklist

Perform the basic steps in Table 2-2 to run a synthesis on the ABI 433A Peptide Synthesizer.

Table 2-2. Tasks Checklist to Run a Synthesis

✓	Instrument Check
	Power on the computer and 433A Peptide Synthesizer
	Calibrate barcode reader, if necessary
	Check waste level
	Check for condensation in the vent line and clear the line
	Change disposable in-line filters, if necessary
	Check volumes of reagents and solvent bottles
	For FastMoc chemistry, check age of HBTU solution
✓	Synthesis Set-Up
	Establish communications between SynthAssist® Software and instrument
	Load Flow Tests 1-18 from SynthAssist Software
	Run Flow Tests A, B, a, and D • Calibrate gas regulators (Module Test Menu) • Check the user-accessible in-line filters for leaks • Check HOBt and DCC lines for blockage, if necessary
	Run Flow Test B again
	Prime lines if you did a bottle change and run appropriate flow test
	Check conductivity background level
	Send flow Tests 19 to 23 and run Flow Test d twice
	Set trace option
✓	Create Run File
	From SynthAssist Software: 1. Create new sequence 2. Select chemistry and save 3. Send Chemistry file to 433A instrument
	From SynthAssist Software: 1. Create a run file 2. Select resin type, enter substitution information, verify cycles and cartridges 3. Name and save the file
	Send Run file to 433A instrument
	Load AA cartridges
	Place empty cartridge under needle
	Open monitoring window, verify window has no data
	Add resin to reaction vessel (RV) - place the RV on vortexer
	From 433A Cycle Monitor Menu respond to: • Resin sampling • Add times • Print events Press Begin to start synthesis

Maintenance Tracking Sheet

Upper Regulator-Flow test 2

Date	Bot Lev	Press	Vol	Vol	Date	Bot Lev	Press	Vol	Vol

Lower Regulator-Flow test 10

Date	Bot Lev	Press	Vol	Vol	Date	Bot Lev	Press	Vol	Vol

Other Flow Tests

Flow test/Date:									
1 RV									
4 RV									
5 RV									
6 RV									
9 RV									
11 Cartridge									
12 Cartridge									
13 Cartridge									
22 Conduc- tivity									

Peptide Synthesis

Date:									
Peptide:									
# of Cycles:									

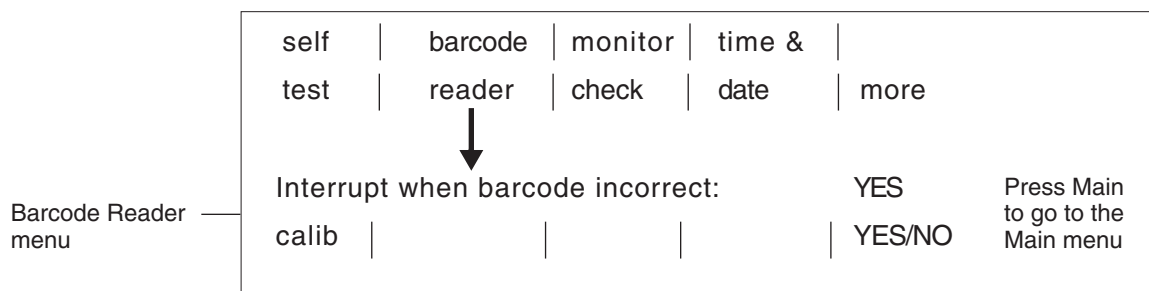
Date:									
Peptide:									
# of Cycles:									

Changed In-Line Filters C=Cartridge T=Top RV B=Bottom RV R=Resin Sampler

Date	Date

Instrument Check

Using the Barcode Reader Menu



Interrupt when barcode incorrect

In the Barcode Reader menu, the **YES/NO** soft key is a toggle switch that changes the response to the barcode interruption option. If you answer **YES**, the ABI 433A instrument controller compares the barcode readings of the labels on each amino acid cartridge during a synthesis to the amino acid sequence you defined in SynthAssist for that run. If the barcode reading for any label does not match the expected sequence, the ABI 433A instrument interrupts synthesis. If you answer **Yes** to both this option and “Print run events?”, the printout displays both the expected amino acid and the barcode readings for each cycle. An asterisk (*) appears on the printout next to the barcode readings that do not match the expected amino acids for the pre-defined sequence.

If you answer **NO** to the barcode interruption option, the ABI 433A instrument controller does not compare the barcode readings for each amino acid cartridge to the pre-defined amino acid sequence. The printout of the synthesis displays only the barcode readings for each cycle. However, the “AA Cart. List” at the top of the Synthesis Report shows the pre-defined peptide sequence in reverse order, with the N-terminal amino acid last on the list.

Barcode calibration

The barcode calibration standardizes the channel readings for accurate translation of the black and white bands on the amino acid cartridge labels. Once you calibrate the barcode reader, you do not have to repeat the calibration except after an instrument re-set, or a memory cartridge replacement. See "Barcode Calibration" on page 6-18 for the calibration procedure.

Checking the Gas Supply

To check the gas supply:

1. Check the pressure supply of the nitrogen tank.

Check the nitrogen tank regulator reading before starting each run. When the tank pressure drops below 400 psi, the tank will soon be empty and you should watch it closely. Check the tank pressure daily to ensure timely replacement of empty tanks and to spot any increase in gas consumption caused by a leak in the nitrogen system.

Inadequate nitrogen delivery affects reagent delivery and the operation of the ejector needle. If a major leak develops, do not operate the synthesizer until the leak has been corrected. If you cannot locate and correct the source of the leak, call the Applied Biosystems Service Department.

2. Check that the regulated pressure reads 65 psi.
3. Change the tank as soon as the tank pressure drops below 300 psi.

WARNING ***GAS TANK EXPLOSION HAZARD. Pressurized gas cylinders are explosive. Attach pressurized gas cylinders firmly to a wall or bench by means of approved brackets, chains, or clamps. Always cap the gas cylinder when not in use.***

WARNING ***DAMAGE TO SYNTHESIZER AND LABORATORY. Do not operate the instrument without gas pressure. Damage can occur to the valves and regulators which could result in damage to the instrument and the laboratory.***

To replace a gas cylinder during a synthesis, pause the synthesizer before changing the nitrogen cylinder.

If your synthesizer has a common source of nitrogen shared with other instruments, ensure that other users know of the damage that can occur to your instrument if they turn off the gas pressure while your instrument is operating. Applied Biosystems recommends that you place a warning sign near your common nitrogen source to notify people to check if your instrument is in operation before they turn off the nitrogen.

To change nitrogen gas tank:

1. Close both the main supply valve on top of the tank and the needle valve on its regulator.
2. Remove the regulator from the empty tank and install it on a full replacement tank.

-
3. Open the main tank supply valve and check for leaks at the regulator connection.
 4. Open the needle valve on the nitrogen regulator at the tank.
 5. Verify that the tank regulator setting returns to its original position.

Testing for Leaks

After changing the nitrogen tank, test the nitrogen supply system for leaks. Immediately repair any leaks disclosed to prevent excessive nitrogen use.

The following procedure tests the gas supply fittings, the vacuum assist input, both regulators, and one port of each brass cylinder of the autosampler assembly. Note that, during these tests, the instrument regulators all read 0 (zero) psi.

Input and Unregulated Internal Pressure Leak Test

To do an input and unregulated internal pressure leak test:

1. Set the manual valves ("Vent Switches") for bottles 9 and 10 to the "vent" position (Switch DOWN). These valves are located to the rear of the right-side panel of the instrument (as viewed from the front).
2. Turn off instrument power.
3. Verify that the regulator is set to 65 psi.
4. Close the compressed gas cylinder valve.
5. Turn the knob of the 65-psi regulator several turns counterclockwise and monitor pressure on the low side of the cylinder regulator. Note that there may be an immediate drop of 2-5 psi; the residual pressure is then 60-65 psi, the baseline pressure for the next step.
6. Observe for at least 5 minutes. The pressure should not drop more than 10 psi total or 2 psi per minute from the baseline pressure.

If the pressure drop exceeds this rate, there is a leak in the system. Do not simply tighten fittings further; instead, recheck all fittings and connections, reset regulator, and repeat this test to ensure that there are no leaks before proceeding.

Caution	<i>Overtightening of fittings can damage the fittings and require replacement.</i>

Checking Waste Level

Check the waste container before starting each synthesis. You can order additional 2.5-gallon polyethylene waste bottles from Applied Biosystems (P/N 140040). Label these containers “Halogenated Waste.” The ABI 433A instrument generates hazardous, halogenated, organic liquid waste. Handle, store, and dispose of this waste in accordance with federal, state, and local hazardous waste regulations.

WARNING	<i>CHEMICAL HAZARDOUS WASTE. Waste produced by the ABI 433A Peptide Synthesizer can be hazardous and can cause injury, illness or death. Handle all liquid, solid, and gaseous waste as potentially hazardous. During transfer, the waste container must be tightly sealed with the waste cap provided. Read all applicable Material Safety Data Sheets. Always handle hazardous materials beneath a fume hood that is connected in accordance with all installation requirements. Dispose of waste in accordance with all local, state, and federal regulations.</i>

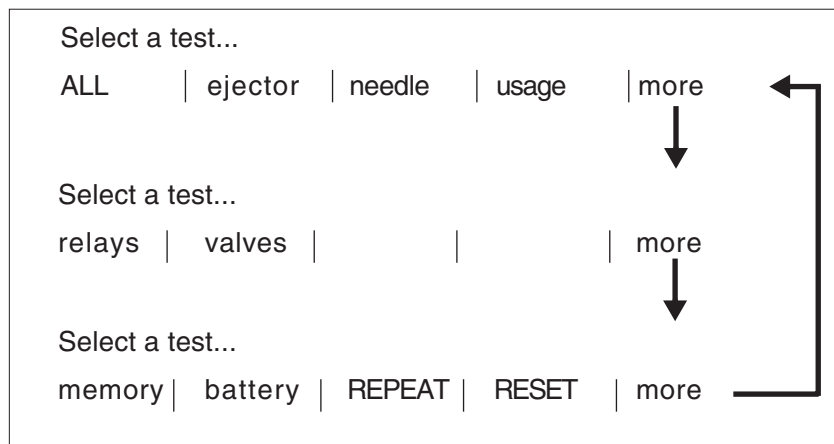
Note	<i>When using TFA (Boc chemistry), always add approximately 150 mL of ethanolamine or 225 mL Applied Biosystems Waste Neutralizer (P/N 400230) to the empty waste container to neutralize the TFA.</i>

If you need to empty the waste container while a synthesis is in progress, the safest method is to set an interrupt in the software. The best place to set an interrupt is prior to module B in step 1 of the subsequent cycle. For more information on setting an interrupt, see page 2-44.

Another way to interrupt a synthesis is to wait until one of the coupling steps begins, then press the **pause** soft key. If you use this method, do not interrupt the synthesis during deprotection, activation, or washing.

Running a Self Test (Optional)

The Self Test menu key appears on page two of the Main Menu (see Figure 2-4). Self Test options include: ALL, ejector, needle, relays, valves, memory, and battery. When you press **ALL**, the instrument automatically performs the tests for ejector, needle, relays, valves, memory, and battery. You can select and separately run the Self Tests.



Changing Disposable In-Line Filters

The ABI 433A instrument contains four user-accessible in-line filters to protect valves, valve blocks, and lines from becoming obstructed with particles. These in-line filters can be found on the right side of the instrument, behind the removable panel, in the following locations:

- One in the amino acid delivery needle line
- Two in the RV line (one top and one bottom)
- One in the line between the 11-port valve block and the resin-sampling valve

Replace the in-line filter in the amino acid delivery needle line at least every 25 cycles. Replace this filter when the amount of solvent delivered during Flow Tests 11 and 12 is less than normal. (See Figure 2-5 on page 2-20 for the SynthAssist modules that correspond to these Flow Tests.)

Note *The amount of solvent delivered during Flow Tests 11 and 12 also decreases if the needle is plugged with septum particles.*

Replace the in-line filters on the bottom of the RV every 50 cycles; replace the in-line filters on the top of the RV every 75 cycles. If the RV drains slowly during a synthesis, the bottom filter needs replacement. Also, when the

amount of fluid in Flow Tests 1, 2, 4, 5, 6, 9 and 10 is low, the bottom in-line filter may be plugged. (See Figure 2-5 on page 2-20 for the SynthAssist modules that correspond to these Flow Tests.)

<i>Note</i>	<i>When changing the top and bottom RV in-line filters, check the flared tubing (P/N 600452) that runs from the valve block to the RV in-line filters or to the conductivity flow cell. The flare next to the in-line filter can become worn and cause leaks. Always check the regulators after changing the reaction vessel in-line filters.</i>

Replace the in-line filter to the resin sampler only when the resin sample is not collected when it should be, or if the resin sample weight is much smaller than normal.

<i>Note</i>	<i>In-line filters are disposable and designed for a single use. Do not attempt to clean, rebuild, or reuse in-line filters.</i>

To order a package of 20 in-line filters, specify part number 401770.

Checking Reagents and Solvent Bottles

WARNING ***PHYSICAL AND CHEMICAL HAZARD. Chemicals reduce the integrity of glass bottles. Re-used bottles are more susceptible to fractures and shattering under pressure. Replace re-used reagent bottles every six weeks.***

Check for sufficient quantities of reagents and solvents before initiating each synthesis. Reagent and solvent usages are given in Table 2-4 (*FastMoc*), Table 2-5 (Fmoc/HOBt/DCC), and Table 2-6 (Boc/HOBt/DCC). The first value is the typical usage if the Flow Tests give average reagent flows. The values in parentheses represent the usage if the flow tests give reagent flows that are in the upper end of the acceptable range. The numbers with asterisks are the reagents that have Add Times associated with them. For a more detailed description of the effect of Add Times, see page 7-54.

Assembling Parallel Bottles Configuration

Note that in the tables for reagent and solvent usage, the information assumes the use of the parallel bottle assembly for the solvent NMP in the *FastMoc* and Fmoc/HOBt/DCC chemistry options and for DCM in the Boc/HOBt/DCC chemistry option. With the parallel bottle assembly, two 4000 mL bottles are connected so that solvent is delivered from both bottles at the same time. This configuration increases the number of cycles that may be run unattended before reagent must be replaced.

IMPORTANT ***When using solvent bottles that are connected with the parallel bottle assembly, always start synthesis with the same amount of fluid in both bottles.***

Caution ***NMP delivery is accomplished by way of a parallel 2-bottle configuration. If one NMP bottle empties, only nitrogen gas from the empty bottle will be delivered instead of NMP.***

Replacing Disposable Reagent Bottle Seals

Reagent bottle seals are disposable and designed only for one-time use. When changing reagent bottles, always replace the polyethylene seals with new seals.

Part numbers for the bottle seals are listed in Table 2-3 on page 2-17.

IMPORTANT ***Never re-use the bottle seal when a new bottle of reagent is added. New bottle seals are required to ensure optimum seal formation.***

Avoiding Synthesis Interruptions

To avoid unnecessary interruptions during synthesis, change bottles before beginning synthesis. After changing a reagent bottle, run the flow test for that bottle to check for leaks and flush the lines.

If a bottle must be changed during a synthesis, first put the instrument into pause mode during a coupling module, but not during the activation, deprotection or washing. It is not possible to run flow tests in the middle of a synthesis.

Table 2-3. Bottle Seal Replacement Part Numbers

Bottle Positions	Bottle Size	Part Number
1, 4, 5, 6	450 mL	400501
2	450 mL	400789
7, 8	200 mL	400790

WARNING ***CHEMICAL HAZARDS.** Chemicals used on the Applied Biosystems ABI 433A Peptide Synthesizer are hazardous and can cause injury. Please familiarize yourself with the information provided in the MSDSs. Always wear chemical-resistant gloves, lab coat, safety glasses, and use proper ventilation when handling chemicals.*

Caution *When using FastMoc chemistries with preloaded or amide resins, remove the DCC reagent from the synthesizer and flush the delivery lines well with DCM to prevent urea crystals from forming in the tubing. The reason for this precaution is that, unlike HMP resin, preloaded and amide resins do not require DMAP-catalyzed DCC attachment of the first residue.*

Table 2-4. FastMoc Chemical Usage: Cycles per Bottle

Bottle Position	Chemical	1.0 mmol Scale	0.25 mmol Scale	0.10 mmol Scale
1	Piperidine*	33 ^b	78 [†] (62 ^b)	225 ^b (180 ^b)
2	—	—	—	—
4	0.1 M DMAP/DMF	N.A.	N.A.	N.A.
5	0.45 M HBTU/HOBt/DMF [‡]	30	100 (95)	100 (95)
6	MeOH	N.A.	N.A.	N.A.
7	2.0 M DIEA/NMP	33	130	130
8	1.0 M DCC/NMP	N.A.	N.A.	N.A.
9**	DCM	N.A.	160 (145)	N.A.
10 ^d	NMP ^{††}	24 ^b	50 ^b (45 ^b)	120 ^b (110 ^b)
	NMP (with r.s.)	20 ^b	45 ^b (40 ^b)	

*Piperidine usage is based on the newer 450 mL bottle.

†These numbers are affected by Add Times and Monitoring Parameters.

^b Values in parentheses represent expected usage when pressure regulator is set at high end of acceptable range.

[‡]Preparation of this reagent is described on page 4-10.

**Bottles 9 and 10 are externally attached bottles.

††The NMP values assume a two-bottle assembly in bottle position 10.

WARNING **CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.**

Table 2-5. Fmoc/HOBt/DCC Chemical Usage: Cycles per Bottle

Bottle Position	Chemical	0.25 mmol Scale	0.10 mmol Scale
1	Piperidine*	78 [†] (62 ^b)	225 ^b (180 ^b)
2	—	—	—
4	0.1 M DMAP/DMF	N.A.	N.A.
5	—	—	—
6	MeOH	45 (41)	112 (100)
7	1.0 M HOBt/NMP	120 (110)	120 (110)
8	1.0 M DCC/NMP	110 (100)	110 (100)
9 [‡]	DCM	72 (65)	200 (180)
10 ^c	NMP**	35 ^b (30 ^b)	90 ^b (80 ^b)

*Piperidine usage is based on the newer 450 mL-sized bottle.

[†]These numbers are affected by Add Times.

^bValues in parentheses represent expected usage when pressure regulator is set at high end of acceptable range.

[‡]Bottles 9 and 10 are externally attached bottles.

**The NMP values assume a two-bottle assembly in bottle position 10.

Table 2-6. Boc/HOBt/DCC Chemical Usage: Cycles per Bottle

Bottle Position	Chemical	0.5 mmol Scale	0.10 mmol Scale
1	DIEA	31 (26)	53 (45)
2	TFA	36 [†] (34 ^a)	110 ^a (108 ^a)
4	Ac ₂ O	200 (170)	200 (170)
5	80% DMSO/NMP (v/v)	135 (90)	450 (300)
6	MeOH	67 (56)	112 (94)
7	1.0 M HOBt/NMP	60 (55)	120 (110)
8	1.0 M DCC/NMP	55 (50)	110 (100)
9 [†]	DCM [‡]	35 ^a (30 ^a)	100 ^a (90 ^a)
10 ^b	NMP	27 ^a (24 ^a)	77 ^a (70 ^a)

*These numbers are affected by Add Times.

^aValues in parentheses represent expected usage when pressure regulator is set at high end of acceptable range.

[†]Bottles 9 and 10 are externally attached bottles.

[‡]The DCM values assume a two-bottle assembly in bottle position 9.

Setting Up Synthesis

Load Flow Tests from SynthAssist Software

Use Flow Tests to measure and calibrate reagent deliveries, check the barcode reader calibration, the conductivity cell, and the conductivity baseline on your instrument.

Flow Tests 1, 2, 4-10, 14, 16, 19-21 require that you place a metering vessel (P/N 400256) in the RV holder. Flow Tests 22 and 23 require a reaction vessel in the RV holder. Flow Tests 11-14, 17 and 18 require that you place a tared, empty, septum-sealed cartridge in the autosampler. Flow test 19 requires a resin-sampling RV and a test tube for the resin-sample line.

IMPORTANT *To prevent accidental chemical spills, place a cartridge in the guideway with the pusher block against it before starting Flow Tests 11, 12, 13, 14, 17 and 18.*

Note *If the instrument has been sitting idle for a while, do Flow Tests 20, 22, and 23 at least twice to get a consistent baseline value.*

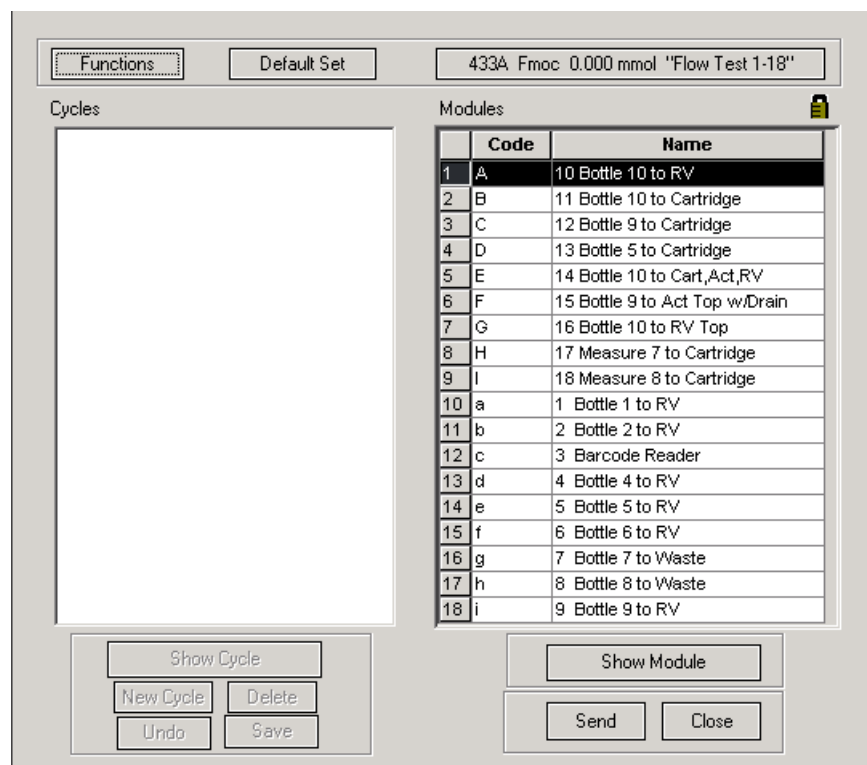


Figure 2-5. Flow tests 1 to 18 and module name

Note *SynthAssist Software allows more than one module to share the same letter name. Flow Tests 1 and 19 are both named Module a; Flow Tests 2 and 20 are both named Module b, c, and so forth.*

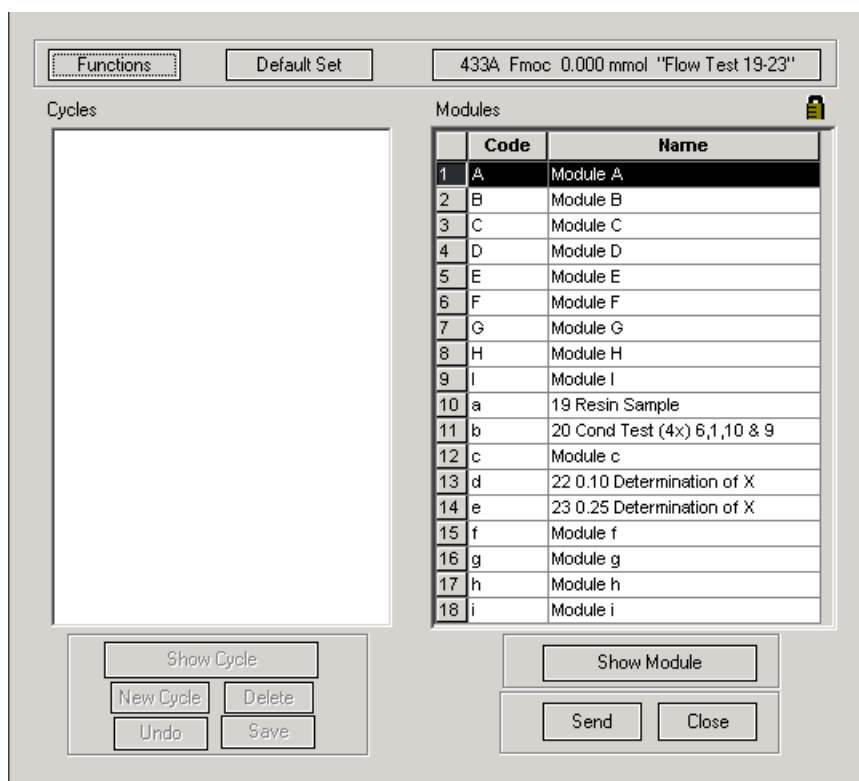


Figure 2-6. Flow tests 19 to 23 and module name

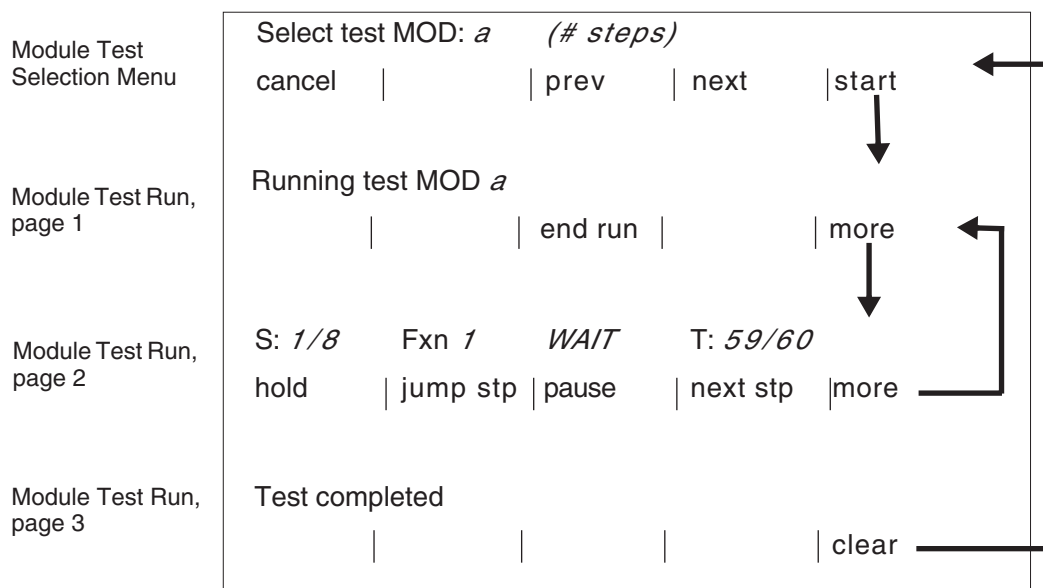
To run a flow test, you must first send the Flow Test modules from SynthAssist Software on the computer to the ABI 433A instrument. Refer to the SynthAssist user guide for the procedure to send Flow Test modules to the ABI 433A instrument.

See "Flow Test Descriptions" on page 6-27 for instructions on adjusting regulators, details on the flow test steps and functions, and running flow tests.

Starting a Flow Test

To select and start a flow test on the ABI 433A Peptide Synthesizer:

1. After you have sent the Flow Test modules to the ABI 433A instrument, press **module test** in the Main Menu.

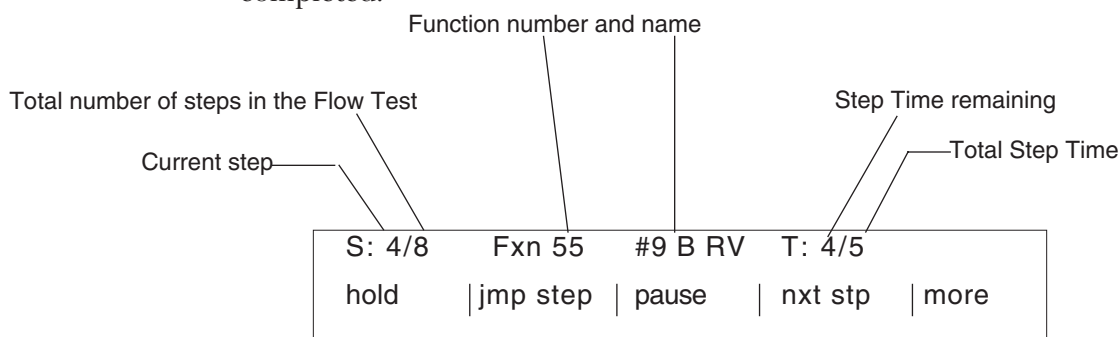


2. Press **prev** or **next** until the module letter-name appears after the words "Select test MOD:" on the LCD.
3. Press **start** to begin the Flow Test. When the Flow Test is completed: press **clear** to return to the Module Test Selection Menu.
4. Press **prev** or **next** to perform another Flow Test. Press **cancel** or press the Main Menu key to return to the Main Menu.

Make copies of the Maintenance Tracking Sheet on page 2-9 and use it to keep a record of the flow tests, regulator pressures, and in-line filter changes. This record is especially useful if the instrument has several users.

Viewing the Flow Test Steps

During flow tests, you may monitor the progress of the test on the LCD. The top line of the LCD displays the Step (**S**) and Function (**Fxn**) that is currently running with the Time (**T**) remaining before the Step is completed.



Controlling Flow Tests

After a Flow Test begins, you can do any of the following:

- Hold, or prolong, the step time.
- Jump to another step in the flow test.
- Pause, or interrupt without stopping, the flow test.
- Proceed to the next step (shorten the current step's time).
- Terminate the flow test.

hold When you press **hold**, an asterisk appears (***hold**). You can press **hold** to prolong a step without changing its programmed time. To hold a step that is 2 seconds or less, press and do not release the **hold** key.

When you hold a step, the Step Time countdown continues until it reaches zero (0). If you continue to hold the step after the countdown reaches zero, the Total Step Time value begins to increase, to reflect how long you hold the step. Synthesis stays at the step until you press ***hold**, and then it proceeds to the next step.

jmp step When you press the **jmp step** key, the message "**Enter step# to jump to:___**" appears. Use the keyboard to select the step number and press **enter**. The new step will begin immediately.

pause Press **pause** to stop the Flow Test momentarily. When you press **pause**, an asterisk appears (***pause**), all valves close, and instrument operation stops. Operation and the step continue when you press ***pause**.

nxt stp When you press **nxt stp**, the current step ends and the next step begins. Use **nxt stp** to cut short the time of the current step.

more Press **more** to return to the previous page of the Module Test menu.

end run When you terminate a Flow Test before all the steps in the module have been completed, harmful chemicals may be left in the instrument line and vessels. If you are using *FastMoc* or Fmoc/HOBt/DCC chemistry, ending Flow Test 1 may leave piperidine in the lines. If you are using Boc chemistry and terminate Flow Test 2, TFA may be in the lines.

Caution *If you press end run in the middle of a Flow Test, chemicals could be left in the lines and vessels. Perform Flow Test 10 to rinse the chemicals out of lines and vessels. If possible, DO NOT terminate Flow Test 2 when TFA is in Bottle 2. If you terminate Flow Test 2 when TFA is in Bottle 2, you must neutralize and clean the metering vessel before it can be removed. To neutralize the metering vessel, start Flow Test 2, jump to step 30, and allow Flow Test 2 to continue to completion.*

Terminating a flow test

To terminate a flow test:

1. Press the **end run** soft key in the Module Test menu.

Running test MOD: *a*

		end run		more

↓

Are you sure you want to end the run?

			no		yes

↓

Select test MOD: *a*

cancel			prev		next		start

2. Press the **yes** soft key in response to the question, “Are you sure you want to end the run?” Press the **no** soft key to continue the Flow Test.
3. Perform Flow Test 10 to rinse chemicals out of the lines and vessels.

IMPORTANT *If you terminate Flow Test 1 with piperidine still in the lines, **do not** follow this immediately with Flow Test 9. The combination of DCM and piperidine can cause the formation of crystals in the lines.*

Calibrate Gas Regulators

Pressurized gas controls timed chemical deliveries on the ABI 433A instrument, so you must correctly calibrate the lower and upper gas regulators. Table 2-7 describes the two Flow Tests that are used to set the regulators.

Table 2-7. Regulator Calibration Volume

Flow Test	Reg.	Volume (mL) in Metering Vessel	Reagent	Delivery Time (sec)	Typical Pressure (psi)
2	upper	2.0 ± 0.05	TFA	18	2.0–3.0
10	lower	2.5 ± 0.1	NMP	5	9.0–11.0

Always check the regulators after changing the reaction vessel in-line filters. To check the regulators, measure a timed delivery of reagent into a 6-mL metering vessel that has been placed in the reaction vessel holder. See page 6-30 for a description of Flow Test 2 and page 6-39 for a description of Flow Test 10. After Flow Test 2, the metering vessel may contain residual TFA, even after thorough washing. Always use caution when handling the vessel.

Lower Regulator (Flow Tests 10 and 11)

The lower regulator controls the pressure to all bottles, except Bottle 2, and the gas delivery to the valve blocks. The lower regulator operating range is approximately 9.0–11.0 psi.

A procedure for adjusting the lower regulator is on page 6-14.

Upper Regulator (Flow Test 2)

The upper regulator controls the gas pressure to Bottle 2, which contains TFA during Boc chemistry. Adjust the upper regulator when the TFA bottle is full, because the rate of TFA delivery decreases slightly when the bottle is nearly empty. See page 6-15 for a procedure for adjusting the upper regulator.

Caution ***DO NOT stop Flow Test 2 when Bottle 2 contains TFA. If Flow Test 2 is terminated while TFA is being used, neutralize and clean the metering vessel before removing it. Neutralize the metering vessel by starting Flow Test 2, jumping to step 30, and then allowing Flow Test 2 to continue to completion.***

Note *The second half of Flow Test 2 washes the lines and the metering vessel with a base. Bottle 1 (DIEA) and Bottle 9 (DCM) must be connected and pressurized to perform Flow Test 2. Flow Test 2 is not necessary when you are using Fmoc/HOBt/DCC (or FastMoc) chemistry.*

The delivery volume of TFA is measured between steps 16 and 25 of Flow Test 2. Delivery volumes should be 2.0 ± 0.05 mL when the regulator is approximately 2.5 psi. If the TFA volume is out of this range, adjust the regulator setting.

Bubbles may be present for the first 6 to 10 seconds of the 18-second TFA delivery to the metering vessel. If a constant stream of bubbles appears as TFA begins to fill the metering vessel, the TFA bottle seal may have a leak. Replace the TFA bottle seal.

In both Flow Test 2 and in module “b” (Boc deprotection), the TFA delivery line is filled with TFA. The TFA bottle is under pressure only immediately before and during TFA delivery. After the delivery, TFA in the line is flushed back into the TFA bottle and the bottle is vented. Do not interrupt Flow Test 2 between step 16 and step 25 when this process occurs.

Checking Liquid Flows

IMPORTANT When using HBTU solution, use Flow Test 13, not Flow Test 5.

Flow Tests 1, 4, 5, 6, 9, and 10

You can check the flow rate of all the other bottles after adjusting the lower regulator. Flow tests 1, 4, 5, 6, 9, and 10 have a 5-second liquid delivery to the metering vessel. Typical delivery volumes and weights are given in Table 2-8. For expected ranges, see Chapter 6 of this guide.

Flow Tests 11 and 12

Bottles 9 and 10 each have an additional flow test that delivers liquid to the cartridge for 5 seconds. This is a good way to test the cartridge in-line filter because a clogged filter restricts the flow. To run the test, place a pre-weighed, used cartridge in the autosampler. Flow Test 11 is described in the preceding section. The expected values are listed in Table 2-8.

Flow Test 13

With the *FastMoc* chemistry option, use Flow Test 13 to check the delivery time of the HBTU/HOBt/DMF solution in Bottle 5. This delivery time determines the weight of HBTU/HOBt/DMF delivered in all *FastMoc* chemistry activation modules. To run Flow Test 13, place a pre-weighed, empty cartridge in the autosampler. For an 8-second delivery, the delivered reagent should weigh 1.9–2.1 g. If the weight of the solution delivered does not fall within this range, see page 6-42. If you must modify the delivery time at Step 4 of Flow Test 13 on your instrument, change the delivery time at Step 23 (Fxn 94, #5 TO CART) in the *FastMoc* activation module “A.” Follow Flow Test 13 with a Flow Test 11 to clear the in-line filter of HBTU.

Table 2-8. Expected Flow Test Deliveries

Flow Test	SynthAssist Module	Boc	Fmoc/HOBt/DCC	FastMoc
1	a	2.0 mL DIEA	1.0 mL Piperidine	1.0 mL Piperidine
4	d	2.0 mL Ac ₂ O	2.0 mL DMAP	2.0 mL DMAP
5	e	1.0 mL DMSO	N.A.	N.A.
6	f	2.25 mL MeOH	same	same
7	g	fill 0.5 mL metering loop	same	same
8	h	fill 0.5 mL metering loop	same	same
9	i	3.2 mL DCM	same	same
10	A	2.5 mL NMP	same	same
11	B	2.1 g NMP	same	same
12	C	3.75 g DCM	same	same
13	D	N.A.	N.A.	1.9 - 2.1 g HBTU
17	H	0.52 - 0.55 g 1 M HOBt/NMP	0.52 - 0.55 g 1 M HOBt/NMP	0.46 - 0.50 g 2M DIEA/NMP
18	I	0.515 - 0.554 g 1 M DCC/NMP	same	same

Checking In-Line Filters for Leaks

After running the flow tests, check the user-accessible in-line filters for solvent leaks. Leakage can occur when either the flared tubing or the in-line filters are worn.

Caution *An in-line filter leak near the conductivity flow cell may short out the electrical contacts to the cell. A short to the electrical contacts results in erratic conductivity signals.*

Checking HOBt and DCC Lines for Blockage

If the instrument is set up to run a chemistry with HOBt/DCC activation and has not been used for a week or more, crystals may form in the lines that carry 1M HOBt/NMP and 1M DCC/NMP. The lines that carry these fluids are part of the metering loop controlled by Angar valves 29, 31, and 32. Check for blockage in these lines by running the appropriate flow tests before beginning a synthesis. If you keep the right side-panel in place on the instrument, run Flow Tests 17 and 18. If you remove the panel on the right side of the ABI 433A instrument, you can observe Flow Tests 7 and 8.

Flow Tests 17 and 18: Flow test 17 delivers 0.5 mL from Bottle 7 (HOBt) and Flow Test 18 delivers 0.5 mL from Bottle 8 (DCC) to the cartridge. Before running either of these tests, place a pre-weighed cartridge in the autodelivery system. During Flow Test 17, function 68 (Measure #7) is activated for 3 seconds. During these 3 seconds, the HOBt solution should

flow through the 0.5 mL loop, through the 11 port valve block, and approximately 8 cm down the waste line from the valve block. The weight of 1 M HOBt in NMP in the cartridge should be 0.52–0.55 g.

During Flow Test 18, function 69 (Measure #8) is activated for 3 seconds, and the DCC solution should flow approximately 12 cm or more down the waste line from the valve block. The weight of 1 M DCC in NMP in the cartridge should be 0.51–0.55 g.

Flow Tests 7 and 8: (If you remove the panel on the right side of the ABI 433A instrument, you can observe Flow Tests 7 and 8.) Fill the 0.5 mL loop, flush the solutions from the line and deliver the contents of the loop to waste. Take off the right side panel to watch the 0.5 mL line fill. If during step 2 of either Flow Test 7 or 8 the line fills in 3 seconds or less, there is no blockage in the line. If the liquid moves very slowly through the line during step 2, there is blockage. A procedure for clearing the blockage is on page 6-16.

Creating a Run File

Choosing a Chemistry File for Conductivity Monitoring of Fmoc Deprotection

A peptide Run file in SynthAssist Software defines the Sequence and designates the Chemistry option for the synthesis. The Chemistry files with a “Mon” in their file name contain a deprotection module for Basic Monitoring of deprotection with feedback. With Basic Monitoring, you can customize the deprotection module by defining the maximum number of Fmoc deprotections per cycle. A monitored loop repeats deprotection until your conditions are met.

The ABI 433A instrument counts the number of deprotection loops that occurred and “feeds back” that number to the coupling module “F.” The coupling loop is repeated the same number of times to assure adequate coupling time.

The pre-defined Chemistry files for Basic Monitoring include:

- *FastMoc 0.10 MonPrevPk*
- *FastMoc 0.10 Mon 1st-X*
- *FastMoc 0.25 MonPrevPk*
- *FastMoc 0.25 Mon 1st-X*
- *FastMoc 1.0 MonPrevPk*

You can change the maximum number of Fmoc deprotections per cycle by modifying the values of “T” in the monitoring functions. Unlike other functions on the ABI 433A instrument, the value of “T” in the monitoring functions does not represent time.

For simplification, this section briefly describes how to modify the deprotection modules for Basic Monitoring for the 0.10 mmol scale. Refer to page 5-6 for a thorough description of the functions and modules used with Basic Monitoring of deprotection. For a description of Conditional Monitoring Chemistry cycles, see page 5-21.

FastMoc MonPrevPk

In this Chemistry file, you can modify two values of “T” in module “B,” the deprotection module, to customize the deprotection:

- **The value of “T” in Function 133** represents the maximum number of monitored loops in a cycle. For most amino acids, 3 loops are sufficient, but you may increase this value when you anticipate a difficult deprotection.

-
- **The value of “T” for Function 134** divided by 10 represents a percentage difference between the last two deprotection peaks in the cycle. If this percentage difference is reached before the maximum number of monitored loops defined in Function 133, the deprotection ends. For most amino acids, Applied Biosystems recommends using a number between 50 and 150 for the value of “T” in this function.

FastMoc Mon 1st-X

In the “*FastMoc 0.10 Mon 1st-X*” file, you can modify the values of “T” in three functions in module “B”:

- **The value of “T” in Function 128** multiplied by 10, represents a baseline conductivity value. This baseline varies from instrument to instrument and may change when you change reagent lot numbers. If you do not know the baseline conductivity value run Flow Test 22 for the 0.10 *FastMoc* and Flow Test 23 for the 0.25 mmol *FastMoc*.
- **The value of “T” in Function 133** represents the maximum number of monitored deprotection loops in a cycle. For most amino acids, 4 loops are sufficient, but you may increase this value when you anticipate a difficult deprotection.
- **The value of “T” for Function 13**, divided by 10 represents a percentage difference between the initial deprotection peak and the last deprotection peak in a cycle. If this percentage difference is reached before the maximum number of deprotection loops in Function 133, the deprotection ends. For most amino acids, Applied Biosystems recommends using a number between 50 and 150 for the value of “T” in this function.

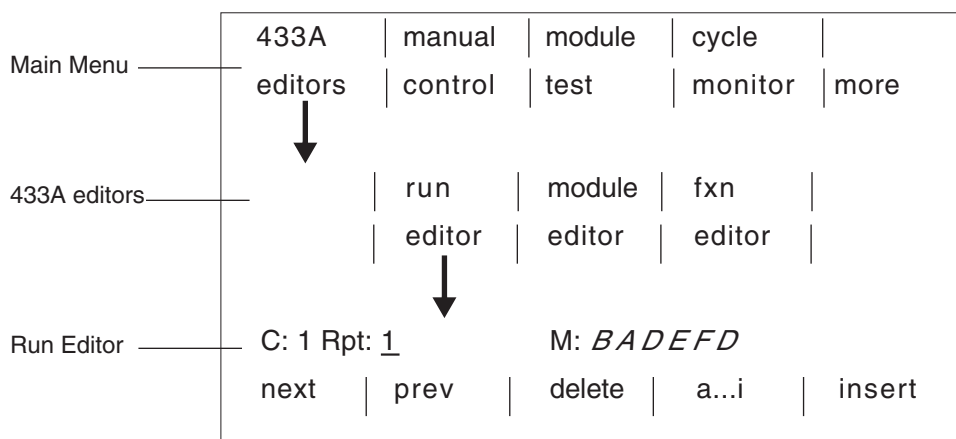
Sending Run File from SynthAssist Software

To use SynthAssist Software to set up a run file:

1. Create the peptide Sequence file.
2. Open and set up a new Run file.
3. Enable communications between the computer and the ABI 433A instrument.
4. Send the chemistry to the ABI 433A instrument.
5. Send the Run file to the ABI 433A instrument.

The section titled “Creating a Peptide Run” in the SynthAssist user guide describes how to perform each of these steps.

After you send the run to the ABI 433A instrument, verify that the 433A Run Editor contains the run.



To check the 433A Run Editor:

1. Press **433A Editors** in the Main menu.
2. Press **run editor** in the 433A editors menu.

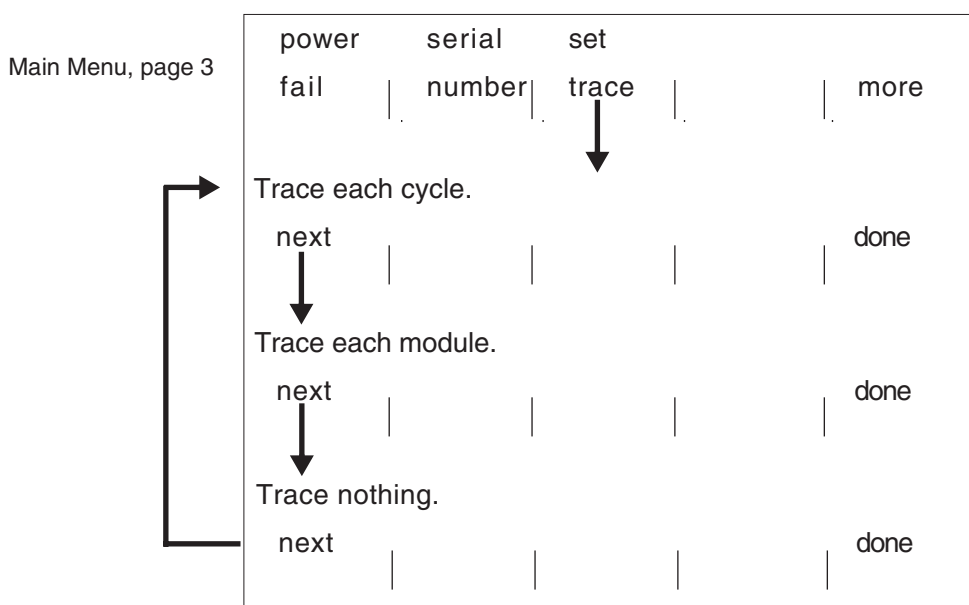
The Run Editor menu displays all the cycles and modules in the run. See page 9-5 for a description of the Run Editor menu.

3. Press the **next** and **prev** keys to review the cycles in the run.

Set Trace Option

You can choose from three levels of detail for the synthesis log:

- **Trace each module** - includes peak and amino acid data, along with a complete listing of all modules in each cycle (maximum detail).
- **Trace each cycle** - includes peak, amino acid, and first module data for each cycle (moderate detail).
- **Trace nothing** - includes only peak and amino acid data (minimum detail).



To select a trace option:

1. Press the **Main Menu** key to return to the Main Menu.
2. Select **set trace** from the 3rd page of the Main Menu.
3. Press the **next** key to cycle among the three available trace activity options.
4. Press **done** when the trace activity option you require displays on the LCD.

Beginning Synthesis

Loading Amino Acid Cartridges

Applied Biosystems provides dry amino acids in pre-loaded cartridges for each chemistry option.

Table 2-9. Applied Biosystems Pre-loaded Amino Acid Cartridges

Chemistry Option	Cartridge Size
<i>FastMoc</i> 1.0 mmol	1 mmol Fmoc
<i>FastMoc</i> 0.25 mmol	1 mmol Fmoc
<i>FastMoc</i> 0.10 mmol	1 mmol Fmoc
Fmoc/HOBt/DCC 0.25 mmol	1 mmol Fmoc
Fmoc/HOBt/DCC 0.10 mmol	1 mmol Fmoc
Boc/HOBt/DCC 0.50 mmol	2 mmol Boc

Each cartridge is labeled with the appropriate three-letter code and barcode. The barcode reader translates the label information for an amino acid printout.

<i>Note</i>	<i>Amino acid cartridges are loaded onto the guideway with the cartridge for the N-terminal amino acid on the extreme left of the guideway, next to the pusher block. If you are not using a pre-loaded resin, the cartridge for the C-terminal amino acid is on the right end of the guideway, closest to the sampling syringe needle.</i>

To load the cartridges:

1. Remove the metal septum tabs from the cartridge top.

Caution	<i>To prevent damage to the syringe needle, remove the metal tab that covers each cartridge septum before the cartridge is placed in the guideway.</i>

2. Move the pusher block to the far left and secure it with the latch.

WARNING	<i>POTENTIAL OPERATOR INJURY. Sudden release of the pusher block causes it to snap forcefully against any cartridges, fingers, or anything else present in the guideway. Always hold the pressure block firmly.</i>

-
3. Place an empty cartridge in the first position in the guideway (under the needles). This cartridge is ejected at the beginning of the cycle (the EJECT CART step at the beginning of the activation module).
 4. Place an amino acid cartridge next to the empty first cartridge. The amino acid designation should face the outside and the barcode should face the instrument.

If you are using pre-loaded resin, the amino acid loaded on the resin is the C-terminal amino acid. The amino acid in the cartridge after the empty first cartridge then represents the amino acid just before the C-terminal amino acid. If you are not using pre-loaded resins, the amino acid cartridge next to the empty first cartridge represents the C-terminal amino acid.

5. Load the rest of the amino acid cartridges in the guideway with the N-terminal amino acid cartridge at the extreme left of the guideway.
6. Place the pusher block against the cartridges.

Caution	<i>If you do not release the pusher block and you answered No to the Barcode Interruption Option on the Barcode Calibration menu (see page 2-10), the instrument does not pause when the barcode reader “reads air.” As a result, chemicals are delivered through to the autosampler assembly and spilled onto the synthesizer.</i>

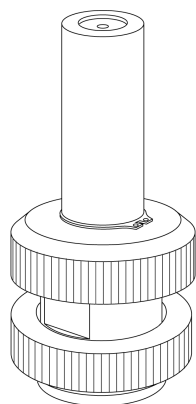
7. Lower the retaining rod.

Loading Test Tubes for Resin Samples (optional)

If resin samples are taken, use the ABI 433A instrument to activate a fraction collector through Relay 0. Use two test tubes for each resin sample, one to collect the resin-sample line wash and another to collect the resin sample. Weigh the second test tube and add 2 to 3 mL of MeOH so that the resin does not stick to the sides of the test tube but settles to the bottom. With Fmoc chemistry, also add 2 to 3 drops of acetic acid to each test tube (see page 3-10).

The volume of the resin sample and the pre-wash are each approximately 3 to 6 mL, so each test tube should hold at least 7 to 8 mL. A 16 x 100 mm test tube, with a capacity of 14 mL, should be sufficient.

Reaction Vessels



0.10 mmol Reaction Vessel

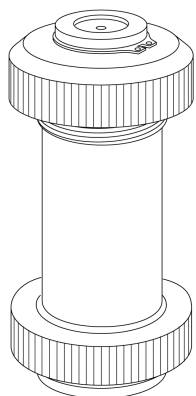
Resin sampling (not shown): P/N 401572

Non-resin sampling (shown): P/N 401571

Volume: 8 mL

Filter: P/N 401524 (Box of 30)

Use with 0.10 mmol *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC cycles.



0.25/0.50 mmol Reaction Vessel

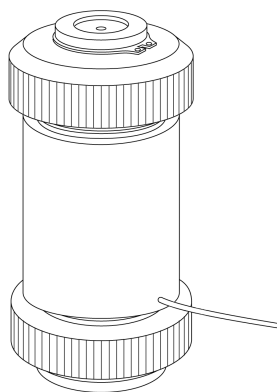
Resin sampling (not shown): P/N 401574

Non-resin sampling (shown): P/N 401573

Volume: 41 mL

Filter: P/N 401524 (Box of 30)

Use with 0.25 mmol *FastMoc*, or Fmoc/HOBt/DCC, and 0.50mmol Boc/HOBt/DCC cycles



1.0 mmol Reaction Vessel

Resin sampling (shown): P/N 401576

Non-resin sampling (not shown): P/N 401575

Volume: 55 mL

Filter: P/N 401524 (Box of 30)

Use with 1.0 mmol *FastMoc* cycles

Resin sampling vessel is used even though resin samples are not taken.

Figure 2-7. Types of reaction vessels

Choosing a Resin for the Reaction Vessel

Your choice of resin determines which chemistry should be used in the first synthesis cycle. Three types of resins are used with *FastMoc* and Fmoc/HOBt/DCC chemistry: pre-loaded, unloaded, or amide. The pre-loaded resin has the C-terminal amino acid in the peptide sequence already attached to the resin. Pre-loaded resin yields a peptide with a C-terminal acid. The unloaded resin does not have an amino acid on it. Unloaded resin yields a C-terminal acid peptide. A peptide synthesized with an amide resin yields a C-terminal amide peptide. Two types of resin are used with Boc/HOBt/DCC chemistry: PAM or MBHA. For further discussions of these resins, see page 3-20 and page 3-21.

Amide and pre-loaded resins may be used with all the ABI 433A instrument chemistry options. These resins are deprotected before the first coupling with standard cycles. Unloaded resins require DMAP-catalyzed DCC coupling (first amino acid only) with *FastMoc* and Fmoc/HOBt/DCC chemistries.

When using unloaded HMP resins with the *FastMoc* chemistry option, run Flow Test 4 and Flow Test 8 before synthesis. DMAP and DCC are used infrequently with *FastMoc* chemistry. Use the flow tests to confirm that these delivery lines are clear.

Refer to Table 2-10 to determine the amount of resin you need for the chemistry option and scale you are using.

Table 2-10. Resin amounts used with Chemistry options

Scale	<i>FastMoc</i>	Fmoc/HOBt/DCC	Boc-HOBt
1.0 mmol	1.0 mmol	N.A	N.A
0.50 mmol	N.A	N.A	0.50 mmol
0.25 mmol	0.25 mmol	0.25 mmol	N.A
0.10 mmol	0.10 mmol	0.10 mmol	0.10 mmol

Examples for calculating amount of resin to add, Boc and Fmoc:

Enter the resin loading (substitution) value in the SynthAssist Chemistry window and SynthAssist Software calculates how many grams of resin you should use to charge the reaction vessel. The following examples show how these calculations are performed.

Boc/HOBt/DCC 0.50 mmol

For Boc-Gly-PAM resin with a loading of 0.78 mmol/g add:

$$\frac{0.50 \text{ mmol}}{0.78 \text{ mmol/g}} = 0.64 \text{ g of Boc-Gly PAM resin}$$

FastMoc 0.25 mmol

For Fmoc-Val resin with a loading of 0.75 mmol/g add:

$$\frac{0.25 \text{ mmol}}{0.75 \text{ mmol/g}} = 0.33 \text{ g of Fmoc-Val resin}$$

Fmoc/HOBt/DCC 0.10 mmol or Boc/HOBt/DCC 0.10 mmol

For a resin with a loading of 0.67 mmol/g add:

$$\frac{0.10 \text{ mmol}}{0.67 \text{ mmol/g}} = 0.149 \text{ g of resin}$$

To add resin to the reaction vessel:

1. Select the desired reaction vessel (RV).

There are three RV sizes (see Figure 2-7 on page 2-35); each is available with or without a resin-sampling line.

IMPORTANT *Using the resin-sampling RV with the non-sampling cycles can adversely affect the success of the synthesis, with the exception of the 1.0 mmol FastMoc chemistry.*

In the resin-sampling cycles, the line is frequently rinsed to prevent residual resin, TFA, or coupling solution from contaminating any subsequent portion of the synthesis. This rinsing does not occur in non-sampling cycles.

WARNING ***CHEMICAL HAZARD. Hazardous solvents—DCM, NMP, or DMF—may squirt out of the resin sampler bulkhead fitting, AT EYE LEVEL, when it is used with a non-sampling RV. To prevent serious chemical burns and eye damage, make sure the sliding cover flap covers the bulkhead fitting when you are using a non-sampling RV. Always wear chemical-resistant gloves, lab coat, and safety glasses.***

2. Hold the RV in a vertical position and place an RV filter on the protruding “knife-edge” found just inside the openings at either end of the RV (see Figure 2-8). The filter forms a seal with the knife edge when the RV cap is screwed in place.

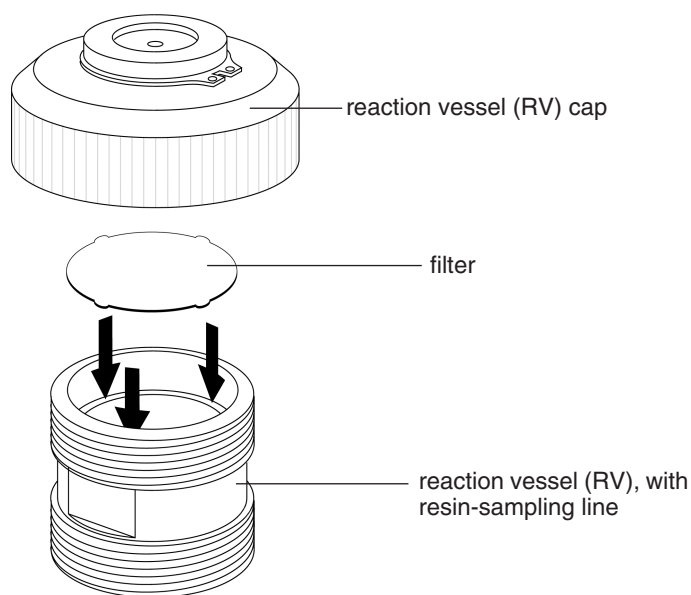


Figure 2-8. Placing RV filter on inner knife edge of reaction vessel

3. Screw on the RV cap, making sure to hold the RV in a vertical position at all times.

Caution *Hold the RV in a vertical position when screwing on the RV cap. If you turn the RV on its side while tightening its cap, the filter may become crooked and form an imperfect seal. As a result, resin may escape and clog the in-line filter.*

Tighten the cap until you feel a firm resistance. This resistance indicates that the primary seal is forming between the filter and the recessed knife edge.

Visually check the filter placement by looking through the open end of the RV. The surface of the filter should be flat and smooth, with no protrusions beyond the knife edge.

Caution *Reaction vessels are designed to be tightened by hand. Use only your hands to tighten the reaction vessel caps.*

-
4. Add the appropriate amount of resin to the reaction vessel (refer to Table 2-10).

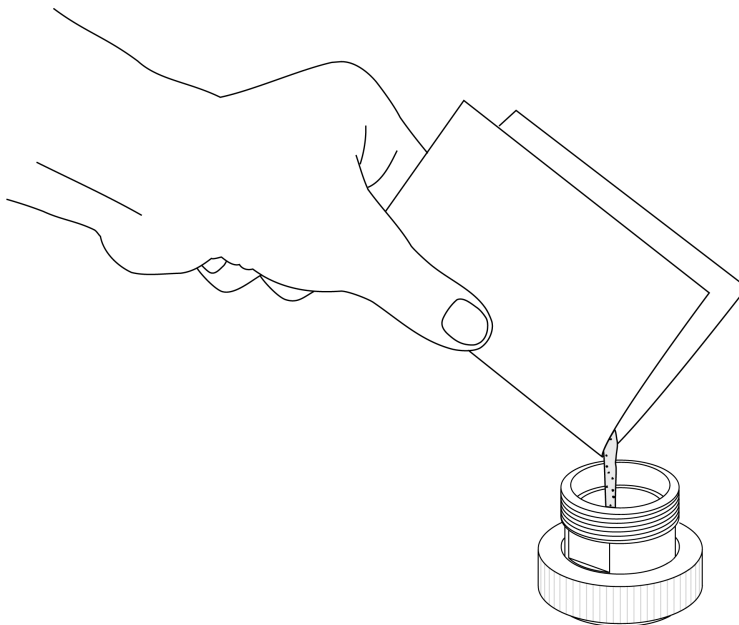


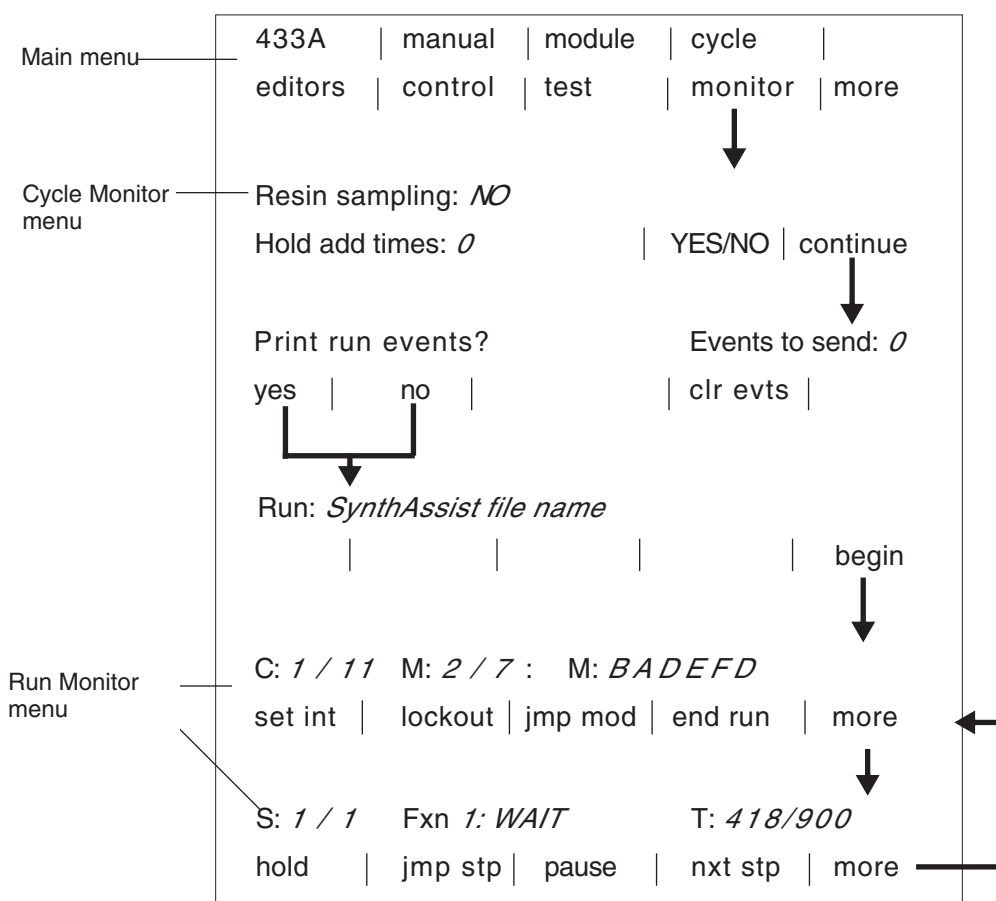
Figure 2-9. Filling the reaction vessel with resin (8-mL RV shown here)

5. Place a filter on the knife edge of the open end of the RV. Tightly screw on the cap, using the procedure described in step 3. Place the RV in the RV holder.
6. Use your fingers to connect the resin-sampler line (if present) to the bulkhead fitting. Then slowly hand-tighten until snug. **Do not over-tighten the fitting.**

Caution	<i>The fitting on the resin-sampling RV line screws easily into the bulkhead. If the fitting does not screw in easily, to prevent stripping the threads, back it off and try again.</i>

Start Synthesis in Cycle Monitor Menu

The Cycle Monitor menu is shown below. Use the Cycle Monitor menu to start a synthesis and then control the synthesis by interrupting, ending, or jumping over module steps.



To begin a synthesis, press *begin* in the Cycle Monitor menu

Resin Sampling and Hold Add Time

The first display in the Cycle Monitor menu asks you for two responses. On the top line, determine if you want resin sampling. On the second line, designate at which cycle, if any, Add Times should be discontinued.

Note *Applied Biosystems recommends you do not discontinue Add Times, except in special circumstances (see page 2-41).*

Resin Sampling

The **YES/NO** soft key is a toggle switch that changes the response after the words "Resin Sampling." Press the **YES/NO** soft key until the appropriate response appears.

Answer **YES** if you want resin samples delivered to the fraction collector at every cycle during synthesis. With resin-sampling, the resin-sampling line is washed with solvent at the appropriate times throughout the synthesis. If you press **YES**, be sure to use an RV with a resin-sampling line and place test tubes in the fraction collector.

Select **NO** if you do not want resin samples and the RV does not have a resin-sampling line. If you press **NO**, the ABI 433A instrument automatically jumps over any resin sampling functions Functions 87 through 93. When the resin-sampling functions occur in the cycles, they appear on the screen for one second, but none of the valves or switches are turned on.

<i>Note</i>	<i>The fraction collector is connected to relay 0 at the rear of the ABI 433A instrument. When this relay is activated, the fraction collector advances. "RELAY 0," or Function 39, is activated during synthesis even when samples are not desired. Disconnect the fraction collector if this is bothersome.</i>

Hold Add Times

Normally, answer 'Hold add times' with a 0 (zero).

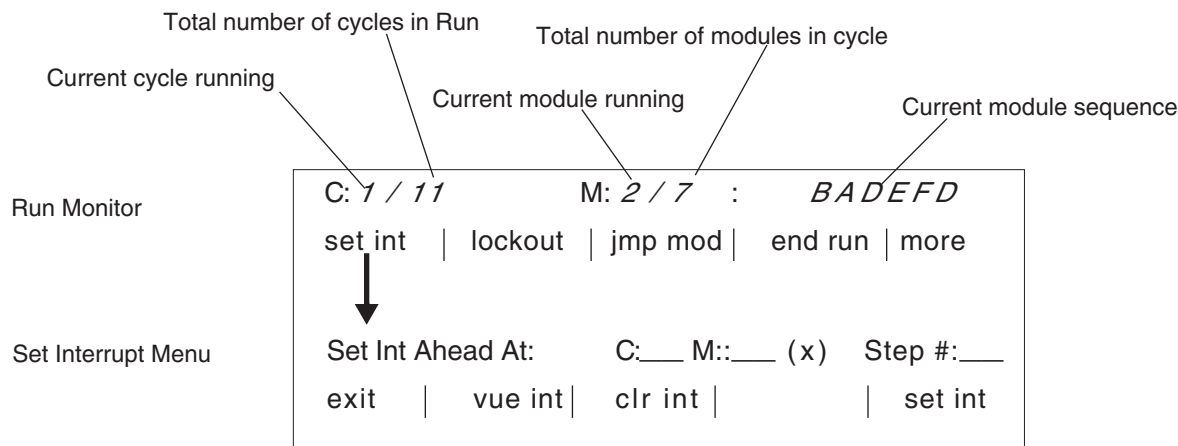
During the synthesis, the weight and volume of the swollen peptide resin increases. To compensate for these increases, the solvent delivery-times increase as synthesis progresses. See "Add Times and Chemical Usage" on page 7-54.

If a Boc/HOBt/DCC 0.50 mmol synthesis is longer than about 35-40 cycles, or when the peptide resin weighs about 3 grams, the vessel can become too full. To allow enough room in the vessel for adequate synthesis reactions, it may be necessary to remove some resin after 25 to 35 cycles. If resin is removed, you may also want to delete the add times and stop solvent delivery increases.

You can stop time additions at a selected cycle by designating the cycle number after the prompt "Hold add times." To stop the add times at cycle 25, for example, enter 25 at this prompt. During synthesis, the steps with add times will increase until cycle 25 is reached. After cycle 25, the times will not change until the end of the synthesis. However, if a value of 0 is entered after this prompt, the step times will increase at each cycle until synthesis stops.

Monitoring and Controlling Synthesis Operations

When you press **begin** in the Cycle Monitor menu, the Run Monitor appears. On the top line, the Run Monitor displays information about the Cycle (C) and Module (M) that the ABI 433A instrument is currently using. The bottom line displays available options for controlling synthesis.



Set Interrupt Menu Press **set int** to interrupt the synthesis at any cycle, module or step.

In the Set Interrupt menu, enter the cycle, module, and step at which you want synthesis to be interrupted. You must set the interruption to occur at least 2 steps ahead of the current step. After entering the information, press **set int**. If you press **clr int**, the interruption is cancelled.

IMPORTANT Do not set an interrupt to occur at a step that contains Fxn 58, INTERRUPT with T=0. Synthesis does not interrupt when the controller reads this function with T=0, unless there is a power failure (see page 8-33).

When the synthesis reaches the designated point of interruption, synthesis temporarily stops. Press the **pause** key to release the interruption and continue synthesis.

vue int When you press this soft key, the LCD indicates if an interruption is pending or if something has caused an interruption.

lockout During a synthesis, you may use this soft key to lock the keyboard. When you press the **lockout** key, the LCD shows the following display.

Enter screen lock combination: _____

lock | | unlock | | cancel

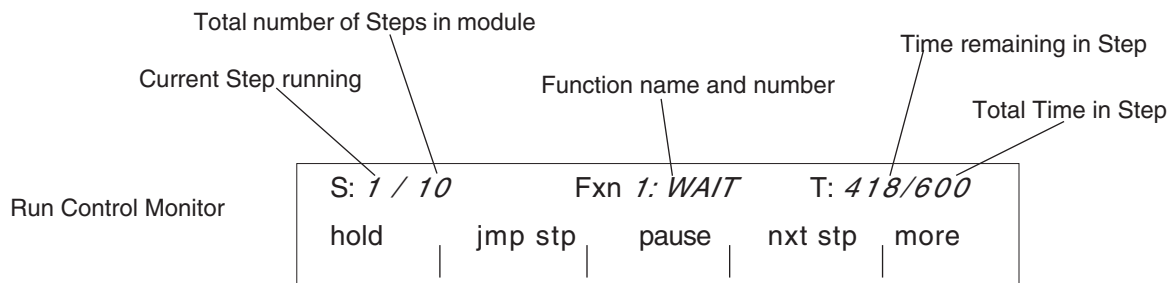
Enter up to 4 numbers and press **lock**. To unlock the keyboard, enter the same 4 numbers and press **unlock**. If you lock the keyboard and then forget the combination, you must turn the synthesizer off and then on to clear the lockout.

jmp mod Press **pause**, then press jump module to go to another module. When you press **jump module**, the menu page displays the message: “**Place cursor on mod:**” with the module sequence. Move the cursor to the desired module and press **enter**. The module selected begins at step number 1. You may press the **cancel** key at any time to return to the previous menu.

end run Press **end run** at any time to terminate synthesis.

Caution *If you press end run in the middle of a cycle, chemicals may be left in the lines and vessels. Rinse the lines and vessels before removing the vessel (See "Rinsing Lines and Vessels After an End Run" on page 2-45).*

The second Run Monitor page When you press **more**, the Run Monitor displays Step (S) information on the top line and more options for synthesis control on the bottom line.



hold When you press **hold**, an asterisk appears (***hold**). Synthesis stays at this step and the time remaining in the step continues to decrease. When the time remaining reaches zero (0), the Total Time in Step value begins to increase. Total Time continues counting seconds until you press the ***hold** key or until the Total Time is 999. You can continue holding the step, but Total Time does not count beyond 999.

When you press the ***hold** key, synthesis continues with the next step. Use the **hold** key to increase the duration of a step without changing its programmed time.

jmp stp When you press **jmp stp**, the message “**Enter step# to jump to: __**” appears. Use the keyboard to select the step number and press **enter**. The new step begins immediately.

nxt stp When you press **nxt stp**, the current step ends and the next step begins. Use this key to cut short the current step time.

pause Press this key to stop synthesis momentarily. An asterisk appears (***pause**). All valves close and instrument operation stops. Synthesis resumes when ***pause** is pressed again.

IMPORTANT *Carefully evaluate the progress of the synthesis and the effect an interruption might have on the process before pressing the **pause** key. An extended interruption during deprotection or activation could seriously compromise the quality of the final peptide product.*

Synthesis Interruptions

In addition to pressing the **pause** key, synthesis can be interrupted in the following ways:

- Add a Function 58 (Interrupt) with a time=1, to a module. When the controller reaches Function 58, time=1, synthesis pauses. To resume synthesis, press the ***pause** key displayed on the screen.
- Synthesis stops automatically if an amino acid cartridge gets caught in the guideway, preventing the needle from going down and the ejector from going out. The screen displays an error message and the keys **continue** and **more** become available. Correct the problem and press **continue** to resume synthesis. Press **more** to return to the Cycle Monitor Menu and terminate synthesis.
- Synthesis pauses automatically if the barcode reader reads the pusher block (Psh = pusher). This pause indicates that there are no amino acid cartridges in the autodelivery system.

To continue the synthesis, place the amino acid cartridges in the autodelivery system and release the pause by pressing the ***pause** key.

Caution *If the pressure block is not released and you have answered **No** to the Barcode Interruption Option (see page 2-10), the barcode reader 'reads air' and does not pause. As a result, chemicals are delivered on to the autosampler assembly.*

- Synthesis is interrupted when you press the **set int** key.

Note *During a synthesis, you can operate the ABI 433A instrument on Manual Control by pressing **pause**, **main menu**, and then **manual control**. After finishing the manual operations, return to **main menu**, press **cycle monitor**, and then press the **pause** key to resume synthesis.*

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- Synthesis is interrupted if you have answered **YES** to the Barcode Calibration menu option “Interrupt when barcode incorrect” (see page 2-10), and the barcode label of the amino acid cartridge for a cycle does not match the expected amino acid for the pre-defined sequence. If you are printing a synthesis report from the synthesizer, the printout displays both the expected amino acid and the incorrect amino acid.
 - To continue synthesis, place the correct amino acid cartridge in the amino acid cartridge guideway so that its barcode will be read when the instrument resumes operation. Release the pause by pressing the ***pause** soft key.
 - Instrument operation is interrupted if the needle is **not** down when a function that opens either valve 12 or 22 is activated. Before these valves can be opened, the controller checks the top needle sensor to make sure the needle is not in the up position. This ensures that fluids are delivered into a cartridge and not onto the surface of the instrument.

If you have written a module that causes this interruption, you must discontinue synthesis and re-write the module.

Rinsing Lines and Vessels After an End Run

If you press **end run** in the middle of a cycle, use the following procedure to ensure that no chemicals remain in the lines and vessels.

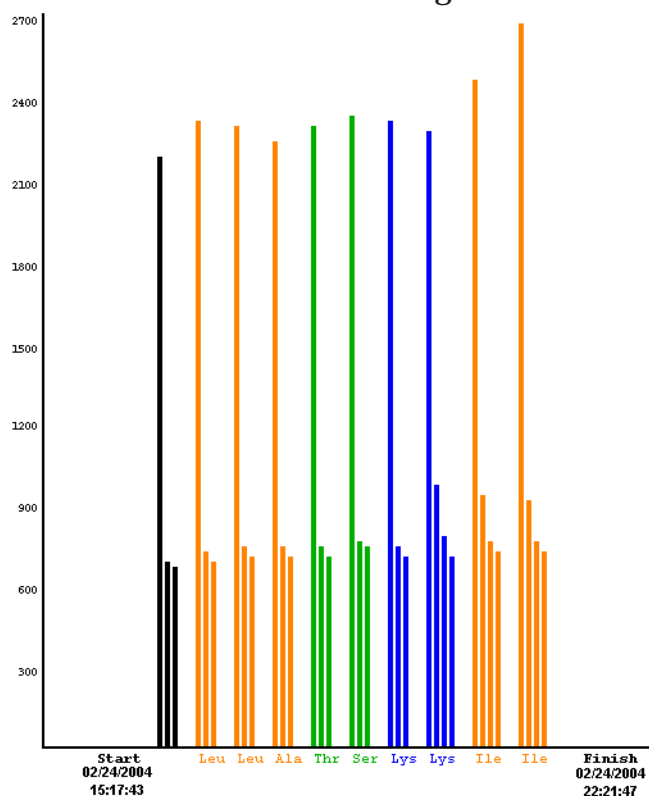
To rinse lines and vessels:

1. Press the Main Menu key to return to the Main Menu.
2. Press the **module test** soft key to enter the Module Test Selection Menu.
3. Run module D (NMP Washes) to completion.
4. Run module c (DCM Washes) to completion.
5. Resin can now be removed from the reaction vessel.

Storing SynthAssist-Generated Synthesis Records

You can save a record of any synthesis on the ABI 433A instrument as a SynthAssist file. Store SynthAssist files on the computer or on CDs. If you have a PC-compatible printer on your computer system, you may also print the contents of SynthAssist windows to generate paper records of your syntheses. Refer to the SynthAssist user guide for more information about these windows.

SynthAssist Software Monitoring Trace



SynthAssist Log

The SynthAssist Log provides a data record of every event that occurred and every peak value collected during the synthesis. The Log Window appears after you choose the Log command in the Window menu of SynthAssist Software.

5	F	8	02/24/2004 19:46:49	4	Sending chemistry file "0.10 MonPrevPk" to SYNTHESIZER.....;
5	F	8	02/24/2004 19:46:53	4	Sending user functions.....;
5	F	8	02/24/2004 19:46:58	4	Sending module 'A' - Activation.....;
5	F	8	02/24/2004 19:47:03	4	Sending module 'B' - Deprotection MonPrevPk.....;
5	F	8	02/24/2004 19:47:08	4	Sending module 'C' - Capping with Ac20 Solution.....;
5	F	8	02/24/2004 19:47:12	4	Sending module 'D' - NMP Washes.....;
5	F	8	02/24/2004 19:47:16	4	Sending module 'E' - Transfer.....;
5	F	8	02/24/2004 19:47:21	4	Sending module 'F' - Coupling etc/feedback.....;
5	F	8	02/24/2004 19:47:26	4	Sending module 'G' - Resin Sampling.....;
5	F	8	02/24/2004 19:47:32	4	Sending module 'H' - Load and Cap.....;
5	F	8	02/24/2004 19:47:36	4	Sending module 'I' - Wait (10 min).....;
5	F	8	02/24/2004 19:47:40	4	Sending module 'a' - Module a.....;
5	F	8	02/24/2004 19:47:44	4	Sending module 'b' - Module b.....;
5	F	8	02/24/2004 19:47:48	4	Sending module 'c' - DCM Washes.....;
5	F	8	02/24/2004 19:47:52	4	Sending module 'd' - Module d.....;
5	F	8	02/24/2004 19:47:56	4	Sending module 'e' - Module e.....;
5	F	8	02/24/2004 19:47:59	4	Sending module 'f' - Module f.....;
5	F	8	02/24/2004 19:48:03	4	Sending module 'g' - Module g.....;
5	F	8	02/24/2004 19:48:07	4	Sending module 'h' - Module h.....;
5	F	8	02/24/2004 19:48:11	4	Sending module 'i' - Module i.....;
5	F	8	02/24/2004 19:48:24	4	Sending run file "Kinstall 24feb04" - Run No: 8 to SYNTHESIZER.....;

Figure 2-10. Example of the SynthAssist Log

If you have not enabled communication between SynthAssist Software and the ABI 433A Peptide Synthesizer, synthesis events and peak data are stored in the ABI 433A instrument memory buffer. The memory buffer may hold information for more than one synthesis. As soon as you enable communication between SynthAssist Software and the ABI 433A instrument, all data stored in the memory buffer is transferred to the computer and becomes a SynthAssist file.

Note *SynthAssist Software cannot distinguish where one synthesis ends and the next one begins. If the ABI 433A instrument memory buffer contains information collected from more than one synthesis, all the data is transferred into one SynthAssist Log file and one SynthAssist Monitor window.*

If you are sure that the memory buffer contains only information that you do not want to save in SynthAssist Software, you can erase all the information in the buffer in the Cycle Monitor menu before you begin synthesis. To clear the memory buffer in the ABI 433A instrument software, press the **clr evts** soft key.

If you intend to use a printout of the SynthAssist Log for your synthesis records, always establish communications between SynthAssist Software and the ABI 433A instrument before each synthesis begins. Event and peak data is then automatically transferred to the SynthAssist Log during the synthesis.

Conversion of *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC Chemistries

FastMoc, Fmoc/HOBt/DCC, and Boc/HOBt/DCC chemistries use different chemicals at five bottle positions (Table 2-11). This section describes the bottle changing procedure for converting from one chemistry to another.

Table 2-11. Bottle Contents for *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC

Chemistry	Bottle 1	Bottle 2	Bottle 4*	Bottle 5	Bottle 7
<i>FastMoc</i>	Piperidine	empty	DMAP	HBTU/DMF	2 M DIEA
Fmoc/HOBt/DCC	Piperidine	empty	DMAP	empty	HOBt
Boc/HOBt/DCC	DIEA	TFA	Ac ₂ O	DMSO	HOBt

* For capping cycles with *FastMoc* chemistry, Bottle 4 contains Ac₂O solution (see page 7-14).

For capping with Boc chemistry, Bottle 4 contains 100% Ac₂O.

Note *Bottles 9 and 10 should contain solvents and be pressurized during all of the chemistry conversion procedures.*

WARNING **CHEMICAL HAZARD.** *Diisopropylethylamine (DIEA) is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! **CHEMICAL HAZARD.** *4-Dimethylaminopyridine (DMAP) is a poison. It may be fatal if absorbed through the skin, and is harmful if swallowed. Exposure causes eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *N,N-Dimethylformamide (DMF) is harmful if inhaled. It is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** Dimethyl sulfoxide (DMSO) may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** (N-[1H-benzotriazol-1-yl] (dimethylamino)methylene]-N-methylanaminium hexafluorophosphate N-oxide (HBTU), formerly 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumhexafluorophosphate, may cause allergic respiratory and skin reactions. Do not breathe the dust, and avoid prolonged or repeated contact with the skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** 1-Hydroxybenzotriazole hydrate (HOBt) has a risk of explosion if heated under confinement. Keep away from heat and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER! **CHEMICAL HAZARD.** Piperidine (hexahydropyridine) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled, swallowed, or absorbed through the skin. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER! **CHEMICAL HAZARD.** Trifluoroacetic acid (TFA) causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Change Bottle 1

If you are switching from Boc/HOBt/DCC to *FastMoc* or Fmoc/HOBt/DCC chemistry and are going to replace the bottle of TFA with a clean, empty bottle, change Bottle 2 before changing Bottle 1. To ensure that you will have DIEA in Bottle 1 to neutralize the TFA.

<i>Note</i>	<i>The delivery line from Bottle 1 will turn brown if piperidine is not adequately washed out before switching to DIEA. However, this brown color does not affect any of the chemistries.</i>

To change Bottle 1:

1. Put a metering vessel in the RV holder.
2. Remove Bottle 1 and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue.
3. Clean the bottle seal and place it with a clean, empty bottle in the number 1 position. Run Flow Test 1. When the flow test is at step 8 (#1 B RV), press **hold**. After 30 seconds, press **end run**.
4. Remove the empty bottle and add 20 mL NMP to the bottle. Replace it in position number 1 and run Flow Test 1. Again, at step 8, press **hold**. Wait until all the NMP has been delivered to the metering vessel, wait 30 seconds more, then press **end run**.
5. Repeat step 4, but use 20 mL DCM instead of NMP.
6. Repeat step 4 again, using 20 mL NMP.
7. Remove the bottle and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue. Add the new reagent and a new bottle seal to Bottle 1. Run Flow Test 1 and when it is at step 8, press **hold**. After 10 seconds, press ***hold** to release the hold and continue the flow test.

Change Bottle 2

You can run Fmoc chemistry with TFA in the number 2 position. If you plan to switch back and forth between Fmoc and Boc, leave TFA in that position. However, if you are not going to use TFA for a week or more, remove it from the instrument and follow the bottle change procedure.

To change Bottle 2:

1. Put a metering vessel in the RV holder.
2. Remove the TFA bottle from position number 2 and replace it with a clean, empty bottle with a regular bottle seal (P/N 400501).

<i>Note</i>	<i>Whenever you run Flow Test 2, DIEA should be in bottle position number 1. If piperidine is in Bottle 1, it slowly reacts with DCM to form a crystalline compound (piperidine hydrochloride).</i>

3. Run Flow Test 2. When it gets to step 14 (#2 B RV), press **hold**.

-
4. After 30 seconds, press **hold** again to release the hold and continue with Flow Test 2 until it reaches step 19 (Flush #2). Press **hold** again for 30 seconds.
 5. After 30 seconds, press **hold** again and continue with Flow Test 2 until it reaches step 21 (Gas-Vent #2). Press **hold** and after 30 seconds press **end run**.

Change Bottle 4

To change Bottle 4:

1. Put a metering vessel in the RV holder.
2. Remove Bottle 4 and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue. Clean the bottle seal and replace it with a clean, empty bottle in the number 4 position.
3. Run Flow Test 4. When the flow test is at step 5 (#4 B RV), press **hold**. After 30 seconds, press **end run**.
4. Remove the empty bottle and add 20 mL NMP to the bottle. Replace it in position number 4.
5. Run Flow Test 4. Again, at step 5, press **hold**. Wait until all the NMP has been delivered to the metering vessel, wait 30 seconds more, then press **end run**.
6. Remove the bottle and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue. Add the new reagent and a new bottle seal to Bottle 4.
7. Run Flow Test 4. At step 5 of Flow Test 4, press **hold**. After 10 seconds, press ***hold** to release the hold and continue the flow test.

Change Bottle 5

To change Bottle 5:

1. Put a metering vessel in the RV holder. Remove Bottle 5 and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue.
2. If applicable, remove the reagent line filter. To backflush the reagent line, first place the end of the line in a test tube. In the Manual Control Menu, activate valves 20, 16, and 15. When a constant flow of NMP enters the test tube, turn off the valves. Open valves 17, 16, and 15 to push out the NMP with gas. Wipe the line with a lint-free tissue.
3. Clean the bottle seal and place it, with a clean, empty bottle, in the number 5 position.

-
4. Run Flow Test 5. When the flow test is at step 4 (#5 B RV), press **hold**. After 30 seconds, press **end run**.
 5. In the Manual Control Menu, activate Function 42 (DRAIN RV) to clear the NMP from the reaction vessel.
 6. Remove the empty bottle and add 20 mL NMP to the bottle. Replace it in position number 5.
 7. Run Flow Test 5. Again, at step 4, press **hold**. Wait until all the NMP has been delivered to the metering vessel, wait 30 seconds more, then press **end run**.
 8. Remove the bottle and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue. If you are filling Bottle 5 with HBTU, firmly press a new reagent line filter on the end of the reagent line. Add the new reagent and a new bottle seal to Bottle 5.
 9. Run Flow Test 13 to check HBTU delivery. Run Flow Test 5 to check DMSO delivery.

Change Bottle 7

To change Bottle 7:

1. Remove Bottle 7 and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue.
2. Clean the bottle seal and place it, with a clean, empty bottle, in the number 7 position.
3. Run Flow Test 7. When the flow test is at step 2 (MEAS #7), press **hold**. After 30 seconds, press **end run**.
4. Remove the empty bottle and add 20 mL NMP to the bottle. Replace it in position number 7.
5. Run Flow Test 7. Again, at step 2, press **hold**. Wait until all the NMP has been removed, wait 30 seconds more, then press **end run**.
6. Remove the bottle and the bottle seal. Wipe the delivery line and the bottom of the cap insert with a lint-free tissue. Add the new reagent and a new bottle seal to Bottle 7.
7. Run Flow Test 7. At step 2, press **hold**. After 10 seconds, press ***hold** to release the hold and continue the flow test.

Shutting the Instrument Down

Proper maintenance, essential for reliable operation of the ABI 433A Peptide Synthesizer, includes clean-up and removal of chemicals before turning the instrument off for an extended amount of time. This section describes shutdown procedures for each of the three chemistry options available on the ABI 433A instrument. During the shutdown procedure, you remove the bottles at each position, back-flush the delivery lines with DCM or NMP followed by DCM, and then dry the delivery lines. Follow the shutdown procedure when the instrument will not be used for more than four weeks or to prepare the instrument for a move.

Required Supplies for All Chemistry Options

Before you begin, have clean, empty bottles on hand for all reagent positions, protective gloves, a 41-mL reaction vessel (RV) with a resin sampling line, and a metering vessel. If using old bottles, rinse them with DCM followed by MeOH and allow the bottles to dry prior to use.

WARNING	CHEMICAL HAZARD. <i>Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING	CHEMICAL HAZARD. <i>Methanol (MeOH) is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING	CHEMICAL HAZARD. <i>N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING ***PHYSICAL AND CHEMICAL HAZARD. Chemicals reduce the integrity of glass bottles. Re-used bottles are more susceptible to fractures and shattering under pressure. Replace re-used bottles every six weeks. For more information about the chemicals used on the ABI 433A Peptide Synthesizer, refer to the Material Safety Data Sheets.***

Check each bottle currently installed on your instrument. Replace any bottles that have been re-used and in use for more than six weeks. When in doubt, replace the bottle.

Reagents for All Chemistry Options

- DCM (P/N 400142)
- MeOH (P/N 400470)
- NMP (P/N 400580)

ABI 433A Instrument Shutdown Procedure for Fmoc/HOBt/DCC Chemistry

<i>Note</i>	<i>Steps 1 and 2 below are provided in case resin sampling was used on the ABI 433A instrument. If resin sampling was not used, proceed with step 3.</i>

WARNING	CHEMICAL HAZARD. Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING	CHEMICAL HAZARD. N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER!	CHEMICAL HAZARD. Trifluoroacetic acid (TFA) causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To shut down the instrument for Fmoc/HOBt/DCC chemistry:

1. Put new reaction vessel filters into the 41-mL, resin-sampling RV, fit the RV in place on the instrument, attach the resin sampler line, and place a test tube in the fraction collector.
2. Run Flow Test 19 twice to flush the reaction vessel and the resin sampler with NMP.
3. Remove the RV and place a metering vessel on the instrument. Have an empty beaker ready to collect NMP rinses.
4. Flush lines 1, 7, and 8 with NMP.
 - a. Ensure that the manual pressure/vent switch for Bottle 10 is in the up position to pressurize the NMP bottle(s).
 - b. Go to the Manual Control menu and open valve 20.

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- c. **Bottle 1:** Remove Bottle 1 and wipe the delivery line with a lint-free tissue. Place an empty beaker at position 1. In the Manual Control menu, open valve 21 for 30 seconds and catch the rinse. After 30 seconds, turn valve 21 off, but leave valve 20 on.
 - d. **Bottle 7:** Remove Bottle 7 and wipe the delivery line with a lint-free tissue. Place an empty beaker at position 7. In the Manual Control menu, open valves 13, 16, and 31 for 30 seconds, then turn off valve 31, but leave valves 13, 16, and 20 on.
 - e. **Bottle 8:** Remove Bottle 8 and wipe the delivery line with a lint-free tissue. Place an empty beaker at position 8. In the Manual Control menu, open valve 32 for 30 seconds, then press **all off** to close all valves.
 5. Flush lines 1, 7, and 8 with DCM. Have an empty beaker ready to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the DCM bottle(s).
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 1:** Place the empty beaker at position 1. In the Manual Control menu, open valve 21 for 30 seconds and catch the rinse. After 30 seconds, turn valve 21 off, but leave valve 18 on.
 - d. **Bottle 7:** Place the empty beaker at position 7. In the Manual Control menu, open valves 13, 16, and 31 for 30 seconds, then turn valve 31 off, but leave valves 13, 16, and 18 on.
 - e. **Bottle 8:** Place the empty beaker at position 8. In the Manual Control menu, open valve 32 for 30 seconds, then press **all off** to close all valves.
 6. Flush lines 2, 4, 5, and 6 with DCM. Have an empty beaker ready to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the DCM bottle(s),
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 2:** Remove Bottle 2, cap securely and set aside. In bottle position 2, install an empty bottle containing the TFA bottle seal. In the Manual Control menu, open valves 26, 7, and 16 for 30 seconds, then turn valve 26 and 7 off, but leave valves 16 and 18 on. Remove the bottle from position 2.
 - d. **Bottle 4:** Remove Bottle 4 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 4. In the Manual Control menu, open valve 14 for 30 seconds, then turn valve 14 off, but leave valves 16 and 18 on.

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- e. **Bottle 5:** Remove Bottle 5 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 5. In the Manual Control menu, open valve 15 for 30 seconds, then turn off valves 15 and 16, but leave valve 18 on,
 - f. **Bottle 6:** Remove Bottle 6 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 6. In the Manual Control menu, open valve 19 for 30 seconds, then press **all off** to close all the valves.
7. Flush all delivery lines with nitrogen. Open valve 17, and then repeat steps 5c to 5e and steps 6c to 6f.

<i>Note</i>	<i>When you repeat steps 5c to 5e and steps 6c to 6f, keep valve 17 open even though the printed procedure instructs you to press all off.</i>

8. Put clean, empty storage bottles in place at positions 1, 2, 4, 5, 6, 7, and 8.
9. Flush delivery lines for the positions 9 and 10.
 - a. Ensure that the manual pressure/vent switches are in the down position to vent the bottles. Unscrew the bottle cap assemblies.
 - b. Place the delivery lines with sintered filters for positions 9 and 10 into a clean waste beaker.
 - c. Open valves 17, 18, and 20 for 30 seconds, then press **all off**.
 - d. If the instrument is being moved, remove the tube assemblies for positions 9 and 10 from the instruments and put blank plugs into the holes in the manifold.

ABI 433A Instrument Shutdown Procedure for *FastMoc* Chemistry

If you are using resin sampling, follow steps 1-2 of the ABI 433A Instrument Shutdown Procedure for Fmoc/HOBt/DCC Chemistry on page 2-55. Continue with the following steps.

If you are not using resin sampling, proceed directly to the following procedure.

WARNING **CHEMICAL HAZARD.** *Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! **CHEMICAL HAZARD.** *Trifluoroacetic acid (TFA) causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

To shut down the instrument for *FastMoc* chemistry:

1. Flush lines 1, 5, and 8 with NMP. Have an empty beaker ready to collect NMP rinses.
 - a. Ensure that the pressure/vent switch for bottle 10 is in the up position to pressurize the NMP bottle(s).
 - b. Go to the Manual Control menu and open valve 20.
 - c. **Bottle 1:** Remove Bottle 1 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 1. In the Manual Control menu, open valve 21 for 30 seconds, then turn valve 21 off, but leave valve 20 on.

-
- d. **Bottle 5:** Remove Bottle 5 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 5. In the Manual Control menu, open valves 15 and 16 for 30 seconds, then turn valve 15 off, but leave valves 16 and 20 on.
 - e. **Bottle 8:** Remove Bottle 8 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 8. In the Manual Control menu, open valves 13 and 32 for 30 seconds, then press **all off** to close all valves.
 2. Flush lines 1, 5, and 8 with DCM. Have an empty beaker ready to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the bottle(s).
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 1:** Place the empty beaker at position 1. In the Manual Control menu, open valve 21 for 30 seconds, then turn valve 21 off, but leave valve 18 on.
 - d. **Bottle 5:** Place the empty beaker at position 5. In the Manual Control menu, open valves 15 and 16 for 30 seconds, then turn valve 15 off, but leave valves 16 and 18 on.
 - e. **Bottle 8:** Place the empty beaker at position 8. In the Manual Control menu, open valves 13 and 32 for 30 seconds, press **all off** to close all valves.
 3. Flush lines 2, 4, 6, and 7 with DCM. Have an empty beaker ready to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the DCM bottle(s).
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 2:** Remove Bottle 2, cap securely and set aside. In bottle position 2, install an empty bottle containing the TFA bottle seal. In the Manual Control menu, open valves 26, 7, and 16 for 30 seconds, then turn valve 26 and 7 off, but leave valves 16 and 18 on. Remove the bottle from position 2.
 - d. **Bottle 4:** Remove Bottle 4 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 4. In the Manual Control menu, open valve 14 for 30 seconds, then turn off valves 14 and 16 only, and leave valve 18 on.
 - e. **Bottle 6:** Remove Bottle 6 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 6. In the Manual Control menu, open valve 19 for 30 seconds, then turn off valve 19, but leave valve 18 on.

-
- f. **Bottle 7:** Remove Bottle 7 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 7. In the Manual Control menu, open valves 13, 16, and 31 for 30 seconds, then press **all off** to close all valves.
 4. Follow steps 8 - 9 of the ABI 433A Instrument Shutdown Procedure for Fmoc/HOBt/DCC Chemistry on page 2-57.

ABI 433A Instrument Shutdown Procedure for Boc/HOBt/DCC Chemistry

If you are using resin sampling, follow steps 1-2 of the ABI 433A Instrument Shutdown Procedure for Fmoc/HOBt/DCC Chemistry on page 2-55. Continue with the following steps.

If you are not using resin sampling, proceed directly to the following procedure.

WARNING **CHEMICAL HAZARD.** *Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! **CHEMICAL HAZARD.** *Trifluoroacetic acid (TFA) causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

To shut down the instrument for Boc/HOBt/DCC Chemistry:

1. Flush lines 1, 2, 4, 5, and 6 with DCM. Have an empty beaker on hand to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the DCM bottle(s).
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 1:** Remove Bottle 1 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 1. In the Manual Control menu, open valve 21 for 30 seconds, then turn off valve 21, but leave valve 18 on.

-
- d. **Bottle 2:**

Remove Bottle 2, cap securely and set aside. In bottle position 2, install an empty bottle containing the TFA bottle seal. In the Manual Control menu, open valves 26, 7, and 16 for 30 seconds, then turn valve 26 and 7 off, but leave valves 16 and 18 on. Remove the bottle from position 2.
 - e. **Bottle 4:** Remove Bottle 4 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 4. In the Manual Control menu, open valve 14 for 30 seconds, then turn valve 14 off, but leave valves 16 and 18 on.
 - f. **Bottle 5:** Remove Bottle 5 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 5. In the Manual Control menu, open valve 15 for 30 seconds, then turn off valves 15 and 16, but leave valve 18 on.
 - g. **Bottle 6:** Remove Bottle 6 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 6. In the Manual Control menu, open valve 19 for 30 seconds, then press **all off** to close all the valves.
2. Flush lines 7 and 8 with NMP. Have an empty beaker ready to collect NMP rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 10 is in the up position to pressurize the NMP bottle(s).
 - b. Go to the Manual Control menu and open valve 20.
 - c. **Bottle 7:** Remove Bottle 7 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 7. In the Manual Control menu, open valves 13, 16, and 31 for 30 seconds, then turn off valve 31, but leave valves 13, 16, and 20 on.
 - d. **Bottle 8:** Remove Bottle 8 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 8. In the Manual Control menu, open valve 32 for 30 seconds, then press **all off** to close all the valves.
 3. Flush lines 7 and 8 with DCM. Have an empty beaker ready to collect DCM rinses.
 - a. Ensure that the manual pressure/vent switch for Bottle 9 is in the up position to pressurize the DCM bottle.
 - b. Go to the Manual Control menu and open valve 18.
 - c. **Bottle 7:** Remove Bottle 7 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 7. In the Manual Control menu, open valves 13, 16, and 31 for 30 seconds, then turn off valve 31, but leave valves 13, 16, and 18 on.

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- d. **Bottle 8:** Remove Bottle 8 and wipe the delivery line with a lint-free tissue. Place the empty beaker at position 8. In the Manual Control menu, open valve 32 for 30 seconds, then press **all off** to close all the valves.
 4. Follow steps 8 - 9 of the ABI 433A Instrument Shutdown Procedure for Fmoc/HOBt/DCC Chemistry on page 2-57.



3 Chemistry

Since its introduction in 1963 by Bruce Merrifield, solid-phase peptide synthesis (SPPS) has become the most successful method for synthesizing peptides. SPPS generally involves five steps:

1. Chain assembly
2. Cleavage from resin and removal of side-chain protecting groups
3. Purification
4. Additional chemical modification
5. Characterization

This chapter provides a basic introduction to the general strategy of chain assembly. More advanced information on all aspects of SPPS can be found in the books and reviews on peptide synthesis, references 1–4, listed at the end of this section.

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Fmoc Chemistry	3-11
Boc Chemistry	3-17
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A General Description of the Synthesis Reaction

Chain Assembly

The two most common strategies for SPPS chain assembly are based on the Boc and Fmoc groups (Figure 3-1).

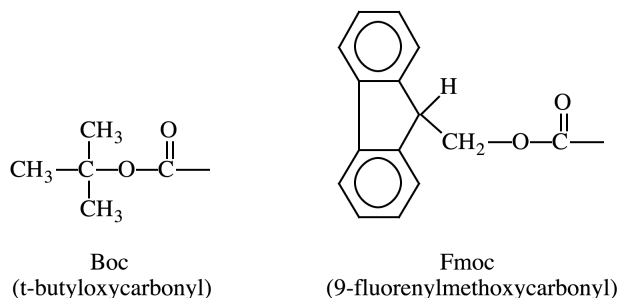


Figure 3-1. Chemical structures of Boc and Fmoc protecting groups

Note In this section of the guide, Fmoc strategy refers to any synthesis chemistry that uses Fmoc protecting groups, including FastMoc™ and Fmoc/HOBt/DCC chemistries.

As shown in Figure 3-2, the Fmoc and Boc groups protect the α-amino group of an amino acid. The ABI 433A instrument can use either the Fmoc or Boc strategy for automated peptide chain assembly.

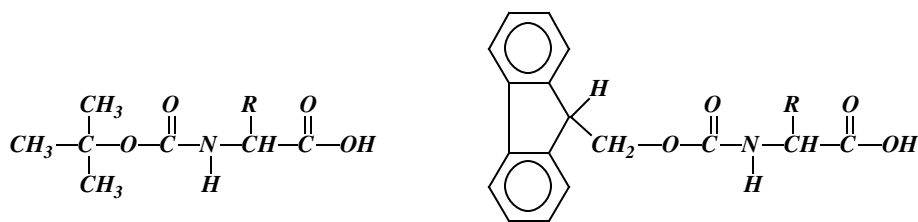


Figure 3-2. Chemical structure of Boc and Fmoc protected amino acids

The peptide is assembled from the C-terminal towards the N-terminal with the α-carboxyl group of the amino acid attached to a solid support, as shown in Figure 3-3.

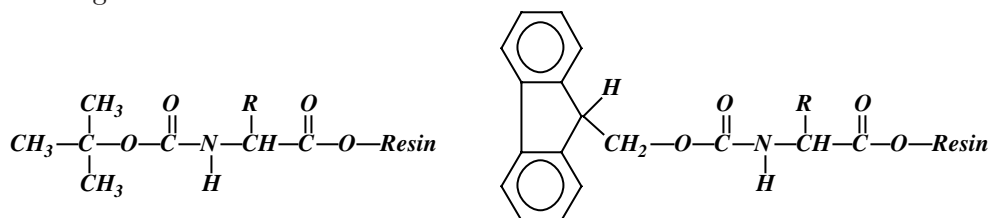
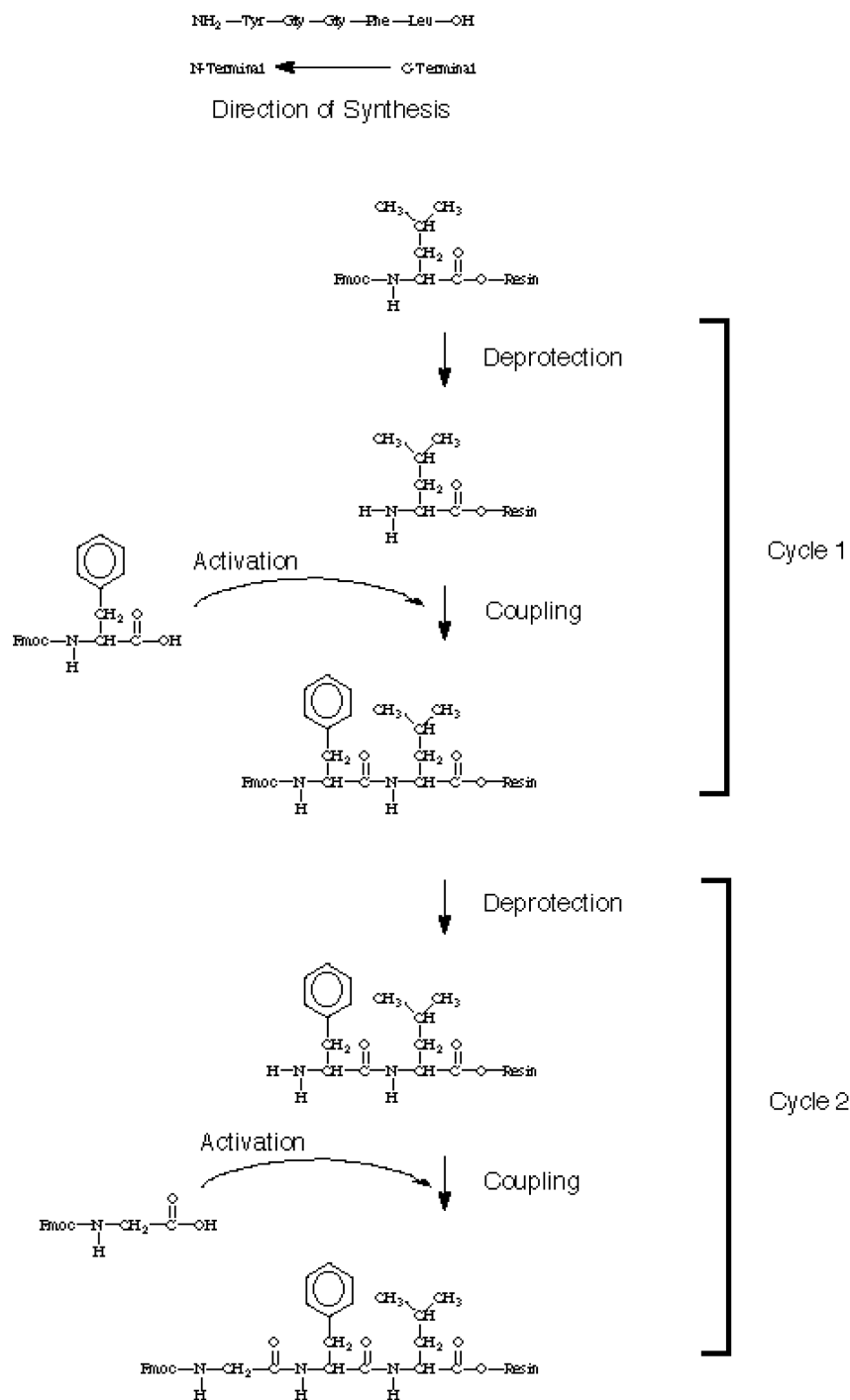


Figure 3-3. Protected amino acids attached to resin

The first step in chain assembly is deprotection, or removal of the protecting group. The Fmoc protecting group is removed by a base, usually piperidine, and the Boc protecting group is removed by an acid, usually TFA.



After deprotection, the next amino acid is coupled to the deprotected amino end of the growing peptide, forming a peptide bond. Chain assembly is thus a series of deprotections and couplings.

Figure 3-4 illustrates the first two cycles of the chain assembly of [Leu⁵]-Enkephalin. For simplification, the α -amino protecting group is shown as “**Fmoc**” and the solid support is shown as “**Resin**.” Note that synthesis begins with the C-terminal of leucine attached to the support.

Solid Support

The resin used on the ABI 433A instrument is a polystyrene bead with 1% divinyl-benzene, a cross-linking agent. The dry resin beads are roughly 40 to 100 microns in diameter. Depending on the type of resin used (see page 3-14 and page 3-20), either 200-400 mesh (38-75 μ) or 100-200 mesh (75-150 μ) resin beads can be used in the reaction vessel. When in contact with solvents such as DCM, DMF, or NMP, the beads swell to approximately 10 times their dry volume. Macroscopically, the resin appears as an insoluble solid support. On the molecular level, however, the resin is “in solution” or fully solvated. This solvation enhances coupling of the peptide resin with the protected amino acids.

Conductivity Monitoring

As the peptide grows within the solvated gel matrix, its sequence influences the conformation and physico-chemical behavior of the resin beads. The conformation of the peptide resin may affect the chemical reactivity of the synthesis through the formation of inter- and intra-chain interactions.^{5,6,7,8} Some peptide resin structures may “bury” the growing N-terminus, thus decreasing reactivity. Inter-chain interactions may increase the effective cross-linking of the matrix, causing the structure to collapse and reducing the diffusion rates through the gel matrix. Currently, we cannot predict when these sequence-dependent phenomena will occur.

The quantitative ninhydrin assay monitors the efficiency of coupling during solid-phase synthesis. However, this assay is “off-line” and cannot offer real-time feedback. The ABI 433A Peptide Synthesizer supports automated conductimetric monitoring of the peptide resin during Fmoc chemistry, with feedback. Monitoring with feedback allows modification of synthesis cycles in progress to accommodate diffusion rate changes in the solvated gel matrix. Channels 2 and 3 are also available for other monitoring options.

Conductivity monitoring works on the principle that in each synthesis cycle the Fmoc group is first removed by treatment with piperidine in NMP, generating a conductive carbamate salt (see Figure 3-5). The extent of deprotection is determined by comparing the conductivity of two samples of deprotection solution. For example, in one algorithm, if the user sets a 4% value for the deprotection loop, the deprotection loop continues until the last conductivity value is within 4% of the previous value.

In a second algorithm, the extent of deprotection is determined by comparing the conductivity of the last sample to that of the first sample in the current cycle. With this algorithm, if the user sets a four percent value for the deprotection loop, the loop continues until the last conductivity value is $\leq 4\%$ the first conductivity value.

Changes in the state of the peptide resin, following the coupling of a particular amino acid residue and a subsequent inefficient deprotection, have also been reflected in the post-coupling washing loop. Conductivity monitoring can also be used for feedback monitoring of the post-coupling.

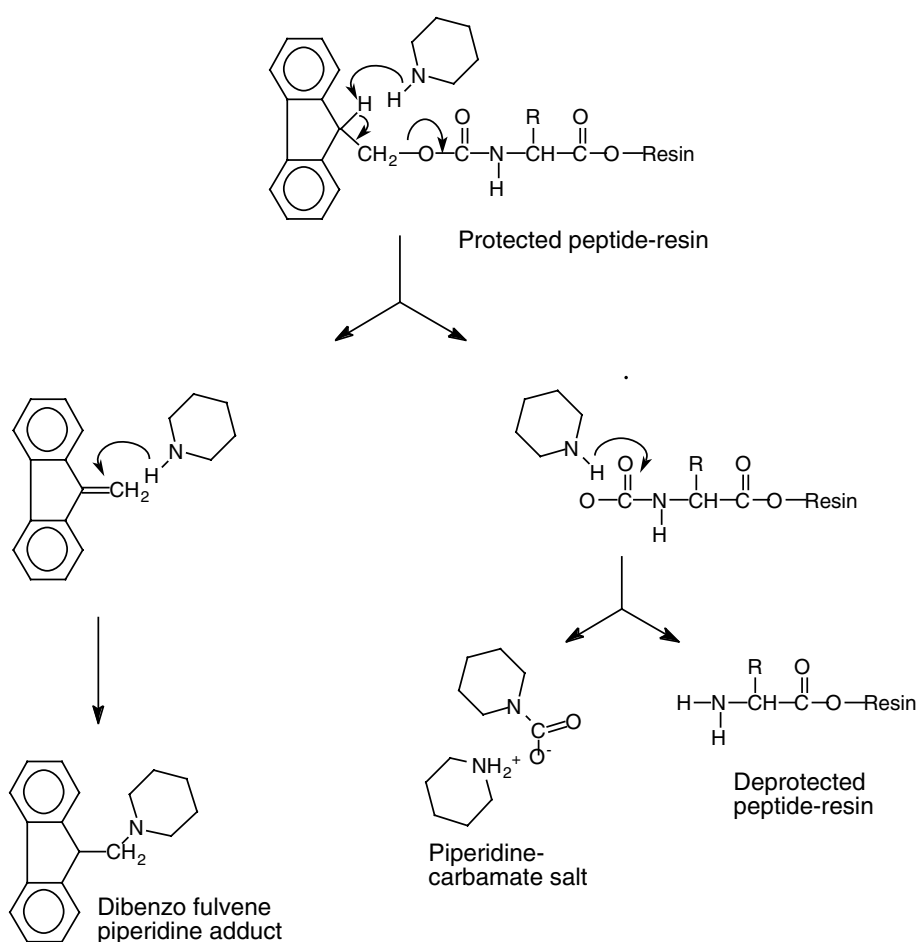


Figure 3-5. Formation of carbamate salt during Fmoc deprotection

Activation

Before coupling, the carboxyl group of the amino acid must be “activated.” Four of the numerous methods of activating the carboxyl group are discussed here:

- HBTU–*FastMoc*
- HOBT/DCC
- Conventional DCC
- Symmetric Anhydride

HBTU Activation

HBTU [2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] is an activating reagent (Figure 3-6) originally developed by Dourtoglou et al.,⁹ and later described by Knorr et al.^{10, 11} *FastMoc*TM is the name Applied Biosystems has given to the combination of HBTU activation with the Fmoc/NMP chemistry, because cycle times with HBTU can be much faster than the HOBT/DCC activation method.

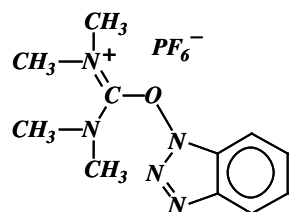


Figure 3-6. HBTU

In the *FastMoc*TM chemistry procedure, HBTU is dissolved in a solution of HOBT, DIEA, and DMF. The amino acid is dissolved in this solution with additional NMP. Activated Fmoc amino acid is formed almost instantaneously and transferred directly to the reaction vessel. Typical results¹² obtained with *FastMoc* cycles have been demonstrated by Fields et al.

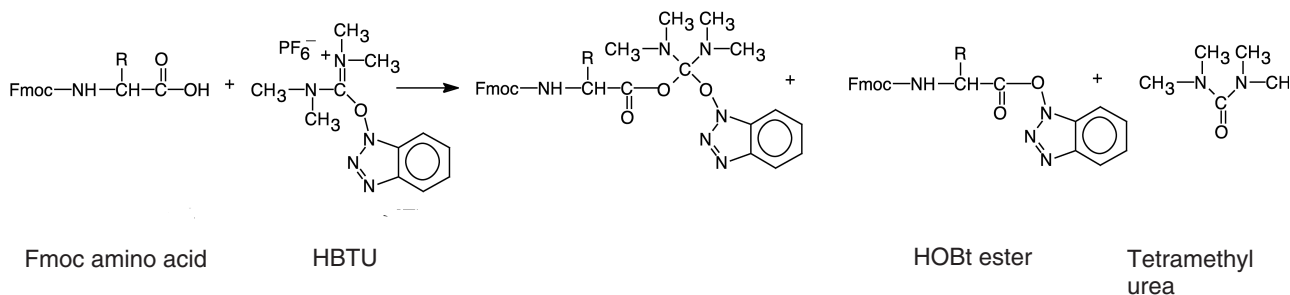


Figure 3-7. HBTU activation, proposed by Henklein et al.¹³

HOBt/DCC Activation

The HOBt (1-hydroxybenzotriazole)/DCC activation method, which produces an active ester of the amino acid, was developed by König and Geiger.¹⁴ Because both the conventional DCC and the symmetric anhydride methods appear to cause dehydration of amides to nitriles, HOBt activation is especially useful for activating unprotected asparagine and glutamine. HOBt activation is also the method of choice for arginine because it appears that the standard DCC or symmetric anhydride methods cause the formation of a lactam.

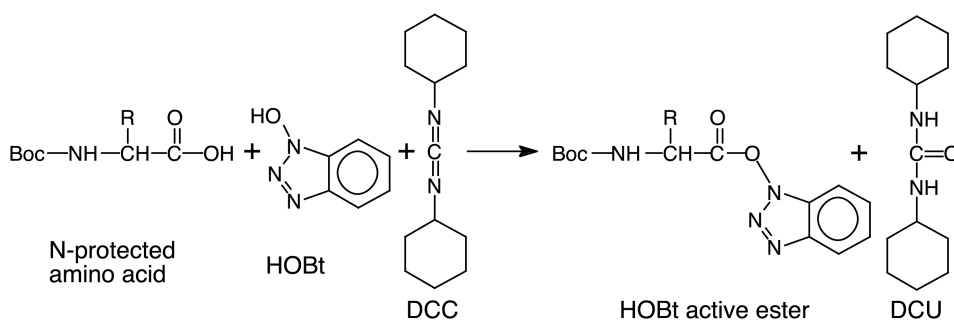


Figure 3-8. HOBt/DCC activation

Activation methods that use N, N'-dicyclohexylcarbodiimide (DCC) produce N, N'-dicyclohexylurea (DCU), a highly insoluble compound. However, DCU is easily separated and removed from the activated amino acid derivative when the solution is transferred from the ACT to the RV.

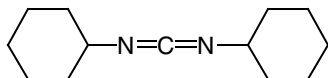


Figure 3-9. Chemical structure of DCC

Symmetric Anhydride Activation

The symmetric anhydride activation method uses two equivalents of amino acid to one equivalent of DCC. When this activation occurs, the protected amino acid and DCC react to form a symmetric anhydride and DCU precipitate. The precipitate is removed before the symmetric anhydride solution is added to the peptide resin. With the Fmoc/HOBt/DCC strategy, the loading cycle uses symmetric anhydride activation.

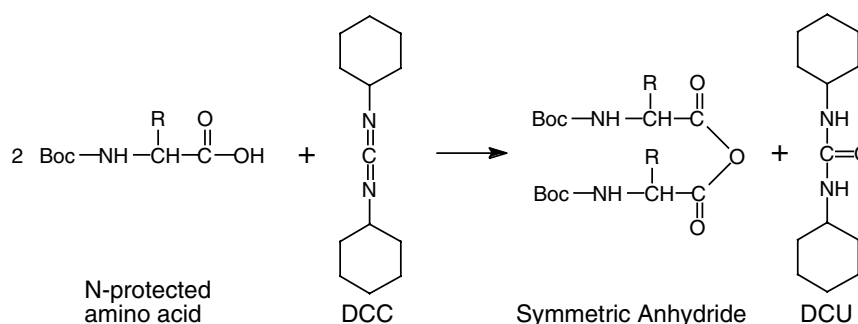


Figure 3-10. Symmetric anhydride activation

Conventional DCC Activation

The conventional DCC activation method uses one equivalent of amino acid with one equivalent of DCC. It also uses a high ratio of DCM to other solvents. This type of activation is efficient for loading the first amino acid on the HMP resin (see page 3-14) with DMAP catalysis. The *FastMoc* loading cycles use conventional DCC activation followed by a capping step with benzoic anhydride.

This method of activation is not used on the ABI 433A instrument for chain assembly because some peptide sequences are not well solvated in DCM. Another disadvantage of conventional DCC activation for chain assembly is that the activated species, O-acylisourea, can rearrange to form unreactive N-acylurea, which results in a loss of activated species. O-acylisourea readily reacts with other N-protected amino acids to give a symmetric anhydride and DCU.

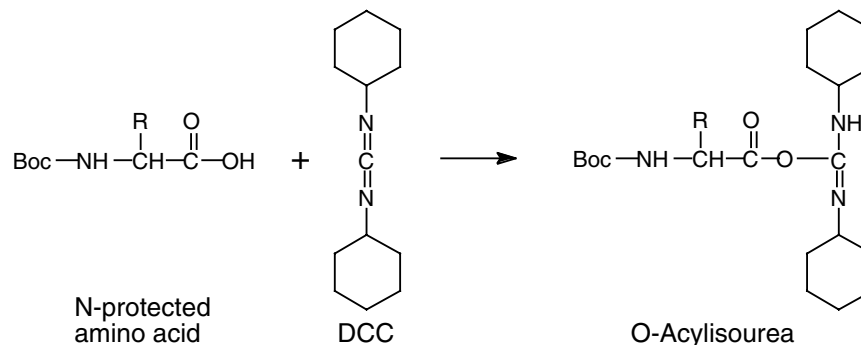


Figure 3-11. Conventional DCC activation

Coupling

In the coupling step, the activated amino acid reacts with the deprotected amino terminal of the growing peptide chain, as shown in Figure 3-12. In this example, the Fmoc derivative is used.

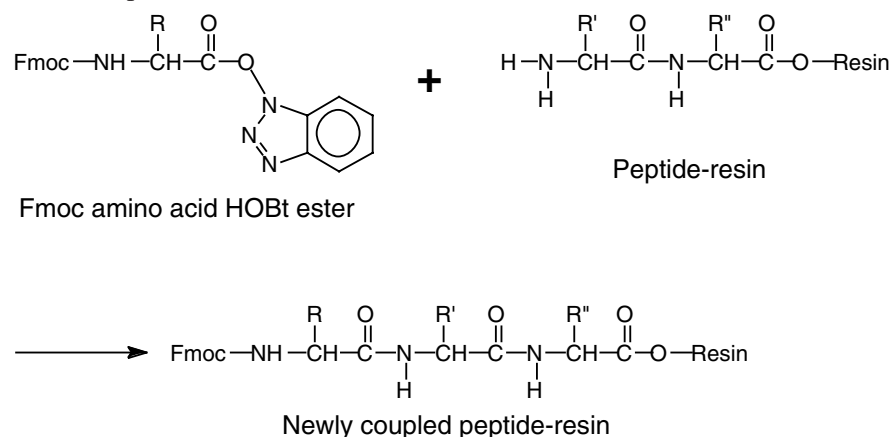


Figure 3-12. Fmoc coupling

With the Fmoc 0.25 mmol cycles, four equivalents of the activated amino acid are added per one equivalent of the growing peptide chain. The objective is to obtain the highest coupling efficiency possible on every step, preferably above 99.0%.

Many couplings in SPPS are above 99.0% efficiency after just a few minutes of reaction, especially for the first few cycles in a chain assembly. Because some coupling reactions are slower, however, coupling reactions in SPPS may last from 10 minutes to two hours.

Even after two hours, a few couplings may have a low efficiency. Low coupling yields seem to correlate with poor solvation of the peptide resin. In most cases, the sequence of the peptide appears to affect coupling efficiency.

One explanation for sequence-dependent coupling problems is the formation of peptide-peptide hydrogen bonds. These hydrogen bonds trigger the formation of peptide aggregates which then block the interaction of the deprotected amino-terminal with activated amino acid.

Therefore, one objective in the coupling step in SPPS is to maximize the peptide resin solvation and minimize peptide-peptide hydrogen bonding. The following are some of the many approaches that have been tried:

- Coupling in a polar, aprotic solvent like dimethylformamide (DMF).
- Adding trifluoroethanol (TFE) to a coupling in dichloromethane (DCM).¹⁵
- Symmetric anhydride coupling in N-methylpyrrolidone (NMP) at an elevated temperature.¹⁶

- Adding dimethylsulfoxide (DMSO) to a DCM coupling.¹⁷
- Adding a base at the end of the coupling, for example, N-methylmorpholine, triethylamine, or diisopropylethylamine (DIEA).¹⁵

DMF (Figure 3-13) appears to correct many sequence-dependent coupling problems and is often used with preformed symmetric anhydrides. Both DMF and N-methylpyrrolidone (NMP [Figure 3-13]) are polar, aprotic, hydrogen-bonding solvents commonly used in SPPS because of their ability to solvate peptides.

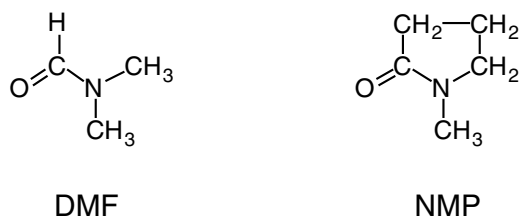


Figure 3-13. Structure of DMF and NMP

To evaluate the solvating ability of several solvents, a study that measured their relative ability to swell peptide resins was performed.¹⁸ The results showed that NMP and DMSO, in general, solvate the side chains of the peptide more thoroughly than others and reduce more peptide-peptide hydrogen bonds. Of course, the σ_2 of a peptide resin varies depending on the length and sequence of the attached peptide.

One of the methods used to measure the coupling efficiency in SPPS is quantitative ninhydrin monitoring. This method was developed for SPPS by Kaiser¹⁹ and was further refined by Sarin et al.²⁰ with the quantitative ninhydrin monitoring procedure.

In order to perform quantitative ninhydrin monitoring, 2 to 10 mg of peptide resin is needed. On the ABI 433A Peptide Synthesizer, a small portion of the resin can be removed automatically by the resin sampler. Refer to Appendix B, *Post-Synthesis Procedures*, for a quantitative ninhydrin procedure.

The quantitative ninhydrin monitoring method described by Sarin is for Boc chain assemblies on PAM resins. However, with two modifications, this method is equally useful for monitoring Fmoc chain assemblies. First, add 2–3 drops of glacial acetic acid to the methanol in the resin sample tube to prevent partial deprotection of the Fmoc group by amines in the NMP. Second, only 5 min of heating is necessary with Fmoc resins because the heating with pyridine initiates the slow removal of the Fmoc group.

Fmoc Chemistry

The use of the Fmoc group (Figure 3-1) as an α -amino protecting group has grown significantly since its introduction by Carpino and Han.^{21, 22} Fmoc methodology adapts to a wide selection of linkers, resins, and cleavage chemistries.

The general Fmoc chemistry protocol for SPPS is illustrated in Figure 3-14. The α -amino protecting group of the amino acid, an Fmoc group (Figure 3-1), is removed at the beginning of every cycle by a secondary amine, typically 20% piperidine.^{23, 24} After deprotection, the resin is washed with NMP to remove the piperidine. The peptide resin is then ready for coupling.

Before coupling, the carboxyl group of the amino acid must be activated. With the ABI 433A Peptide Synthesizer, two different types of activation are available for Fmoc chemistry: HBTU or HOBt/DCC. Activation with HBTU is also known as *FastMoc* chemistry.

In the coupling step, the activated Fmoc amino acid reacts with the amino-terminal of the growing peptide chain to form a peptide bond. In the Fmoc/HOBt/DCC 0.25 mmol cycles on the ABI 433A instrument, four equivalents of the activated amino acid are added to each one equivalent of the growing peptide chain.

When coupling is complete, the resin is washed with NMP. At this point, a resin sample can be taken. Deprotection and coupling steps are repeated with each subsequent amino acid until the chain assembly has been completed.

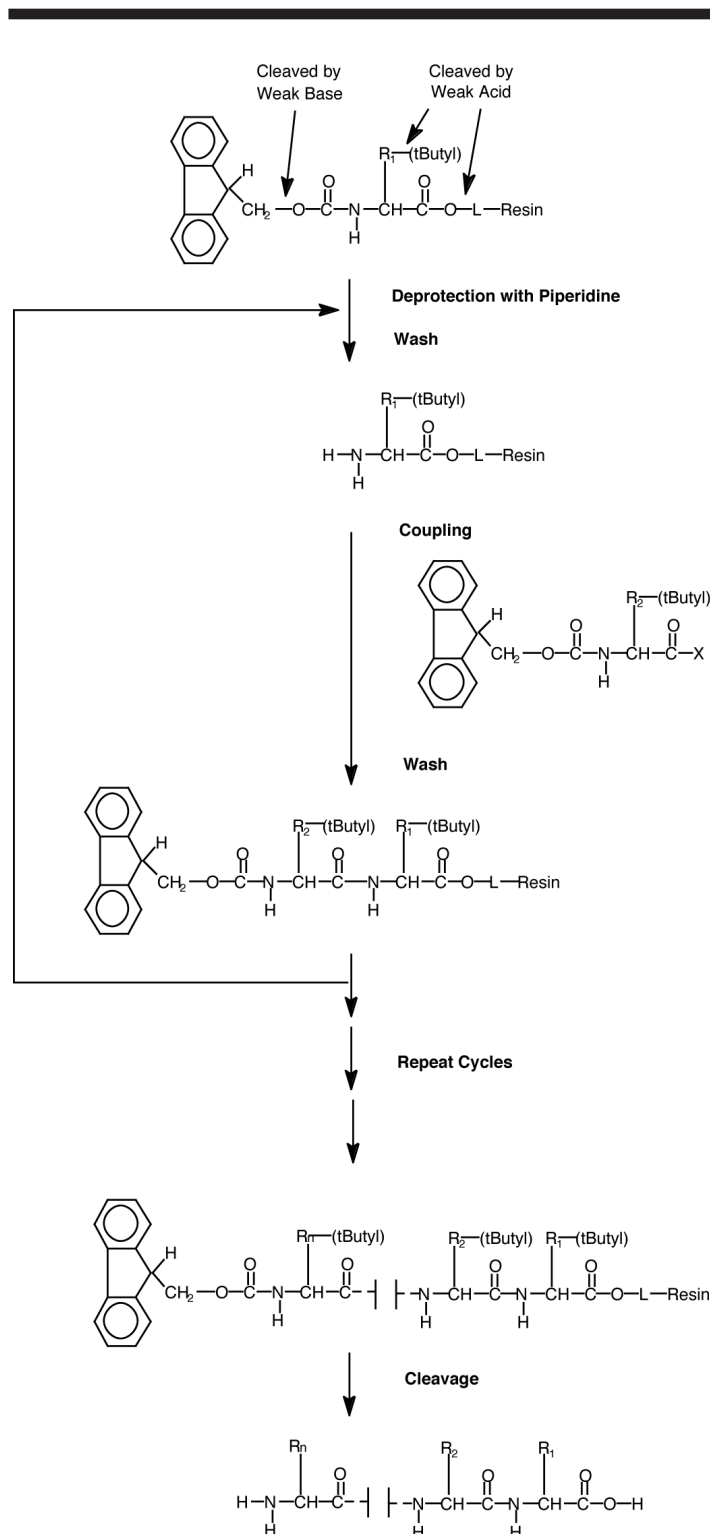


Figure 3-14. General Protocol Fmoc Chemistry using HOBt esters. "L" represents the linker between the peptide and the resin. "X" represents the active ester portion of the amino acid.

FastMoc Protocol

In the *FastMoc* cycles, amino acids are activated with HBTU (see Figure 3-7 on page 3-6). In the activation step of 0.25 mmol and 0.10 mmol *FastMoc* cycles, 1.0 mmol of dry protected amino acid in a cartridge is dissolved in a solution of HBTU, DIEA, and HOBt in DMF with additional NMP added. The *FastMoc* 1.0 mmol cycles use 3 one-millimole cartridges, or a total of 3 mmol Fmoc amino acid. The activated Fmoc amino acid is formed almost instantaneously and the solution is transferred directly to the reaction vessel. Table 3-1 summarizes chain assembly times with the *FastMoc* protocol.

Table 3-1. ABI 433A Instrument *FastMoc* Chain Assembly Time[†] (min)

Operation and Reagents	1.0 mmol	0.25 mmol	0.10 mmol
Deprotection	29	15	10
Washes with NMP	12	5	3
Coupling	20	21	9
Washes with NMP	9	4	2
Resin sample (optional)	(2)	(2)	(2)
Total Time (without resin sample)	70*	45*	24*

[†] Conductivity monitoring with feedback may change cycle times
 *Resin sampling adds 2 more minutes to the total times.

Fmoc/HOBt/DCC Protocol

Activation by HOBt/DCC with the resulting activated carboxylic acid derivatives is illustrated in Figure 3-15.

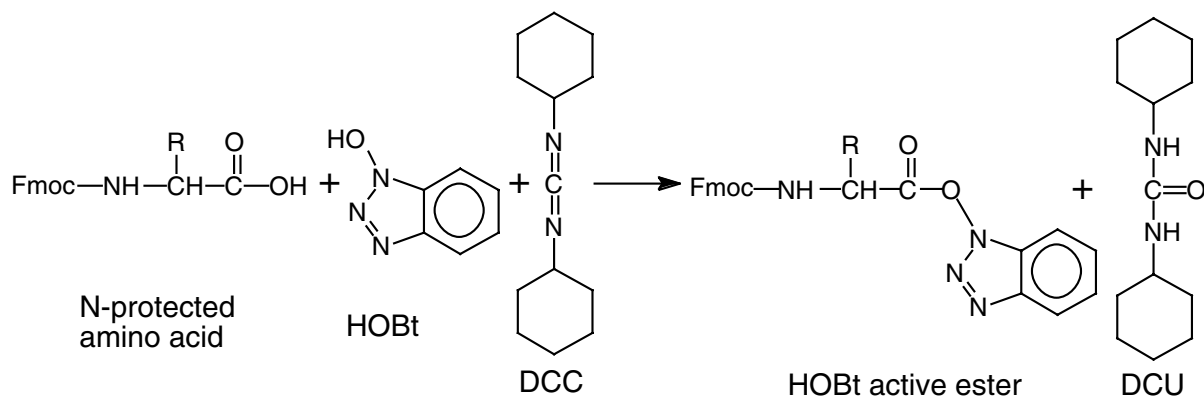


Figure 3-15. Activation using HOBt/DCC

In the activation step, 1.0 mmol of dry, protected amino acid in a cartridge is dissolved with a solution of NMP and 1 mL of 1M HOBt in NMP. This solution is then transferred to the activator vessel (ACT) where 1 mL of 1 M DCC in NMP is added. The 0.5 mL measuring loop controls these

calibrated deliveries. After approximately 40 to 50 minutes of activation, the HOBt active ester is transferred to the RV while the DCU precipitate remains in the activator vessel.

Table 3-2 summarizes the ABI 433A Peptide Synthesizer protocol for chain assembly with Fmoc chemistry. The Fmoc/HOBt/DCC 0.25 mmol cycles use 1 mmol Fmoc-amino acid, HOBt-active ester with 0.25 mmol resin. Each 0.25 mmol cycle lasts 108 minutes. The Fmoc/HOBt/DCC 0.10 mmol cycles use 1 mmol Fmoc-amino acid, HOBt-active ester with 0.10 mmol resin. Each 0.10 mmol cycle lasts 60 minutes. Because the ABI 433A instrument can be programmed by the user, the chemistry is easily modified.

Table 3-2. ABI 433A Instrument Fmoc/HOBt/DCC Chain Assembly Time (min)

Operation and Reagents	0.25 mmol	0.10 mmol
Deprotection (20% Piperidine in NMP)	21	14.0
Washes with NMP	9	4.5
Coupling Fmoc-AA-HOBt ester in NMP	71	37.0
Washes with NMP	7	4.5
Resin sample (optional)	(2)	(2.0)
Total Time (without resin sample)	108	60.0

Fmoc Resins

This section discusses three kinds of resins that can be used with the Fmoc protocol on the ABI 433A Peptide Synthesizer. Each resin provides a unique feature to the final product:

- HMP resins—a carboxylic acid terminal peptide
- Amide resins—an amide terminal peptide
- Super acid-labile resins—protected side-chains with a carboxylic acid terminal peptide

HMP Resins

Applied Biosystems supplies the HMP resin (4-hydroxymethyl-phenoxy-methyl-copolystyrene-1% divinylbenzene resin) developed by Wang²⁵ (Figure 3-16). HMP resin is also known as Wang resin or p-alkoxybenzyl alcohol resin. After cleavage with 95% TFA, the final peptide has a free carboxylic acid terminal.

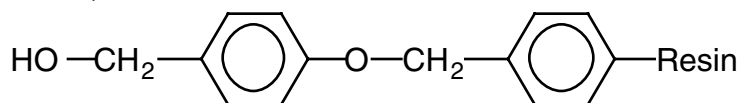


Figure 3-16. HMP resin

You may use either preloaded or unloaded HMP resins. The preloaded form already has the first amino acid attached to the resin (Figure 3-17).

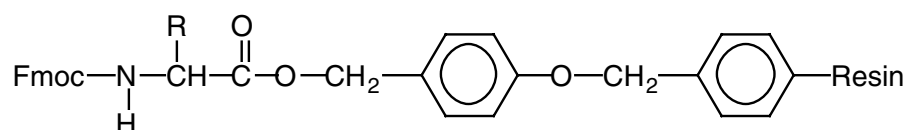


Figure 3-17. Preloaded HMP resin. 'R' refers to the amino acid side chain

With unloaded HMP resin, the first amino acid can be coupled to the HMP resin by using DCC with DMAP catalysis and the loading cycles on the instrument. The standard loading cycles can be used for all the Fmoc amino acids except Arg, Asn, His and Gln.

To load Arg, Asn, Gln or His requires minor changes to module **h**. These variations are described in "Advanced Operations" on page 7-1. Because the carboxamide side chain of Fmoc-Asn and Fmoc-Gln dehydrates during DCC activation, many peptide chemists prefer to use a protected derivative such as Fmoc-Asn(Trt).²⁶ Another method of making Asn and Gln C-terminal peptide is discussed in the following section on amide resins.

An unusual situation occurs with Fmoc-Pro. Although Fmoc-Pro can be loaded to HMP resin quantitatively, a potential diketopiperazine side reaction occurs during the chain assembly which can drastically reduce the yield of final peptide resin.²⁷

Because the loading may not be 100% complete, the loading cycle should be followed by a capping cycle, especially when using HBTU activation. Capping can be accomplished by using acetic anhydride or benzoic anhydride with DMAP catalysis.

Amide Resins

With the Fmoc procedure, it is possible to make N-terminal amide peptides by using a modified benzhydrylamine resin that contains ortho- and para-electron donating methoxy groups.^{28, 29, 30} These resins usually have the amine protected with the Fmoc group. To remove the Fmoc group with piperidine/NMP at the start of the synthesis, use the ABI 433A instrument cycles that are designed for use with preloaded resins.

IMPORTANT *Even though these amide resins do not have the first amino acid already attached, use them with the cycles designed for pre-loaded resins. **DO NOT** use amide resins with the loading cycle, *h e f*.*

TFA cleavage removes the amide peptide from the resin. The exact protocol varies according to the resin used.

A clever use of these Fmoc amide resins employs Fmoc-Asp(α -OtBu) and Fmoc-Glu(α -OtBu) attached to resin by the COOH side-chain group. The final product after chain assembly is completed has a C-terminal asparagine or glutamine.^{31, 32}

Substitution Determination

The substitution value of the Fmoc-AA resin, expressed in mmol/gram, is usually printed on the reagent bottle. If you are using unloaded HMP resin to start the synthesis, you can calculate the new substitution on SynthAssist® Software or refer to the description of post-synthesis calculations found in Appendix B, “Post-Synthesis Procedures” in Volume 2.

A convenient way to confirm the substitution of an Fmoc-AA resin at any point in the synthesis procedure uses the absorbance of N-(9-fluorenylmethyl) piperidine at 301 nm ($\epsilon=7800$).³⁴ Accurately weigh 4 to 8 mg of resin into a test tube and treat it with 0.5 mL 20% piperidine in DMF, as described in the following example.

Example:

Into a test tube containing 5.05 mg of Fmoc-Gly resin, add 0.5 mL 20% piperidine in DMF. Use 0.5 mL 20% piperidine in DMF in an empty test tube as a blank.

Over the next 15 minutes, swirl the test tube with the Fmoc-Gly resin two or three times to make sure all the resin has come in contact with the piperidine solution. Add DMF to both tubes to bring to a volume of 50 mL. Zero the spectrophotometer at 301 nm with the Blank. The absorbance of the solution is 0.526.

Substitution calculation:

$$\frac{A_{301} \times \text{Vol}(\text{mL})}{7800 \times \text{wt}(\text{g})} = \frac{(0.526) \times (50)}{7800 \times 0.00505(\text{g})} = (0.67 \text{ mmol})/\text{g}$$

Boc Chemistry

The general protocol for SPPS using the Boc/HOBt/DCC chemistry is illustrated in Figure 3-19. The Boc group that protects the α -amino group is removed at the beginning of every cycle by a weak acid, typically 50% TFA in DCM. After deprotection, the resin is washed with DCM to remove most of the TFA. Any remaining TFA and the protonated amino groups are neutralized with a dilute DIEA solution. The resin is then washed with NMP, the same solvent used in the coupling reaction.

Boc/HOBt/DCC Protocol

Before coupling, the carboxyl group of the amino acid must be “activated.” HOBt/DCC is used for standard activation of Boc amino acids on the ABI 433A Peptide Synthesizer. Activation by HOBt/DCC is illustrated with resulting activated carboxylic acid derivatives in Figure 3-18.

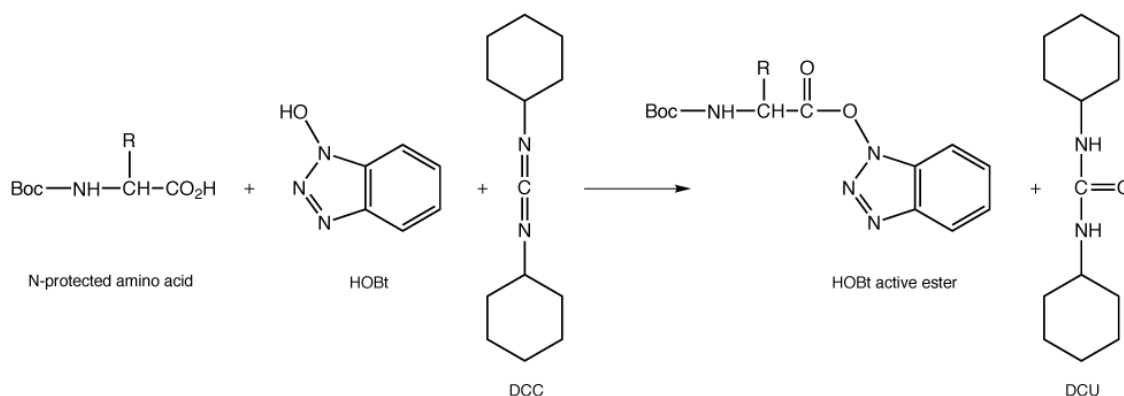


Figure 3-18. Activation using HOBt/DCC

In the cartridge during the Boc 0.50 mmol reaction, 2.0 mmol of dry, protected amino acid is dissolved with a solution of NMP and 2 mL of 1M HOBt in NMP. This solution is then transferred to the activator vessel where 2 mL of 1M DCC in NMP is added. The 0.5 mL measuring loop measures the calibrated deliveries of 1M HOBt in NMP and 1M DCC in NMP.

After approximately 40 to 50 minutes of activation, the HOBt-active ester is transferred to the RV. The DCU precipitate stays in the activator vessel.

During coupling, the activated Boc amino acid reacts with the amino terminal of the growing peptide chain, as shown in Figure 3-20. In the Boc 0.50 mmol cycles, the ABI 433A instrument uses four equivalents of the activated amino acid per one equivalent of the growing peptide chain.

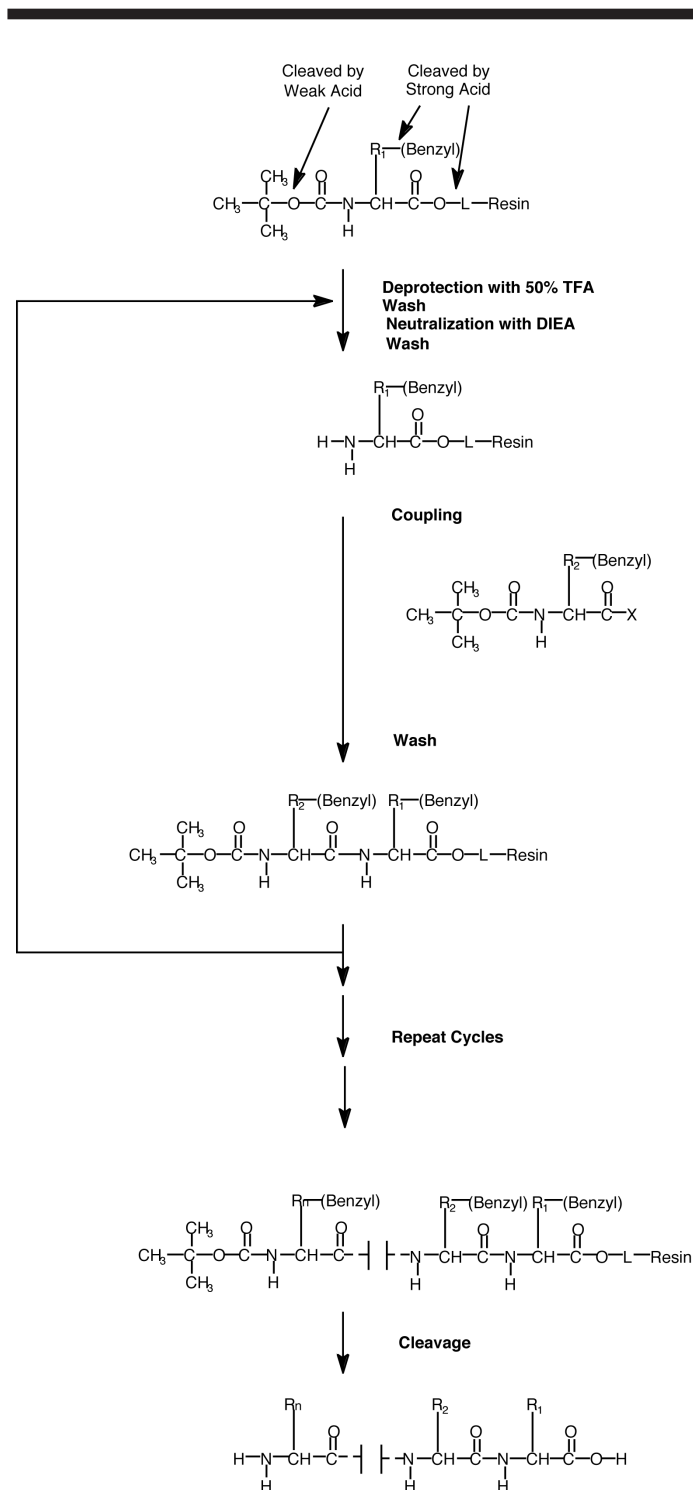


Figure 3-19. General Protocol Boc Chemistry Using HOBt Active Esters. “L” represents the linker between the peptide and the resin. “X” represents the active ester portion of the amino acid.

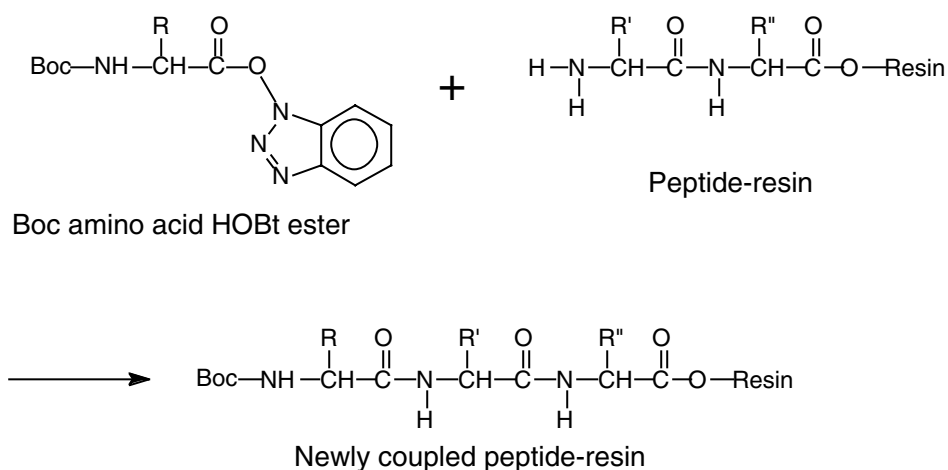


Figure 3-20. Boc coupling

There are three coupling stages, defined by the solvent added at each step:

- 100% NMP
- DMSO
- DIEA

During the NMP stage, coupling reactions may exceed 99% completion, although in some cases, the coupling may be much less. DMSO is added to enhance and improve solvation of the peptide resin and increase coupling for difficult sequences. Enough DMSO is added to produce a solution with 15% DMSO and 85% NMP. During the last coupling stage, DIEA is added to further disrupt peptide-peptide hydrogen bonds and increase solvation. The DIEA also neutralizes any protonated amine groups which helps accelerate the coupling reaction.

A resin sample can be taken after coupling is finished. Once the resin sample is removed, the capping reaction begins.

Capping uses a mixture of acetic anhydride, DIEA, and NMP to acetylate any unreacted amines and make them unavailable to react in future coupling cycles. This process simplifies the purification process because it is easier to remove the shorter, capped peptides than the longer deletion peptides. Table 3-3 summarizes the operations and reagents involved in Boc/HOBt/DCC chain assembly on the ABI 433A Peptide Synthesizer.

Boc 0.50 mmol cycles use 2 mmol Boc amino acid HOBt-active ester with 0.50 mmol resin. Each Boc 0.50 mmol cycle lasts 104 minutes. Boc 0.10 mmol cycles use 1 mmol Boc-amino acid HOBt-active ester with 0.10 mmol resin. A Boc 0.10 mmol cycle lasts 65 minutes.

Table 3-3. Chain Assembly Time (min) Using Boc/HOBt/DCC Chemistry

Operations and Reagents	0.50 mmol	0.10 mmol
TFA deprotection		
30% TFA in DCM	3	3
50% TFA in DCM	16	11
Washes and neutralizations		
DCM washes (5X)	3	2
5% DIEA	4	2
NMP washes (6X)	5	3
Coupling		
Boc-AA-HOBt ester in NMP	39	23
DMSO to make 15% DMSO/85% NMP	16	8
3.8 equiv. DIEA	5	4
Wash and resin sample NMP wash		
Resin sample (optional)	(2)	(2)
Capping		
10% Ac ₂ O, 5% DIEA in NMP	9	5
Washes		
DCM washes (6X)	4	4
Total Cycle Time (without resin sample)	104	65

Boc Resins

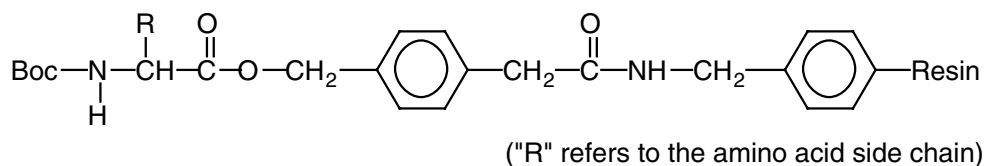
PAM and MBHA resins can be used with the Boc chemistry on the ABI 433A instrument.

Each resin provides a unique feature to the final product.

- PAM resins—a carboxylic acid terminal peptide
- MBHA resins—an amide terminal peptide

PAM Resins

PAM resins were developed to minimize the loss of peptide chains during SPPS.³⁵ The name “PAM” is derived from the linker, 4-(oxymethyl)-phenylacetamidomethyl. A PAM resin structure is shown in Figure 3-21.

**Figure 3-21. PAM resin**

With the conventional Merrifield-type resin, up to 0.7% of the peptide can be lost with each TFA treatment. In comparison, with the PAM resin only 0.007% loss occurs per cycle.³⁶

Besides increased acid stability, properly synthesized PAM resins offer two additional advantages: they contain few aldehydes and no extra hydroxymethyl sites. Aldehyde functionalities, when found in conventional resins, can cause Schiff's base formation with the peptide amine, resulting in a deletion peptide.³⁷ Furthermore, conventional resins may have hydroxymethyl sites which, through trifluoroacetylation, can cause chain termination.³⁸

MBHA Resins

The structure of MBHA (methylbenzhydrylamine) resin in the free amine form is shown in Figure 3-22. Even though it may not be indicated on the label, it is always purchased as the hydrochloride salt.

The first amino acid can easily be attached to the MBHA resin using the normal coupling cycle. Cleavage with a strong acid (such as HF or TFMSA) is necessary to remove the amide peptide from MBHA resins.

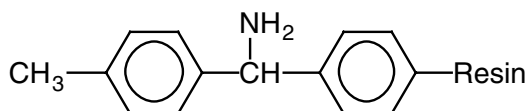


Figure 3-22. MBHA resin

A clever use of MBHA resins incorporates Boc-Asp(α -OBzl) or Boc-Glu(α -OBzl) anchored to MBHA resin by its side chain. After cleavage with HF or TFMSA, the peptide contains C-terminal asparagine or glutamine.³⁸

Cleavage

The booklet entitled *Introduction to Cleavage Techniques* presents a comprehensive discussion of cleavage procedures and scavengers for both Fmoc and Boc chemistry.

This booklet is available on the web: www.appliedbiosystems.com > **Service and Support** > **Product & Service Literature** and enter **343901** in the Document title box. It is 71 pages long. A shorter reference "Cleavage, Deprotection and Isolation of peptides after Fmoc synthesis" can be obtained on the same web site using document number 123507 and is 12 pages long.

Although the peptide resins obtained with *FastMoc* chemistry are not specifically discussed in the booklet, they are Fmoc-peptide resins. You can use the same cleavage techniques on the products of *FastMoc* chemistry as those used with the products of Fmoc chemistry with HOBt/DCC.

Note When you use the derivatives *Fmoc-Asn(Trt)* or *Fmoc-Gln(Trt)*, with more than 0.5 g of peptide resin, use twice the amount of cleavage mixture recommended.

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4 Chemistry Options

This chapter describes seven chemistry options that you can use on the ABI 433A Peptide Synthesizer. The reagents, protocols, and modules related to these chemistries are also included.

Contents

Introduction	4-2
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Introduction

There are seven chemistry options on the ABI 433A Peptide Synthesizer:

- *FastMoc*[™] 0.25 mmol
- *FastMoc* 0.10 mmol
- *FastMoc* 1.0 mmol
- Fmoc_HOBt_DCC 0.25 mmol
- Fmoc_HOBt_DCC 0.10 mmol
- Boc_HOBt_DCC 0.50 mmol
- Boc_HOBt_DCC 0.10 mmol

The numbers in millimoles refer to the scale of the synthesis, which is determined by the amount of starting resin in the reaction vessel.

ABI Chemistry, a folder in the SynthAssist[®] Software software package, contains fifteen Chemistry files: seven for the chemistry options listed above and ten additional *FastMoc*[™] chemistry files for conductivity monitoring. Four Basic Monitoring files provide conductivity monitoring of deprotection with feedback for coupling. Four Conditional Monitoring files contain modules for extended deprotection and coupling, with optional capping and monitored NMP washes, and conditional double coupling.

The ten *FastMoc* chemistry files for conductivity monitoring:

- *FastMoc* 0.10 Mon 1st-X
- *FastMoc* 0.10 MonPrevPk
- *FastMoc* 0.25 Mon 1st-X
- *FastMoc* 0.25 MonPrevPk
- *FastMoc* 1.0 MonPrevPk
- *FastMoc* 0.10 CondMon 1-X
- *FastMoc* 0.10 CondMonPrevPk
- *FastMoc* 0.25 CondMon 1-X
- *FastMoc* 0.25 CondMonPrevPk
- *FastMoc* 1.0 CondMonPrevPk

Modules

Each synthesis cycle is made up of a combination of modules. Each module consists of a group of steps that are necessary to complete a specific chemical task. For example, *FastMoc* module “A” contains the steps necessary to activate an amino acid. All modules can hold up to 99 steps. You can copy or modify any module with the procedures on page 9-6.

Table 4-1, Table 4-1, and Table 4-1 list the modules provided by ABI for the *FastMoc* chemistry option. Table 4-4 lists the modules for the Fmoc/HOBt/DCC, and Boc/HOBt/DCC chemistry options in the SynthAssist Software.

Table 4-1. *FastMoc* modules: 1.0, 0.25, and 0.10 mmol

Module letter	Synthesis task	Note
A	Activation	Dissolves amino acid
B	Deprotection	Piperidine deprotection
C	Capping with Ac ₂ O solution	
c	DCM Washes	
D	NMP Washes	
E	Add DIEA and transfer to RV	
F	Clean, Couple, Drain, & Wash	Clean cartridge, coupling, drain, and NMP washes
G	Resin Sampling	
H	Load and Cap	
I	Wait	

Table 4-2. *FastMoc* modules with Basic Monitoring: 1.0, 0.25, and 0.10 mmol

Module letter	Synthesis task	Note
A	Activation	Dissolves amino acid
B	Deprotection/ <i>algorithm</i>	MonPrevPk or Mon 1stPk-X*
C	Capping with Ac ₂ O solution	
c	DCM Washes	
D	NMP Washes	
E	Transfer	
F	Coupling, etc/feedback	
G	Resin Sampling	
H	Load and Cap	
I	Wait	

* not for 1mmol scale

Table 4-3. *FastMoc* modules with Conditional Monitoring

Module letter	Synthesis task	Note
A	Activation	Dissolves amino acid
B	Deprotection/ <i>algorithm</i>	
C	Capping with Ac ₂ O	
D	NMP Washes	
E	Transfer	
F	Coupling etc/SkipMod	
G	Resin Sampling	
H	Load and Cap	
I	Wait	
a	DoMonMod (activation/transfer)*	Conditional module
b	DoMonMod (deprotection/ <i>algorithm</i>)	Conditional module
c	DCM Washes	Conditional module
d	DoMonMod (capping/wash)	
f	DoMonMod (coupling)	
g	DoMonMod (resin sampling)	Conditional module
h	Module h	
i	Cart (eject/advance)/SkipMod*	

*Not for 1mmol scale

Table 4-4. *Fmoc/HOBt/DCC* and *Boc/HOBt/DCC* modules: 0.50/0.25 and 0.10 mmol

Module	Synthesis tasks	Variations
a	Activation	Piperidine in Fmoc, TFA in Boc
b	Deprotection	
c	DCM washes	
d	NMP wash	DIEA neutralization with Boc
e	Transfer contents of activator to RV, clean cartridge and activator	
f	Coupling	Times vary, DMSO added in Boc/HOBt/DCC 0.50 mmol
g	Drain, NMP wash, Resin sample	DIEA addition in Boc/HOBt/DCC 0.50 mmol, DMSO addition in Boc/HOBt/DCC 0.10 mmol, Capping in both scales Boc/HOBt/DCC
h	HMP resin load in Fmoc/HOBt/DCC Coupling in Boc/HOBt/DCC 0.50 mmol DCM wash in Boc/HOBt/DCC 0.10 mmol	
i	Wait	

Cycles

During each cycle, one amino acid is added to the peptide-resin by using a series of modules you specify. For example, a typical Fmoc/HOBt/DCC cycle could include modules “a f g b d e f.” Table 4-5 lists the cycles provided in SynthAssist Software for Fmoc/HOBt/DCC and Boc/HOBt/DCC chemistries.

Table 4-5. Pre-programmed SynthAssist Cycles for Fmoc/HOBt/DCC and Boc/HOBt/DCC

Chemistry	First Cycle	Middle Cycle	Last Cycle
Fmoc 0.25 mmol	a i b d e f	a f g b d e f	a f g b d e f f f g b d c
Fmoc 0.10 mmol	a i b d e	a f g b d e	a f g b d e f f f g b d c
Boc 0.50 mmol	a i b c d e f	a g c b c d e f	a g c b c d e f h g c
Boc 0.10 mmol	a i b c d e	a g c b c d e	a g c b c d e f g h

FastMoc modules have been designed to be uniform, regardless of the scale of the synthesis. For example, *FastMoc* module “A” always dissolves and activates amino acid; *FastMoc* module “B” always removes the Fmoc protecting group. *FastMoc* cycles for Basic conductivity monitoring contain a module “B” that “feeds back” to the coupling module “F.” *FastMoc* cycles for Conditional conductivity monitoring contain conditional modules for extended deprotection, extended coupling, capping, and double coupling. Some examples of *FastMoc* cycles are listed in Table 4-6. See *Monitoring a Synthesis* on page 5-1, for more information on monitoring modules.

Table 4-6. Pre-programmed FastMoc cycles on SynthAssist Software

Synthesis conditions	First Cycle	Middle Cycle	Last Cycle
Pre-loaded resin with resin sampling	BADEFG	BADEFG	BIDC
Amide resin without resin sampling	cDBADEF	BADEF	BIDC
HMP resin without resin sampling or final deprotection	HF	BADEF	DC
Pre-loaded resin with monitoring (1st Pk-X) and final deprotection	cD	BADEFD	BIDc
Pre-loaded resin with monitoring (PrevPk) and final deprotection	cD	BADEF	BIDc
Amide resin with conditional extended deprotection, coupling, and capping, and final deprotection	cDBbADEFfd	BbADEFfd	BIDc

The Run Editor can hold up to 30 lines. Modules can be rearranged to create shorter or longer cycles, or double couple cycles. It is also possible to start a synthesis with advanced add times. For some descriptions of user-defined cycles, see Chapter 7, on page 7-1.

Monitoring

With *FastMoc* chemistry on the ABI 433A Peptide Synthesizer, feedback monitoring regulates deprotection by measuring either conductive or spectrophotometric species. Functions 128-149 set the monitoring conditions and check the monitoring data to see if it meets the conditions. Table 4-7 lists the Monitoring Functions. See Section 5, Monitoring a Synthesis, for more details about these functions.

Table 4-7. ABI 433A Instrument Monitoring Functions

Function Number	Function Name	T represents	Action
128	Mon 1st Pk-X	Conductivity $\div$ 10	Alternate to Fxn 130, sets conductivity baseline ($X=T\div 10$)
129	Mon1stPk	Fxn active if T=1	Applies spectrophotometric monitoring algorithm
130	MonPrevPk	Fxn active if T=1	Applies conductivity monitoring algorithm
131	Mon Stop	Fxn active if T=1	Stops examining data, finds peak X ($X=10 \times T$)
132	Save MonPk	Fxn active if T=1	Saves data peak
133	MonBegLoop	Repetitions	Sets maximum number of loops
134	MonEndLoop	Percentage	Counts loops, determines deprotection endpoint for Fxns 128, 129, and 130
135	Mon Reset	Channel 1, 2, 3	Clears and resets monitoring data to zero
136	SkipModMon	Fxn active if T=1	Skips module steps if Fxn 133 T=total number of loops
137	Do Mod Mon	Fxn active if T=1	Performs module if Fxn 133 T=total number of loops
138	Int MaxMon	Fxn active if T=1	Interrupts module if Fxn 133 T=total number of loops
139	IntConduct	Conductivity	Interrupts synthesis if $(10 \times T) \geq$ conductivity
140	Int Chnl 2	Absorbance or other	Interrupts synthesis if $(10 \times T) \geq$ absorbance (spectrophotometric or other)
141	Int Chnl 3	Other source	Interrupts synthesis if $(10 \times T) \geq$ data value
142	MonDrain X	Conductivity	Sets maximum conductivity of RV drain
143	MonRVWaste	Seconds	Sets maximum time for RV drain to waste
144	MonRVtoAux	Seconds	Sets maximum time for RV drain to auxiliary waste
145	Test X> Pk	Data value	Compares peak X in Fxn 132 to $10 \times T$
146	Test X< Pk	Data value	Compares peak X in Fxn 132 to $10 \times T$
147	SkipOnTest	Fxn active if T=1	Skips module steps if Fxn 145 or 146 active
148	Do On Test	Fxn active if T=1	Performs module if Fxn 145 or 146 active
149	Int On Test	Fxn active if T=1	Interrupts module if Fxn 145 or 146 active

FastMoc Chemicals, Protocols, and Modules

The *FastMoc* protocol uses the Fmoc/NMP cycles with HBTU [2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] activation. HBTU is dissolved in a solution of HOBt in DMF. The amino acid is dissolved in this solution with additional NMP and DIEA (diisopropylethylamine). This solution is then transferred directly to the reaction vessel. HBTU activation is highly efficient, especially when difficult couplings are predicted, such as with Arg and the branched side chains of Ile, Val, and Leu.

The *FastMoc* 1.0 mmol cycles use three 1.0-mmol Fmoc-amino acid cartridges in the 55-mL reaction vessel. The *FastMoc* 0.25 mmol cycles use one 1.0-mmol Fmoc-amino acid cartridge in the 41-mL reaction vessel. The *FastMoc* 0.10 mmol cycles use one 1.0 mmol amino acid cartridge in the 8-mL reaction vessel. Table 4-8 and Table 4-9 summarize the protocols for all three scales on the ABI 433A instrument.

Table 4-8. ABI 433A Instrument *FastMoc* Chain Assembly Time (in minutes)

Operation and Reagents	1.00 mmol cycles	0.25 mmol cycles	0.10 mmol cycles
Deprotection	29	15	10
Washes with NMP	12	5	3
Coupling	20	21	9
Washes with NMP	9	4	2
Resin Sample (optional)	(2)*	(2)*	(2)*
Total Time [†]	70	45	24

* Resin sampling adds 2 more minutes to the total cycle time of the 0.25 mmol cycles and the 0.10 cycles.

[†] Chain assembly time with conductivity monitoring and conditional cycles varies and is sequence dependent

Table 4-9. Comparison of ABI 433A Instrument *FastMoc* Scales (without monitoring)

	Resin (mmol)	Amino Acid (mmol)	AA:Resin	Reaction Vessel	Waste per Cycle (mL)
1.00 mmol	1.00	3.0	3:1	55 mL	270
0.25 mmol	0.25	1.0	4:1	40 mL	100
0.10 mmol	0.10	1.0	10:1	8 mL	50

IMPORTANT *FastMoc modules must be used for the FastMoc chemistry. The modules in the FastMoc cycles differ from those in the Fmoc/HOBt/DCC cycles in both order and content.*

Chemicals Required for *FastMoc* Chemistry

Table 4-10 lists the reagents and solvents used in *FastMoc* chemistry and their bottle positions on the synthesizer.

Table 4-10. Reagents and Solvents for *FastMoc* Chemistry on the ABI 433A Instrument

Bottle #	Reagent	Part Number
1	Piperidine	Aldrich 57, 126-1
2	_____	
4*	0.1 M DMAP in DMF	400631
5	0.45 M HBTU/HOBt in DMF (must be prepared)	401132
6*	Methanol	400470
7	2 M Diisopropylethylamine (DIEA) in N-Methylpyrrolidone (NMP)	401517
8*	1.0 M Dicyclohexylcarbodiimide (DCC) in N-Methylpyrrolidone (NMP)	400663
9	Dichloromethane	400142
10	N-Methylpyrrolidone (NMP)	400580

* Reagents in Bottles 4, 6, and 8 are used only in module F

DANGER! ***CHEMICAL HAZARD. 1.0 M N,N-Dicyclo-hexylcarbodiimide/N-Methyl-pyrrolidinone (DCC/NMP) is a combustible liquid and vapor. It may be fatal if it is inhaled. Exposure causes eye, skin, and respiratory tract burns and it is a possible developmental hazard. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.***

WARNING ***CHEMICAL HAZARD. Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.***

DANGER! ***CHEMICAL HAZARD. 2 M N,N-Diisopropylethylamine/1-Methyl-2-Pyrrolidinone (DIEA/NMP) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract burns and it is a possible developmental hazard. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.***

WARNING **CHEMICAL HAZARD.** *Dimethylaminopyridine/Dimethylformamide (DMAP/DMF) is a flammable liquid and vapor. It may be fatal if it is absorbed through the skin. Exposure causes eye, skin, and respiratory tract burns and it is harmful if inhaled or swallowed. Exposure may cause liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *(N-[1H-benzotriazol-1-yl](dimethylamino)methylene]-N-methylanaminium hexafluorophosphate N-oxide (HBTU), formerly 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumhexafluorophosphate, may cause allergic respiratory and skin reactions. Do not breathe the dust, and avoid prolonged or repeated contact with the skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *1-Hydroxybenzotriazole hydrate (HOBt) has a risk of explosion if heated under confinement. Keep away from heat and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *Methanol is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! **CHEMICAL HAZARD.** *Piperidine (hexahydropyridine) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled, swallowed, or*

absorbed through the skin. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Bottle 5 (HBTU) Preparation

Purchase HBTU as part of the HBTU activation kit (P/N 401132). The activation kit contains a bottle of solid HBTU (100 mmol), a bottle of 0.5 M HOBt in DMF, and two HBTU delivery line filters. Store the reagents in the HBTU activation kit at 0–4 °C until you are ready to use them. Mix the reagents together just prior to use.

WARNING **CHEMICAL HAZARD.** (N-[1H-benzotriazol-1-yl] (dimethylamino)methylene]-N-methylanaminium hexafluorophosphate N-oxide (HBTU), formerly 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumhexafluorophosphate, may cause allergic respiratory and skin reactions. Do not breathe the dust, and avoid prolonged or repeated contact with the skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare 0.45 M HBTU/HOBt/DMF:

Note Do not attempt to use the 1 M HOBt/NMP for this preparation. HBTU does not readily dissolve in NMP at this concentration.

1. Pour 200 mL of 0.5 M HOBt in DMF into the 450 mL bottle containing 100 mmol dry HBTU.
2. Recap bottle and shake until HBTU is dissolved. The increased volume due to HBTU reduces the concentration of the two species from 0.5 M to 0.45 M.
3. Locate the Bottle 5 delivery line and thoroughly dry it with a lint-free tissue.
4. Firmly press a new, polyethylene, reagent line filter onto the end of the Bottle 5 delivery line. Replace the bottle seal and screw Bottle 5 into the ratchet cap on the peptide synthesizer.

The 0.45 M HBTU/HOBt/DMF solution is stable at room temperature for at least 6 weeks, as determined by use testing. After a few days, the solution turns yellow. This color change does not have any adverse effect on the efficiency of the reagent.

Required Amino Acid Derivatives

With the exception of Fmoc-Gln and Fmoc-Asn, use the Fmoc-amino acid derivatives sold by Applied Biosystems to synthesize with *FastMoc* chemistry.

The coupling efficiencies and solubilities of Fmoc-Gln and Fmoc-Asn are greatly enhanced when the side chains are protected. Based on their coupling performance and ease of removal during TFA cleavage, Applied Biosystems recommends the trityl-protected derivatives. You can also use Fmoc-Asn(Trt) and Fmoc-Gln(Trt) with the modified loading cycles to load HMP resin.

Amino Acid	Part number (1 mmol cartridge)	
	each	box of 10
N- α -Fmoc-(trityl)-L-asparagine	401089	411089
N- α -Fmoc-(trityl)-L-glutamine	401090	411090

Loading and Capping

When loading amino acids on HMP resin, run Flow Test 4 and Flow Test 8 before synthesis. Since DMAP and DCC are used infrequently with *FastMoc* chemistry, you must pressurize the reagent bottles and flush the delivery lines with reagent. Accurate deliveries of these two reagents are essential for efficient loading.

IMPORTANT When using HBTU solution, use Flow Test 13, not Flow Test 5.

You may observe minor side reaction with *FastMoc* chemistry when loading is not efficient. HBTU-activated amino acid can react with some of the remaining hydroxyl sites on the HMP resin, producing peptides with deletions at the C-terminus that appear as small impurities in chromatograms and mass spectra.

Note In commercially prepared, loaded resins, the hydroxyl sites have been capped.

If you find these minor impurities unacceptable, Applied Biosystems recommends that you follow loading to HMP resin by capping with benzoic anhydride. Fill a dry, clean cartridge with 3 mmol (0.60-0.70 g) benzoic anhydride. Use the capped resin with the standard loading protocols. This procedure is described on page 7-16.

If you want to load the amino acid on HMP resin and then follow this with acetic anhydride capping, you must interrupt synthesis to change bottle 4. This procedure is described on page 7-15.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the peptide-resin (See *References* on page 3-22, reference 23).

Examples of *FastMoc* Modules for Run Editor

The examples shown here work for *FastMoc* 1.0, 0.25, and 0.10 mmol chemistry without conductivity monitoring or conditional modules. When you change the scale of the synthesis, you must transfer the appropriate Chemistry file from SynthAssist Software to the ABI 433A instrument. For more examples, see "Advanced Operations" on page 7-1.

Example 1: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on HMP resin, with removal of the final Fmoc group and resin samples, the run editor is:

Cy: 1	Rpt: 1	M: HF
Cy: 2	Rpt: 9	M: BADEFG
Cy: 11	Rpt: 1	M: BIDC

Example 2: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on HMP resin, with removal of the final Fmoc group and no resin samples, the run editor is:

Cy: 1	Rpt: 1	M: HF
Cy: 2	Rpt: 9	M: BADEF
Cy: 11	Rpt: 1	M: BIDC

Example 3: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on Fmoc-Leu resin with removal of the final Fmoc group and no resin samples, the run editor is:

Cy: 1	Rpt: 9	M: BADEF
Cy: 10	Rpt: 1	M: BIDC

FastMoc 1.0 mmol

Chemical Usage

Table 4-14 summarizes the chemical usage of the *FastMoc* 1.0 mmol cycles for average flow rates.

See page 4-8 for chemistry warnings.

Table 4-11. Chemical Usage of *FastMoc* 1.0 mmol Cycles

Bottle	Chemical	mL / Bottle	Cycle 1 mL / cycle	Cycle 10 mL /cycle	Cycle 20 mL /cycle	Cycle 30 mL /cycle	Cycles per bottle***
1	Piperidine	450	9	10.5	12	14	33
4	DMAP [†]	200					
5	HBTU/ HOBt	230	6	6	6	6	30
6	MeOH [†]	450					
7	2 M DIEA	200	5	5	5	5	33
8	DCC [†]	200					
9	DCM ^{††}	4000					
10	NMP ^{**}	8000 ^{**}	249	269	326	394	24
Waste	—	9463	270 [*]	292 [*]	350 [*]	420 [*]	26 [*]

[†] DMAP, DCC, and MeOH are only used during the loading cycle

^{††} DCM is used only in the loading cycle (Module H) and in the final resin wash (Module C).

^{*} This value is for non resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

^{**} Based on the two-bottle configuration of NMP.

^{***} These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than those cited, the number of cycles/bottle will be proportionately lower.

WARNING *CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.*

Reaction vessel with *FastMoc* 1.0 mmol Cycles

Use the large 55 mL reaction vessel with a resin sample line (part number 401576) the 1.0 mmol cycles.

The large reaction vessel is available in both a resin sampling and a non-resin sampling version. However, Applied Biosystems has found that the resin sampling version works better with the 1.0 mmol scale synthesis, because the extra steps required when clearing the resin sampling line help the resin to vortex. Even though you use a resin sampling vessel, you do not need to take resin samples. If you want to take resin samples, include a module G in the Run Editor. If you do not want to take resin samples, do not include a module G in the Run Editor.

Reaction Vessel Filter Requirement

When using the 1.0 mmol cycles, you *must* use two reaction vessel filters on the bottom cap because the larger amount of resin used in the 1.0 mmol cycles causes a single filter to collapse. The top cap needs only a single reaction vessel filter.

Bottle #7 (2.0 M DIEA)

The 1.0 mmol cycles use 2.0 M DIEA in NMP in bottle #7. This is the same as the *FastMoc* 0.25 and 0.10 cycles described in User Bulletin 35. The use of DIEA at any other molarity is not recommended.

Addition of HBTU

Applied Biosystems recommends adding less than one equivalent of HBTU. A report shows that, following Fmoc removal, the newly-exposed NH_2 groups can react with HBTU, resulting in a guanidinium-like adduct, which can terminate synthesis¹. Avoid this side reaction by adding only 0.9 mmol HBTU to the 1 mmol Fmoc amino acid, and by adding the DIEA to the HBTU and Fmoc-amino acid solution to initiate the activation before transferring the activated amino acid to the reaction vessel.

For example:

- If Flow Test 13 is 2.3–2.5 g, then use 7 sec for Fxn 94 in Module A.
- If Flow Test 13 is 2.1–2.3 g, then use 8 sec for Fxn 94 in Module A.
- If Flow Test 13 is 1.9–2.1 g, then use 9 sec for Fxn 94 in Module A.

FastMoc 1.0 mmol Modules

The large, 55-mmol reaction vessel with a resin-sampling line (P/N 603225) must be used with the *FastMoc* 1.0 mmol cycles. The 55-mmol reaction vessel is available with or without a resin-sampling line; however, we have found that the extra steps required to clear the resin-sampling line help the resin to vortex. Resin samples need not be taken when you use an RV with a resin-sampling line. If you do not want to take resin samples, do not include a module G in the Run Editor.

¹. Gausepohl, H., U. Piesles, and R.W. Frank. 1992. Schiff base analog formation during in situ activation by HBTU and TBTU. p. 523–524. // J.A. Smith and J.E. Rivier (eds.), Proceedings of the Twelfth American Peptide Symposium, 1992, ESCOM, Leiden.

Module A - Dissolving Amino Acid

Total time = 23.6 minutes

At the beginning of module A, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 g) and 0.9 mmol of 0.45 M HBTU/HOBt in DMF (2.0 g) are added to the cartridge. The amino acid is dissolved by mixing for 5.5 minutes and transferred to the activator vessel. This process is repeated two more times. The module includes flushing with NMP and gas dry with nitrogen to assure complete removal of HBTU.

Module B - Piperidine Deprotection

Total time = 7.0 minutes

This module begins with one NMP wash of the resin. A 15% piperidine/NMP solution is introduced and allowed to deprotect for 2 minutes. The RV is drained and a second treatment with 15% piperidine is performed. The valve blocks are then rinsed thoroughly. The resin will continue to deprotect for an additional 23.6 minutes during module A.

Module C - DCM Washes

Total time = 9.5 minutes

The resin is washed 6 times with DCM. After the final wash, the DCM in the resin sample line is removed. This module is used only at the end of the synthesis.

Module D - NMP Washes

Total time = 11.6 minutes

The RV is drained and the resin is washed 6 times with NMP. During each NMP wash the resin sample line is rinsed with NMP. There is an add time of 1 in the loop function, so after cycle 4, the number of washes is 7, and after cycle 14, the number of washes is 8, and so forth.

Module E - Add DIEA and Transfer to RV

Total time = 3.0 minutes

At the beginning of the module, 3 mL of 2 M DIEA in NMP is added to the cartridge, to initiate the activation of the amino acid. The activated amino acid is then transferred from the activator to the RV.

**Module F - Clean cartridge, Couple,
Drain and NMP Washes**

Total time = 24.4 minutes

During this module, the amino acid cartridge is washed 4 times with NMP. This NMP is transferred to the activator vessel and used later in the module to wash the resin in the RV. Coupling occurs during this cartridge washing, and the coupling then continues for another 15 minutes. The RV is drained and the resin is washed with the NMP from the activator vessel. The cartridge is washed an additional 2 times with NMP, which also used to wash the resin.

Module G - Resin Sample

Total time = 2.2 minutes

This module takes a resin sample. Place a blank tube between each resin sample tube. The tared resin sample test tube should contain 2-3 mL MeOH and 2-3 drops of acetic acid.

Module H - Loading and Capping

Total time = 90 minutes

Use this module to load the first amino acid onto the HMP resin. The contents of three 1.00-mmol Fmoc amino acid cartridges are dissolved, one at a time, in an NMP/DCM mixture and transferred to the RV. This is followed with 3 mL of 1.0 M DCC/NMP and 0.1 equivalent of DMAP. The resin is mixed for 45 minutes. The RV is drained and the resin is washed 2 times with 50% MeOH and 50% DCM and 4 times with DCM. The resin is then capped with 3 mmol benzoic anhydride (0.60-0.70 g) which has been placed in one cartridge.

The loading and capping is done with modules HF. It requires 3 Fmoc-amino acid cartridges and 1 cartridge containing 3.0 mmol (0.60-0.70 g) of benzoic anhydride.

Module H is modified for Arg, Asn, Gln and His the following way:

	Fmoc-His(Bum)	Fmoc-Arg(Mtr) Fmoc-Arg(Pmc)	Fmoc-Gln(Trt) Fmoc-Asn(Trt)
Step 14 (#9 CART)	0 sec	0 sec	4 sec
Step 15 (#10 CART)	8 sec	7 sec	4 sec

IMPORTANT Do not load with unprotected Fmoc-Asn or Fmoc-Gln. Loading with His(Trt) can cause racemization.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the peptide-resin (see "References" on page 3-22, reference 23).

Module I - 15 Min. Wait

Total time = 15 minutes

Module I (same as Module i) is used in the last cycle to extend the total deprotection time to 22 minutes.

Modules for the Run Editor

Synthesis process	Modules	Requires
Single coupling with resin sampling, with C-terminal amide or pre-loaded resins	BADEFG	3 amino acid cartridges
Single coupling without resin sampling, with C-terminal amide or pre-loaded resins	BADEF	3 amino acid cartridges
Double coupling with resin samples	BADEIADEFG	6 amino acid cartridges
Double coupling without resin sampling	BADEIADEF	6 amino acid cartridges
Final deprotection	BIDC	
Loading and capping initial resin	HF	3 amino acid cartridges
use suggested module H modifications, shown on page 4-16, with Arg, Asn, Gln, and His derivatives		+ 1 cartridge containing 3 mmol benzoic anhydride (0.60-0.70 g)

If you want to load the C-terminal amino acid to an HMP resin and follow this with a capping step, the first cycle should be: HF.

Place the amino acid cartridges in the first three cartridge positions. After the third cartridge, place a cartridge that contains 3.0 mmol (0.60-0.70 g) benzoic anhydride.

For all subsequent cycles use: BADEF (G)

FastMoc 0.25 mmol

Chemical Usage

Table 4-14 summarizes the chemical usage of the *FastMoc* 0.25 mmol cycles for average flow rates.

See page 4-8 for chemistry warnings.

Table 4-12. Chemical Usage of *FastMoc* 0.25 mmol Cycles

Bottle	Chemical	mL /Bottle	Cycle 1 mL / cycle	Cycle 10 mL /cycle	Cycle 20 mL /cycle	Cycle 30 mL /cycle	Cycles per bottle***
1	Piperidine	450	5.0	5.5	6.0	6.5	78
4	DMAP [†]	200					
5	HBTU/ HOBt	230	2.0	2.0	2.0	2.0	110
6	MeOH [†]	450					
7	2 M DIEA	200	1	1	1	1	130
8	DCC [†]	200					
9	DCM ^{††}	4000					
10	NMP	8000**	92	107	121	136	50
Waste	—	9463	100*	115*	130*	145*	65*

[†] DMAP, DCC, and MeOH are only used in the loading cycle, HF

^{††} DCM is used only in the loading cycle (Module H) and in the final resin wash (Module C).

*This value is for non resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

** Based on the two-bottle configuration of NMP.

***These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than these cited, the number of cycles/bottle will be proportionately lower.

Table 4-13. Chemical Usage of *FastMoc* 1.0 mmol Cycles

Bottle	Chemical	mL / Bottle	Cycle 1 mL / cycle	Cycle 10 mL /cycle	Cycle 20 mL /cycle	Cycle 30 mL /cycle	Cycles per bottle***
1	Piperidine	450	9	10.5	12	14	33
4	DMAP [†]	200					
5	HBTU/ HOBt	230	6	6	6	6	30
6	MeOH [†]	450					
7	2 M DIEA	200	5	5	5	5	33
8	DCC [†]	200					
9	DCM ^{††}	4000					
10	NMP**	8000**	249	269	326	394	24
Waste	—	9463	270*	292*	350*	420*	26*

[†] DMAP, DCC, and MeOH are only used during the loading cycle

^{††} DCM is used only in the loading cycle (Module H) and in the final resin wash (Module C).

*This value is for non resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

** Based on the two-bottle configuration of NMP.

***These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than those cited, the number of cycles/bottle will be proportionately lower.

FastMoc 0.25 mmol Modules

The *FastMoc* 0.25 mmol cycles are used with either the resin sampling or non-resin sampling, 41-mL reaction vessel. The Fmoc-amino acid is dissolved with 2.1 g NMP, 2.0 g of 0.45 M HBTU/HOBt in DMF, and 2 M DIEA, then transferred to the reaction vessel. For examples of *FastMoc* cycles and Runs you can create with these modules, see "FastMoc 0.25 mmol and 0.10 mmol Cycles" on page 7-3.

Module A - Dissolving Amino Acid

Total time = 7.6 minutes

At the beginning of module A, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 g) and 0.9 mmol of 0.45 M HBTU/HOBt in DMF (2.0 g) are added to the cartridge. The amino acid is dissolved by mixing for 6 minutes. The module includes flushing with NMP and gas dry with nitrogen to assure complete removal of HBTU.

Module B - Piperidine Deprotection

Total time = 8.8 minutes

This module begins with one NMP wash of the resin. A solution of 18% piperidine/NMP is introduced and allowed to deprotect for 3 minutes. The RV is drained and a second treatment with 20% piperidine is performed. The valve blocks are then rinsed thoroughly. The resin continues to deprotect for an additional 7.6 minutes during module A.

Module C - DCM Washes

Total time = 7.6 minutes

The resin is washed 6 times with DCM. After the final wash, the DCM in the resin sample line is removed. This module is used only at the end of the synthesis.

Module D - NMP Washes

Total time = 4.6 minutes

The RV is drained and the resin is washed 5 times with NMP. During each NMP wash the resin sample line is rinsed with NMP. There is an add time of 1 in the loop function, so after cycle 4, the number of washes is 6, and after cycle 14, the number of washes is 7, and so forth.

Module E - Add DIEA and Transfer to RV

Total time = 2.2 minutes

At the beginning of the module, 1 mL of 2 M DIEA in NMP is added to the cartridge, to initiate the activation of the amino acid. The activated amino acid is then transferred from the cartridge to the RV.

**Module F - Clean cartridge, Couple,
Drain and NMP Washes**

Total time = 22.2 minutes

During this module, the amino acid cartridge is washed 3 times with NMP. This NMP is transferred to the Activator Vessel and used later in the module to wash the resin in the RV. Coupling occurs during this cartridge washing, and the coupling is then continued for another 15 minutes. The RV is drained and the resin is washed with the NMP from the activator vessel.

Module G - Resin Sample

Total time = 1.7 minutes

This module takes a resin sample. Place a blank tube between each resin sample tube. The tared resin sample test tube should contain MeOH and a few drops of acetic acid.

Module H - Loading and Capping

Total time = 54 minutes

This module is used to load the first amino acid onto the HMP resin. The 1.00 mmol Fmoc amino acid is dissolved in an NMP/DCM mixture and transferred to the RV, followed with 1 mL of 1.0 M DCC/NMP and 0.1 equivalent of DMAP. The resin is mixed for 30 minutes. The RV is drained and the resin is washed 2 times with 50% MeOH and 50% DCM and 5 times with DCM. The resin is then capped with 3 mmol benzoic anhydride (0.60-0.70 g) which has been placed in one cartridge.

The loading and capping is done with modules HF. It requires 1 Fmoc-amino acid cartridge and 1 cartridge containing 3.0 mmol (0.60-0.70 g) of benzoic anhydride.

Module H is modified for Arg, Asn, Gln and His the following way:

	Fmoc-His(Bum)	Fmoc-Arg(Mtr) Fmoc-Arg(Pmc)	Fmoc-Gln(Trt) Fmoc-Asn(Trt)
Step 14 (#9 CART)	0 sec	0 sec	4 sec
Step 15 (#10 CART)	8 sec	7 sec	4 sec

IMPORTANT Do not load with unprotected Fmoc-Asn or Fmoc-Gln. Loading with His(Trt) can cause racemization.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the final peptide-resin (see "References" on page 3-22, reference 23).

Module I - 15 Min Wait

Total time = 15 minutes

Module I (same as Module i) is used in the last cycle to extend the total deprotection time to 22 minutes.

FastMoc 0.10 mmol

Chemical Usage

FastMoc 0.10 mmol cycles are usually used with the non-resin-sampling, 8-mL reaction vessel. The Fmoc-amino acid is dissolved and activated in the cartridge in a mixture of 2.0 g of 0.45 M HBTU/HOBt in DMF, 2 M DIEA, and 0.8 mL NMP.

See page 4-8 for chemistry warnings.

Note *To take resin samples, use a resin-sampling, 8 mL reaction vessel.*

Table 4-14. Chemical Usage of 0.10 mmol *FastMoc* Cycles

Bottle	Chemical	mL per Bottle	Cycle 1 mL / cycle	Cycle 10 mL /cycle	Cycle 20 mL /cycle	Cycle 30 mL /cycle	Cycles per bottle***
1	Piperidine	450	2.0	2.0	2.0	2.0	225
4	DMAP†	2					
5	HBTU/ HOBt	230	2.0	2.0	2.0	2.0	110
6	MeOH†	450					
7	2 M DIEA	200	1	1	1	1	130
8	DCC†	200					
9	DCM††	4000					
10	NMP	8000**	45	53	61	69	120
Waste	—	9463	50	58	66	74	120

† DMAP, DCC, and MeOH are only used in the loading cycle.

†† DCM is used only twice during a peptide synthesis with 0.10 mmol cycles: once when the first amino acid is loaded and again, 15 mL, in the final washes.

*This value is for non resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

** Based on the two-bottle configuration of NMP.

***These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than these cited, the number of cycles/bottle will be proportionately lower.

WARNING **CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.**

FastMoc 0.10 mmol Modules

For examples of *FastMoc* cycles and Runs you can create with these modules, see "FastMoc 0.25 mmol and 0.10 mmol Cycles" on page 7-3.

Module A - Dissolving Amino Acid

Total time = 7.6 minutes

At the beginning of module A, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 g) and 0.9 mmol of 0.45 M HBTU/HOBt in DMF (2.0 g) are added to the cartridge. The amino acid is dissolved by mixing for 6 minutes. The module includes flushing with NMP and gas dry with nitrogen to assure complete removal of HBTU.

Module B - Piperidine Deprotection Total time = 2.9 minutes

This module begins with one NMP wash of the resin. A 22% piperidine/NMP solution is introduced and allowed to deprotect for 2 minutes. The RV is drained and a second treatment with 22% piperidine is performed. The valve blocks are then rinsed thoroughly. The resin will continue to deprotect for an additional 7.6 minutes during module A.

Module C - DCM Washes Total time = 6.3 minutes

The resin is washed 6 times with DCM. After the final wash, the DCM in the resin sample line is removed. This module is used only at the end of the synthesis.

Module D - NMP Washes Total time = 2.5 minutes

The RV is drained and the resin is washed 4 times with NMP. During each NMP wash the resin sample line is rinsed with NMP. There is an add time of 1 in the loop function, so after cycle 4, the number of washes is 5, and after cycle 14, the number of washes is 6, and so forth.

Module E - Add DIEA and Transfer to RV Total time = 2.1 minutes

At the beginning of the module, 1 mL of 2 M DIEA in NMP is added to the cartridge, to initiate the activation of the amino acid. The activated amino acid is then transferred from the cartridge to the RV.

Module F - Clean Cartridge, Couple, Drain and NMP Washes Total time = 9.3 minutes

During this module, the amino acid cartridge is washed 2 times with NMP. This NMP is transferred to the Activator Vessel and used later in the module to wash the resin in the RV. Coupling occurs during this cartridge washing, and the coupling is then continued for another 4.5 minutes. The RV is drained and the resin is washed with the NMP from the Activator Vessel.

Module G - Resin Sample Total time = 1.4 minutes

This module takes a resin sample. Place a blank tube between each resin sample tube. The tared resin sample test tube should contain MeOH and a few drops of acetic acid.

Module H - Loading and Capping Total time = 51 minutes

This module is used to load the first amino acid onto the HMP resin. The 1.00 mmol Fmoc amino acid is dissolved in an NMP/DCM mixture and transferred to the RV, followed with 1 mL of 1.0 M DCC/NMP and 0.1

equivalent of DMAP. The resin is mixed for 30 minutes. The RV is drained and the resin is washed 2 times with 50% MeOH and 50% DCM and 5 times with DCM. The resin is then capped with 3 mmol benzoic anhydride (0.60-0.70 g) which has been placed in one cartridge.

The loading and capping is done with modules HF. It requires 1 Fmoc-amino acid cartridge and 1 cartridge containing 3.0 mmol (0.60-0.70 g) of benzoic anhydride.

Module H is modified for Arg, Asn, Gln and His the following way:

	Fmoc-His(Bum)	Fmoc-Arg(Mtr) Fmoc -Arg(Pmc)	Fmoc-Gln(Trt) Fmoc-Asn(Trt)
Step 14 (#9 CART)	0 sec	0 sec	4 sec
Step 15 (#10 CART)	8 sec	7 sec	4 sec

IMPORTANT Do not load with unprotected Fmoc-Asn or Fmoc-Gln. Loading with His(Trt) can cause racemization.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the final peptide-resin (see "References" on page 3-22, reference 23).

Module I - 15 Min Wait

Total time = 15 minutes

Module I (same as Module i) is used in the last cycle to extend the total deprotection time to 17 minutes.

Fmoc/HOBt/DCC Chemicals, Protocols and Modules

Chemicals Required for Fmoc/HOBt/DCC Chemistry

Table 4-15 lists the reagents and solvents that are used on the ABI 433A Peptide Synthesizer, according to bottle number, for synthesis with Fmoc chemistry with HOBt/DCC activation. Table A-1 in Appendix 2 lists all the chemicals that can be used for synthesis on the ABI 433A instrument with the Fmoc/HOBt/DCC protocol.

Table 4-15. Reagents and solvents for Fmoc/HOBt/DCC chemistry

Bottle #	Reagent	Part Number
1	Piperidine	Aldrich 57, 126-1
2	_____	
4*	0.1 M Dimethylaminopyridine (DMAP) in N,N-dimethylformamide (DMF)	400631
5	_____	
6	Methanol	400470
7	1.0 M 1-Hydroxybenzotriazole (HOBt) in NMP	400662
8	1.0 M Dicyclohexylcarbodiimide (DCC) in N-Methylpyrrolidone (NMP)	400663
9	Dichloromethane	400142
10	N-Methylpyrrolidone (NMP)	4344551 (4 bottles of 400580)

* Bottle 4 with DMAP/DMF is used only in the loading cycle, HF

Refrigerate 1.0 M 1-Hydroxybenzotriazole in N-Methylpyrrolidone at 4°C or store it in a freezer at -15 °C. This HOBt solution slowly decomposes to give a slightly yellow solution.

DANGER! ***CHEMICAL HAZARD. 1.0 M N,N-Dicyclo-hexylcarbodiimide/N-Methyl-pyrrolidinone (DCC/NMP) is a combustible liquid and vapor. It may be fatal if it is inhaled. Exposure causes eye, skin, and respiratory tract burns and it is a possible developmental hazard. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.***

WARNING ***CHEMICAL HAZARD. Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a***

potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER! *CHEMICAL HAZARD. Dimethylaminopyridine/Dimethylformamide (DMAP/DMF) is a flammable liquid and vapor. It may be fatal if it is absorbed through the skin. Exposure causes eye, skin, and respiratory tract burns and it is harmful if inhaled or swallowed. Exposure may cause liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING *CHEMICAL HAZARD. Methanol is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING *CHEMICAL HAZARD. N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! *CHEMICAL HAZARD. Piperidine (hexahydropyridine) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled, swallowed, or absorbed through the skin. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Fmoc/HOBt/DCC Protocols

Fmoc/HOBt/DCC 0.25 mmol cycles use 1.0 mmol Fmoc-amino acids with 0.25 mmol resin in the 41-mL reaction vessel. Fmoc/HOBt/DCC 0.10 mmol cycles use the 1.0 mmol amino acid cartridge with 0.10 mmol resin and the 8 mL reaction vessel. Table 4-16 and Table 4-17 summarize the protocols for both scales on the ABI 433A instrument.

Table 4-16. ABI 433A Instrument Fmoc/HOBt/DCC Chain Assembly Time (in minutes)

Operation and Reagents	0.1 mmol	0.25 mmol
Deprotection	21	14.0
Washes with NMP	9	4.5
Coupling	71	37.0
Washes with NMP	7	4.5
Resin sample (optional)	(2)	(2.0)
Total time	108*	60.0*

*Resin sampling adds two more minutes to the total cycle times of 108 and 60.0 minutes.

Table 4-17. Comparison of ABI 433A Instrument Fmoc/HOBt/DCC Scales

	Resin (mmol)	Amino Acid (mmol)	AA: Resin	Reaction Vessel	Waste per Cycle (mL)
0.25 mmol	0.25	1.00	4:1	41 mL	273
0.10 mmol	0.10	1.00	10:1	8 mL	95

Required Amino Acid Derivatives

The coupling efficiencies and solubilities of Fmoc-Gln and Fmoc-Asn are greatly enhanced when the side chains are protected. Based on their coupling performance and ease of removal during TFA cleavage, Applied Biosystems recommends the trityl-protected derivatives. Fmoc-Asn(Trt) and Fmoc-Gln(Trt) can also be used with the modified loading cycles to load HMP resin.

Amino Acid	Part number (1 mmol cartridge)	
	each	box of 10
N- α -Fmoc-(trityl)-L-asparagine	401089	411089
N- α -Fmoc-(trityl)-L-glutamine	401090	411090

Fmoc/HOBt/DCC 0.25 mmol

Chemical Usage

Before starting a synthesis, be sure that the Synthesizer has an adequate supply of chemicals. Table 4-18 shows the chemical usage per cycle for the Fmoc/HOBt/DCC 0.25 mmol synthesis of a 10-residue peptide at average flow rates. Note that some reagents are consumed faster than others. Also, due to add times, the usage per cycle of some chemicals, such as piperidine and NMP, will be greater on longer peptides. Approximately 34 unattended Fmoc/HOBt/DCC 0.25 mmol cycles can be performed with two 4-liter bottles in position 10 (NMP).

See page 4-24 for chemistry warnings.

Table 4-18. Chemical Usage Per Cycle Fmoc/HOBt/DCC Chemistry, 0.25 mmol resin, 10-residue peptide

Bottle	Chemical	mL/Bottle	mL/Cycle	Cycles/Bottle**
1	Piperidine	450	4.5	92*
2	_____	_____	_____	_____
4††	DMAP	200	NA	NA
5	_____	_____	_____	_____
6	MeOH	450	10	45
7	HOBt	200	1.6	120
8	DCC	200	1.8	110
9	DCM	4000	55	72
10	NMP	8000	200	35*†
Waste bottle		9463	273	34

†Based on a two bottles of NMP in parallel assembly.

†† DMAP is used only during the loading cycle, HF

*For a detailed description of the affect of the add times, see page 1-37.

**These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than these cited, the number of cycles/bottle will be proportionately lower.

Table 4-19. Chemical Usage of FastMoc 1.0 mmol Cycles

Bottle	Chemical	mL / Bottle	Cycle 1 mL / cycle	Cycle 10 mL /cycle	Cycle 20 mL /cycle	Cycle 30 mL /cycle	Cycles per bottle***
1	Piperidine	450	9	10.5	12	14	33
4	DMAP†	200					
5	HBTU/ HOBt	230	6	6	6	6	30
6	MeOH†	450					
7	2 M DIEA	200	5	5	5	5	33
8	DCC†	200					
9	DCM††	4000					
10	NMP**	8000**	249	269	326	394	24
Waste	—	9463	270*	292*	350*	420*	26*

† DMAP, DCC, and MeOH are only used during the loading cycle

†† DCM is used only in the loading cycle (Module H) and in the final resin wash (Module C).

*This value is for non resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

** Based on the two-bottle configuration of NMP.

***These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and piperidine, 1.0 mL/5 sec. If flow rates on a ABI 433A instrument are greater than those cited, the number of cycles/bottle will be proportionately lower.

WARNING **CHEMICAL HAZARD.** *Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.*

Fmoc/HOBt/DCC 0.25 mmol Modules

For examples of cycles and runs you can create with these modules, see "Fmoc/HOBt/DCC Cycles" on page 7-20.

Module **a** - Activation

Total time = 24 minutes

At the beginning of module "a," the amino acid cartridge name is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 mL) and 1M HOBt (1 mL) are added to the cartridge.

After 15 minutes of intermittent mixing, the dissolved amino acid is transferred to the ACT where 1M DCC (1 mL) is added.

Module **b** - Piperidine deprotection

Total time = 23 minutes

The first operation in module "b" is an NMP wash, followed by two 20% piperidine/NMP treatments; one 3- minute treatment followed by a 15-minute treatment. The module ends in a drain and an NMP wash.

Module **c** - DCM washes

Total time = 6 minutes

Module "c" consists of 8 DCM washes in the RV. The first and last washes also include a rinse of the resin sample line. Module **c** is only used after the last coupling in an Fmoc/HOBt/DCC synthesis to remove the remaining NMP and facilitate resin drying.

Module **d** - NMP washes

Total time = 7 minutes

Module "d" consists of 6 NMP washes in the RV. The first and last washes also include a resin-sample line rinse.

Module **e** - Transfer and washing

Total time = 7.5 minutes

The first part of module "e" consists of transferring the activated amino acid from the ACT to the RV. Next, the cartridge is washed with DCM. The DCM wash is mixed with MeOH in the ACT to dissolve the DCU. Then the ACT is drained and thoroughly rinsed with DCM.

Module f - Coupling

Total time = 20 minutes

This module consists of approximately 20 minutes of RV vortexing, mixing, and ACT mixing.

Note

Total coupling time is 71 minutes. The peptide-resin is in the presence of activated amino acid during module "e," "f," and "a."

Module g - Resin sampling

Total time = 8 minutes

The RV drains, the resin is washed with NMP, and a resin sample is taken.

Module h - Auto loading

Total time = 23.5 minutes

This module is used to load the first amino acid onto the HMP resin. The amino acid is dissolved in an NMP/DCM mixture and transferred to the ACT where 1M DCC (0.5 mL) is added to perform a symmetric anhydride activation. At the end of this module, approximately 0.1 equivalent of DMAP is added to the RV to act as a coupling catalyst when the activated amino acid reaches the RV.

This module is used in combination with other modules to perform the complete resin loading process. Module h can also be used for capping with benzoic anhydride.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the final peptide-resin (see "References" on page 3-22, reference 23).

To load Asn, Gln, Arg and His, refer to page 7-6.

Module i - Wait

Total time = 15 minutes

Module i consists of 1 step which provides a 15-minute waiting period.

Fmoc/HOBt/DCC 0.10 mmol

Chemical Usage

Before starting a synthesis, be sure that the Synthesizer has an adequate supply of chemicals. Table 4-20 shows the chemical usage per cycle for the Fmoc/HOBt/DCC 0.10 mmol synthesis of a 10-residue peptide. Note that some reagents are consumed faster than others. Also, due to add times, the usage per cycle of some chemicals, such as NMP, is greater on longer peptides. Approximately 90 unattended Fmoc/HOBt/DCC 0.10 mmol cycles can be performed with two 4-liter bottles in position 10 (NMP).

See page 4-24 for chemistry warnings.

Table 4-20. Chemical Usage Per Cycle, Fmoc/HOBt/DCC Chemistry, 0.10 mmol resin, 10-residue peptide

Bottle	Chemical	mL/Bottle	mL/Cycle	Cycles/Bottle
1	Piperidine	450	2	225
2	—	—	—	—
4	DMAP	200	NA	NA
5	—	—	—	—
6	MeOH	450	4	112
7	HOBt	200	1.6	120
8	DCC	200	1.8	110
9	DCM	4000	20	200
10	NMP	8000	66	90*
Waste bottle		9463	95	100

* See *Add Times and Chemical Usage* on page 7-54 for a detailed description of the effect of add times

WARNING ***CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.***

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the final peptide-resin (see "References" on page 3-22, reference 23).

Fmoc/HOBt/DCC 0.10 mmol Modules

Fmoc/HOBt/DCC 0.10 mmol modules are very similar to the Fmoc/HOBt/DCC 0.25 mmol modules in their basic function. However, each one has been modified to use less solvent and less time. For examples of cycles and Runs you can create with these modules, see "Fmoc/HOBt/DCC Cycles" on page 7-20.

Module a - Activation

Total time = 21 minutes

At the beginning of module "a," the amino acid cartridge name is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 mL) and 1M HOBt (1 mL) are added to the cartridge.

After 16 minutes of intermittent mixing, the dissolved amino acid is transferred to the ACT where 1M DCC (1 mL) is added.

Module b - Piperidine deprotection

Total time = 17 minutes

The first operation in module "b" is an NMP wash, followed by two 20% piperidine/NMP treatments: one 3-minute treatment followed by an 11-minute treatment. The module ends with a drain and an NMP wash.

Module c - DCM washes

Total time = 6 minutes

Module "c" consists of 8 DCM washes in the RV. The first and last washes include a resin-sample line rinse. After the last rinse, the DCM is cleared from the line. Module c is only used after the last coupling in an Fmoc/HOBt/DCC synthesis to remove the remaining NMP and facilitate resin drying.

Module d - NMP washes

Total time = 3 minutes

Module "d" consists of 5 NMP washes in the RV. The first, second and last washes include a rinse of the resin-sample line.

Module e - Transfer and washing

Total time = 6 minutes

The first part of module "e" consists of transferring the activated amino acid from the ACT to the RV, done in four transfers.

Next, the cartridge is washed with DCM. The DCM wash is mixed with MeOH in the ACT to dissolve the DCU. Then the ACT is drained and thoroughly rinsed with DCM.

Module f - Coupling

Total time = 8.5 minutes

This module consists of RV vortexing, mixing, and ACT mixing.

Note

Total coupling time is approximately 37 minutes. The peptide-resin is in the presence of activated amino acid during modules "a," "e," and "f."

Module g -Resin sampling

Total time = 5 minutes

The RV is drained, a resin sample is taken, and the resin is washed with NMP.

Module h - Auto loading

Total time = 23 minutes

This module is used to load the first amino acid onto the HMP resin and, in combination with other modules, to perform the complete resin loading process. Module "h" should not be used with Asn, Gln, Arg or His because of the solubility properties of these amino acids. See page 7-6 for directions for loading these amino acids.

You can use Module "h" for capping with benzoic anhydride.

Amino acid is dissolved in an NMP/DCM mixture and transferred to the ACT. Then 1M DCC in NMP (0.5 mL) is added to perform a symmetric anhydride activation. Finally, approximately 0.1 equivalent of DMAP is added to the RV. DMAP acts as a coupling catalyst when the activated amino acid reaches the RV.

Although it is possible to load Fmoc-Pro onto the HMP resin, the formation of a diketopiperazine during synthesis drastically reduces the yield of the final peptide-resin (see "References" on page 3-22, reference 23).

Module i -Wait

Total time =15 minutes

Module i programs a 15-minute waiting period.

Boc/HOBt/DCC Chemicals, Protocols and Modules

Chemicals Required for Boc/HOBt/DCC Chemistry

Table 4-21. Reagents and solvents for Boc/HOBt/DCC chemistry

Bottle #	Reagent	Part Number
1	N, N-Diisopropylethylamine (DIEA)	400136
2	Trifluoroacetic acid (TFA)	400137
4	Acetic anhydride	400660
5	Dimethyl sulfoxide/N-methylpyrrolidone (DMSO/NMP, 80/20)	400661
6	Methanol	400470
7	1.0 M 1-hydroxybenzotriazole in N-methylpyrrolidone (HOBt in NMP)	400662
8	1.0 M Dicyclohexylcarbodiimide in N-methylpyrrolidone (DCC in NMP)	400663
9	Dichloromethane (DCM)	400142
10	N-methylpyrrolidone (NMP)	400580
Waste	150 mL waste neutralizer	400230

Table 4-21 lists all chemicals used for synthesis on the ABI 433A Peptide Synthesizer using the Boc/HOBt/DCC Protocol.

A special gasket designed for Bottle 2 prevents splatter of TFA when you change the bottle. Replace this gasket each time you change Bottle 2.

Bottle 5 is formulated as a 80% DMSO/20% NMP solution, which is liquid at 8 °C (48 °F) or above. If the laboratory falls below this temperature, increase the percentage of NMP in the DMSO, and the cycle times for activation and coupling.

Refrigerate 1.0 M HOBt in NMP at 4 °C or store it in a freezer at –15 °C. This solution slowly decomposes to give a slightly yellow solution.

DANGER! ***CHEMICAL HAZARD. Acetic Anhydride is a combustible liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled and may cause allergic reactions. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.***

WARNING ***CHEMICAL HAZARD. 1.0 M N,N-Dicyclo-hexylcarbodiimide/N-Methyl-pyrrolidinone (DCC/NMP) is a combustible liquid and vapor. It may be fatal if it is inhaled. Exposure causes***

eye, skin, and respiratory tract burns and it is a possible developmental hazard. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** Diisopropylethylamine (DIEA) is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** Dimethylsulfoxide/N-methylpyrrolidone (DMSO/NMP) is a combustible liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** 1 M 1-Hydroxybenzotriazole/N-Methylpyrrolidone (HOBt/NMP) is a combustible liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental hazard. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** Methanol is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD.** *N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

DANGER! **CHEMICAL HAZARD.** *Trifluoroacetic acid (TFA) causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.*

Before you start synthesis, place **waste neutralizer in the waste container** to neutralize the TFA when you use Boc/HOBt/DCC chemistry. When using the waste neutralizer supplied by Applied Biosystems, add approximately 150 mL of waste neutralizer to an empty waste container.

Boc/HOBt/DCC Protocols

Boc/HOBt/DCC 0.50 mmol cycles use 2.0 mmol Boc-amino acids with 0.50 mmol resin in the 41 mL reaction vessel. Boc/HOBt/DCC 0.10 mmol cycles use the 1.0 mmol amino acid cartridge with 0.10 mmol resin and the 8 mL reaction vessel. Table 4-22 and Table 4-23 summarize these steps in both Boc/HOBt/DCC protocols.

Table 4-22. ABI 433 Instrument Boc/HOBt/DCC chain assembly time (minutes)

Operation and Reagents	0.50 mmol	0.10 mmol
TFA deprotection		
25% TFA in DCM	3	3
50% TFA in DCM	16	11
Washes and neutralizations		
DCM washes	3	2
5% DIEA washes	4	2
NMP washes	5	3
Coupling		
Boc-AA-HOBt ester in NMP	39	23
DMSO to make 15% DMSO/85%NMP	16	8
3.8 equiv. DIEA	5	4
Wash and resin sample		
NMP wash		
Resin sample	(2)	(2)
Capping		
10% Ac ₂ O, 5% DIEA in NMP	9	5
Washes		
DCM washes	4	4
Total Cycle Time	104*	65*

* Resin sampling adds two more minutes to the total cycle times of 104 and 65.0 minutes.

Table 4-23. Comparison of ABI 433A instrument Boc/HOBt/DCC scales

	Resin (mmol)	Amino Acid (mmol)	AA: Resin	Reaction Vessel	Waste per Cycle (mL)
0.50 mmol	0.50	2.00	4:1	41 mL	370
0.10 mmol	0.10	1.00	10:1	8 mL	120

Boc/HOBt/DCC 0.50 mmol

Chemical Usage

Before starting a synthesis, be sure that the synthesizer has an adequate supply of chemicals. Table 4-24 shows the chemical usage per cycle for the Boc/HOBt/DCC 0.50 mmol synthesis of a 10-residue peptide flow rates. Note that some reagents are consumed faster than others. Also, due to add times, the usage per cycle of some chemicals, such as TFA, DCM and NMP, is greater on longer peptides. (For a description of the affect of add times, see "Add Times and Chemical Usage" on page 7-54).

See page 4-33 for chemistry warnings.

Table 4-24. Chemical usage per cycle, Boc/HOBt/DCC chemistry, 0.50 mmol resin, 10-residue peptide

Bottle	Chemical	mL/Bottle	mL/Cycle	Cycles/Bottle†
1	DIEA	175	5.5	31
2	TFA	450	10	36*
4	Ac ₂ O	450	2.2	200
5	DMSO	450	3.3	135
6	MeOH	450	6.7	67
7	HOBt	200	3.3	60
8	DCC	200	3.6	55
9	DCM	8000	178	35***
10	NMP	4000	140	27
waste**		9463	363	26

** These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and TFA, 2.0 mL/18 sec. If flow rates on a ABI 433A instrument are greater than these cited, the number of cycles/bottle will be proportionately lower.

**The waste bottle should also contain 150 mL of ethanalamine as a neutralizer for TFA.

***DCM usage for 20 couplings = 200 mL/cycle. Cycles / bottle value based on two-bottle configuration of DCM.

WARNING ***CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.***

Boc/HOBt/DCC 0.50 mmol Modules

For examples of cycles and Runs you can create with theses modules, see "Boc/HOBt/DCC Cycles" on page 7-36.

Module a - Activation

Total time = 15 minutes

At the beginning of module "a," the name on the new amino-acid cartridge is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced.

NMP (3.3 mL) is added to the cartridge. After 6 minutes of intermittent mixing, 1M HOBt (2 mL) is added. After 3 minutes, the dissolved amino acid is transferred to the ACT where 1M DCC (2 mL) is added.

Because the peptide-resin is coupling in the RV during all but the first activation, the RV is vortexed during module “a.”

Module b - TFA Deprotection

Total time = 20 minutes

The first operation in module “b” is a DCM wash of the resin. This is followed by a 3-minute treatment with 25% TFA and a 16-minute treatment with 50% TFA. The module finishes with an RV drain.

Module c - DCM Wash

Total time = 3 minutes

Module “c” consists of 5 DCM washes. A resin-sample line wash is included in the first and the last washes. Module “c” is used two times in a synthesis cycle: once after the TFA deprotection (“b”) and once after the capping (“g”).

Module d - DIEA Neutralization and NMP Wash

Total time = 7.5 minutes

There are two treatments of approximately 5% DIEA in NMP. DIEA is added from the bottom, top, and from the resin-sample line to fully neutralize the entire vessel.

There are 6 NMP washes. A wash of the resin-sample line is included in the third and sixth washes.

Module e - Transfer and Washing

Total time = 8.5 minutes

In the first part of module “e,” the activated amino acid is transferred from the ACT to the RV in 4 portions.

Next, the cartridge is washed two times with DCM. This DCM is added to the ACT, along with MeOH, to dissolve the DCU. The DCM/MeOH solution is drained and the ACT is thoroughly washed with DCM.

Module f - Coupling and DMSO Addition

Total time = 30 minutes

In the first 29 minutes of module “f,” the RV is vortexed repeatedly at 12-second intervals while the peptide-resin is coupling in NMP. During the last minute of the module, DMSO is added to make a 15% DMSO solution. The coupling reaction is then continued with 15% DMSO/85% NMP.

Module g - DIEA Addition, Drain, Resin Sample and Capping

Total time = 16.5 minutes

DIEA is added to the RV and the coupling reaction continues in the first 5 minutes of module “g.” The RV is then drained, and NMP is added. Between steps 38 and 65, a resin sample is removed. Finally, the unreacted amines are capped with acetic anhydride in a mixture of NMP and DIEA.

Module h - Coupling

Total time = 20 minutes

Module “h” consists of a series of 12-second vortexes. Module “h” is used only once: in the last line of modules, in place of module “a.”

Module i - Wait

Total time = 15 minutes

Module “i” is only one step, a 15-minute wait. It is used only once: in the first module line, in place of modules “g” and “c.”

Boc/HOBt/DCC 0.10 mmol

Chemical Usage

Table 4-25 summarizes the chemical usage per cycle for the Boc/HOBt/DCC 0.10 mmol synthesis of a 10-residue peptide. Note that some reagents are consumed faster than others. Also, because of add times, the usage per cycle of some chemicals, mainly TFA, DCM and NMP, will be greater on longer peptides.

See page 4-33 for chemistry warnings.

Table 4-25. Chemical Usage Per Cycle, Boc/HOBt/DCC chemistry, 0.10 mmol resin, 10-residue peptide

Bottle	Chemical	mL/Bottle	mL/Cycle	Cycles/Bottle***
1	DIEA	175	3.3	53
2	TFA	450	3.3	110*
4	Ac ₂ O	200	1.0	200
5	DMSO	450	1.0	450
6	MeOH	450	4.0	112
7	HOBt	200	1.6	120
8	DCC	200	1.8	110
9	DCM [†]	8000	62	100*
10	NMP	4000	42	77
waste **		9463	120	79

* For a detailed description of the effect of add times, see "Add Times and Chemical Usage" on page 7-54.

** The waste bottle should also contain 150 mL of ethanolamine as a neutralizer for TFA

*** These figures are based on chemical usage with average flow rates, i.e., NMP, 2.5 mL/5 sec; DCM, 3.4 mL/5 sec; and TFA, 2.0 mL/18 sec. If flow rates on a ABI 433A instrument are greater than these cited, the number of cycles/bottle will be proportionately lower.

[†] DCM cycles/bottle value based on two-bottle configuration.

WARNING **CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak hazardous chemicals. Secure each four-liter bottle in a low-density polyethylene safety container. Fasten the cover on the safety container and lock the handles in an upright position.**

Boc/HOBt/DCC 0.10 Modules

For examples of cycles and Runs you can create with these modules, see "Boc/HOBt/DCC Cycles" on page 7-36.

Module a - Activation

Total time = 17 minutes

At the beginning of module "a," the old cartridge is ejected and the new cartridge is advanced.

NMP (1.7 mL) is added to the cartridge followed by 1M HOBt (1mL). After 11 minutes, the dissolved amino acid is transferred to the ACT where 1M DCC (1 mL) is added.

Because the peptide-resin is coupling in the RV during all but the first activation, the RV is vortexed during module "a."

Module b - TFA Deprotection

Total time = 16 minutes

The first operation in module "b" is a DCM wash of the resin. This wash is followed by a 3-minute treatment with 25% TFA and a 11-minute treatment with 50% TFA. The module finishes with an RV drain.

Module c - DCM Wash

Total time = 1.5 minutes

Module "c" consists of 4 DCM washes with a resin-sample line wash added to the first wash. Module c is used two times in a synthesis cycle: once after the TFA deprotection (module "b") and once after capping (module "g").

Module d - DIEA Neutralization and NMP Wash

Total time = 4.5 minutes

There are two treatments of approximately 5% DIEA in NMP. DIEA is added from the bottom, the top and from the resin-sample line to fully neutralize the entire vessel.

There are 5 NMP washes with a resin-sample line wash added to the third and fifth washes.

Module e - Transfer and Washing

Total time = 5 minutes

In the first part of module "e" the activated amino acid is transferred from the ACT to the RV.

The cartridge is washed with DCM, which is then added to the ACT, along with MeOH, to dissolve the DCU. The DCM/MeOH solution is drained and the ACT is thoroughly rinsed with DCM.

Module f - Coupling

Total time = 20 minutes

Module "f" consists of 20 minutes of coupling. In the Boc/HOBt/DCC 0.10 mmol cycles it is used only during the last cycle.

**Module g - DMSO Addition, DIEA Addition,
Drain, Resin Sample, and Capping** Total time = 16.5 minutes

DMSO is added to the RV to make up a 15% DMSO solution and then coupling continues for 8 minutes. DIEA is added next and 4 more minutes of coupling follow. The RV is then drained and NMP is added. If you are taking a resin sample, it is removed at this point. Finally, any unreacted peptides are capped with acetic anhydride in a mixture of NMP and DIEA.

Module h - Final DCM Wash Total time = 4 minutes

Module “h” consists of 8 DCM washes. With the last wash, DCM is cleared out of the resin-sampling line. This module is used only after the last coupling.

Module i - Wait Total time = 15 minutes

Module “i” is only one step, a 15-minute wait. It is used only once, in the first module string in place of modules “g” and “c.”

5 Monitoring a Synthesis

This chapter describes both basic and conditional monitoring of a synthesis, including Monitoring options, related functions, and modules.

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An Overview of the *FastMoc*TM Monitoring Cycles

The pre-defined Chemistry files in SynthAssist[®] Software with “Mon” in the name (for example, MonPrevPk, Mon1st-X, CondMonPrevPk and CondMon1-X) contain modules that allow conductivity monitoring of deprotection with feedback control of coupling. Conductivity monitoring works on the principle that, during deprotection, a conductive carbamate salt is generated when the Fmoc group is removed by treatment with piperidine in NMP (see page 3-6). The ABI 433A Peptide Synthesizer monitors the extent of deprotection in a cycle by comparing the conductivity of two samples of deprotection solution. As deprotection goes to completion, less and less Fmoc is removed, with correspondingly lower conductivity readings.

Difficult deprotections during a peptide synthesis are generally sequence-dependent and result from the characteristics of the peptide-resin matrix. With every coupling, the peptide grows longer and becomes more likely to acquire the features associated with secondary structure, such as random coils and beta sheeting. The conformation of the peptide-resin may affect the chemical reactivity of the synthesis through the formation of inter- and intra-chain interactions. Some peptide-resin structures may hinder the growing N-terminus and this hindered structure decreases reactivity. Inter-chain interactions may increase the effective cross-linking of the matrix, causing the structure to collapse and reducing the diffusion rate through the gel.

Extending deprotection time allows more time for NMP and piperidine to diffuse through the gel and react with more of the available N-terminal amino acids. Peptide chemists at Applied Biosystems have observed that when a peptide-resin requires extended deprotection time to improve chemical reactivity, the coupling time should also be extended.

How Conductivity Monitoring Cycles Work

With the pre-defined Chemistry files for conductivity monitoring, the deprotection module contains a “deprotection loop.” This loop contains all the functions required to deliver deprotection reagents to the reaction vessel and vortex and drain the vessel. At the end of each deprotection loop, the deprotection solution flows out of the reaction vessel into a conductivity flow cell and then to the waste bottle. While the deprotection solution is in the flow cell, a conductivity reading is taken once every second. The highest conductivity value is stored as a peak value in the ABI 433A Peptide Synthesizer software.

You can define the criteria that determine how many times the deprotection loop can be repeated. An algorithm manages the process of analyzing the data collected at the end of each deprotection loop. When the user-defined criteria are met, the deprotection module ends.

The number of times the deprotection loop was repeated is saved in the ABI 433A instrument software. With Basic Monitoring, as synthesis proceeds, the controller feeds back the number during the coupling module. As a result of the feedback, the loop in the coupling module is repeated for the same number of loops that were required for complete deprotection.

Conductivity monitoring with feedback allows real-time modification of synthesis cycles in progress, so that cycle times vary to accommodate diffusion rate changes in the peptide-resin matrix. In this manner, the rate of the synthesis chemistry controls the ABI 433A instrument.

Pre-defined Chemistry Files for Conductivity Monitoring

There are two categories of SynthAssist Chemistry files for conductivity monitoring:

- Basic Monitoring
- Conditional Monitoring

With Basic Monitoring, the number of monitored deprotection loops performed in module “B” determines the number of feedback coupling loops performed in module “F.” The other modules in the cycle are not affected by monitoring.

With Conditional Monitoring, the number of deprotection loops performed in module “B” is compared to a user-defined limit. Conditional modules become active when the number of deprotection loops reaches the user-defined limit. You can use conditional modules for steps such as capping or double coupling that are performed only when the user-defined limits are reached.

Monitoring Functions

Monitoring functions are designed to perform various tasks necessary for monitoring. Table 5-1 displays the monitoring functions used in the monitoring modules. Unlike other functions, “T” in the monitoring functions does not represent time. Instead, “T” may represent a conductivity baseline, the maximum number of monitoring loops, a percentage, or a monitoring channel.

IMPORTANT *With the exception of Function 133 (MonBegLoop), always assign some value other than zero for “T” in the monitoring functions. The controller ignores a monitoring function when “T”=0.*

Table 5-1. Monitoring Functions in the Monitoring Modules

Function Number	Function Name	Description
128	Mon 1stPk -X	Sets baseline ($X/10=T$) and begins conductivity data collection. Algorithm compares last peak minus baseline to first peak minus baseline.
130	MonPrevPk	Begins conductivity data collection without setting baseline. Algorithm compares last two sequential peaks. "T"=1, perform function.
131	Mon Stop	Stops data collection. "T"=1, perform function.
132	Save MonPk	Saves highest value collected during deprotection. "T"=1, perform function.
133	MonBegLoop	Repeats monitored loop until either the percentage difference (in Fxn 134) or a set number of loops occurs. "T" = number of loops.
134	MonEndLoop	Determines conditions that end monitored deprotection loop. "T" = % x 10.
135	Mon Reset	Eliminates previous peak values stored. "T"=1, the channel for conductivity.
136	SkipModMon	Skips module steps when conditions not met. "T"=1, the channel for conductivity
137	Do ModMon	Performs module steps when conditions not met. "T"=1, the channel for conductivity

The rest of this section explains how you can use these functions to customize deprotection and define the conditions that add capping or double coupling modules when they are needed.

Basic Monitoring

Four SynthAssist Chemistry files contain modules for monitoring and extending deprotection and coupling only. These are the Basic Monitoring Chemistry files:

- *FastMoc* 0.10 Mon 1st-X
- *FastMoc* 0.25 Mon 1st-X
- *FastMoc* 0.10 MonPrevPk
- *FastMoc* 0.25 MonPrevPk

With Basic Monitoring, module “B” (Deprotection) monitors the conductivity of the deprotection solution. It repeats the deprotection loop until certain pre-defined conditions are met. The number of deprotection loops in module “B” is counted and ‘feeds back’ to module “F.” Module “F” (Coupling, etc./Feedback) performs coupling with the feedback. As a result, the coupling loop in module “F” is performed the same number of times as the monitored deprotection loops in module “B” to ensure adequate coupling time after multiple deprotections.

Table 5-2 lists the tasks performed by the modules used in the Basic Monitoring Chemistry files.

Table 5-2. *FastMoc* modules with Basic Monitoring

Module letter	Synthesis task
A	Dissolves amino acid
B	Deprotection/ MonPrevPk
B	Deprotection/ Mon 1stPk-X
C	Capping with Ac ₂ O solution
c	DCM wash
D	NMP washes
E	Transfer
F	Coupling, etc./feedback
G	Resin sample
H	Load and Cap
I	Wait

***FastMoc* Basic Monitoring Cycles**

Table 5-3 displays the cycles and modules that compose the pre-defined Basic Monitoring Chemistry files. The name of the Monitoring Chemistry file contains the name of the algorithm used in module “B” (Deprotection). See page 5-7 for a description of the module “B” algorithms.

Table 5-3. Cycles in FastMoc Basic Monitoring Chemistry files

Cycle	MonPrevPk	Mon 1st -X
Single Couple (SC)	BADEF	BADEFD
Cycle 1-Amide	cDBADEF	cDBADEFD
Single Couple with Resin Sample (RS)	BADEFG	BADEFDG
Cycle 1-SC/RS	cDBADEFG	cDBADEFDG
Single Couple/Ac ₂ O capping	BADEFCD	BADEFCD
Cycle 1-SC/Ac ₂ OCapping	cDBADEFCD	cDBADEFCD
Double Couple (DC)	BADEIADEF	BADEIADEFD
Cycle 1-DC	cDBADEIADEF	cDBADEIADEFD
Double Couple/2 RS	BADEIADGEFDG	BADEIADGEFDG
Cycle 1-DC/2 RS	cDBADEIADGEFDG	cDBADEIADGEFDG
Double Couple/Ac ₂ O capping	BADEIADEFCD	BADEIADEFCD
Cycle 1-DC/Ac ₂ O Capping	cDBADEIADEFCD	cDBADEIADEFCD
Final Deprotection	BDc	BDc
Final Acetylation	BIDCDc	BIDCDc
Loading & Benzoic Anhydride Capping	HF	HF
Complete Wash	cD	cD
NMP Wash	D	D
DCM Wash	c	c

- Use the Cycle 1- cycles as the first cycle in a synthesis that begins with an amide resin.
- Use the Complete Wash cycle as the first cycle with a preloaded resin.
- Use the Loading & Benzoic Anhydride Capping cycle as the first cycle with HMP resins.

Table 5-4 displays the cycles that define the Default Sets for the Basic Monitoring Chemistry files.

Table 5-4. Default sets for FastMoc Basic Monitoring Chemistry files

AA	Cycle	MonPrevPk	Mon 1st -X
Default	Single Couple	BADEF	BADEFD
Preload	Complete Wash	cD	cD
Load	Load & Benzoic Anhydride Capping	HF	HF
Amide	Cycle1-amide	cDBADEF	cDBADEFD
Other	<<None>>		
End	Final Deprotection	BDc	BDc

Choosing a Monitoring Option

With conductivity monitoring, an algorithm analyzes two pieces of information to determine the efficiency of deprotections in each cycle:

- The number of times the deprotection loop has been repeated
- The difference between the last deprotection peak and an earlier peak, expressed as a percentage

When these conditions match pre-determined conditions defined by the user, the ABI 433A instrument controller ends the deprotection module.

Two different algorithms are available for conductivity monitoring of deprotection on the ABI 433A instrument. When you choose a monitoring file, you automatically use the algorithm assigned to it.

Note *One algorithm manages each deprotection module “B.” Because there are two possible algorithms to choose from, there are two deprotection modules “B” for the 0.10 mmol Basic Monitoring Chemistry file and two deprotection modules “B” for the 0.25 mmol Basic Monitoring Chemistry file. The name of the algorithm is found in the function name and is repeated in the module name, and in the Basic Monitoring file name (Table 5-5).*

Table 5-5. Monitoring Algorithms in Basic Monitoring “B” Modules

Basic Monitoring File	Deprotection Module	Monitoring Algorithm
0.10 Mon1st-X	B-Deprotection/ Mon1stPeak-X	Fxn 128, Mon 1stPk-X
0.25 Mon1st-X	B-Deprotection/ Mon1stPeak-X	Fxn 128, Mon 1stPk-X
0.10 MonPrevPk	B-Deprotection/ MonPrevPk	Fxn 130, MonPrevPk
0.25 MonPrevPk	B-Deprotection/ MonPrevPk	Fxn 130, MonPrevPk
1.0 MonPrevPk	B-Deprotection/ MonPrevPk	Fxn 130, MonPrevPk

IMPORTANT *In the monitoring functions, which include Function 128 through Function 149, “T” does not represent time. Instead, “T” may represent a conductivity baseline, the maximum number of monitoring loops, a percentage, or a monitoring channel.*

Function 130: Monitor Previous Peak

The algorithm applied by Function 130, Monitor Previous Peak, compares the last two peaks a series of deprotections. The value of “T” for Function 130 is 1, which simply directs the controller to perform the function.

You can influence the way Function 130 operates by changing the value of “T” for Functions 133 (MonBegLoop) and 134 (MonEndLoop). See page 5-13 for an explanation of how the monitoring functions work in the Deprotection/MonPrevPk module.

Function 130, Monitor Previous Peak

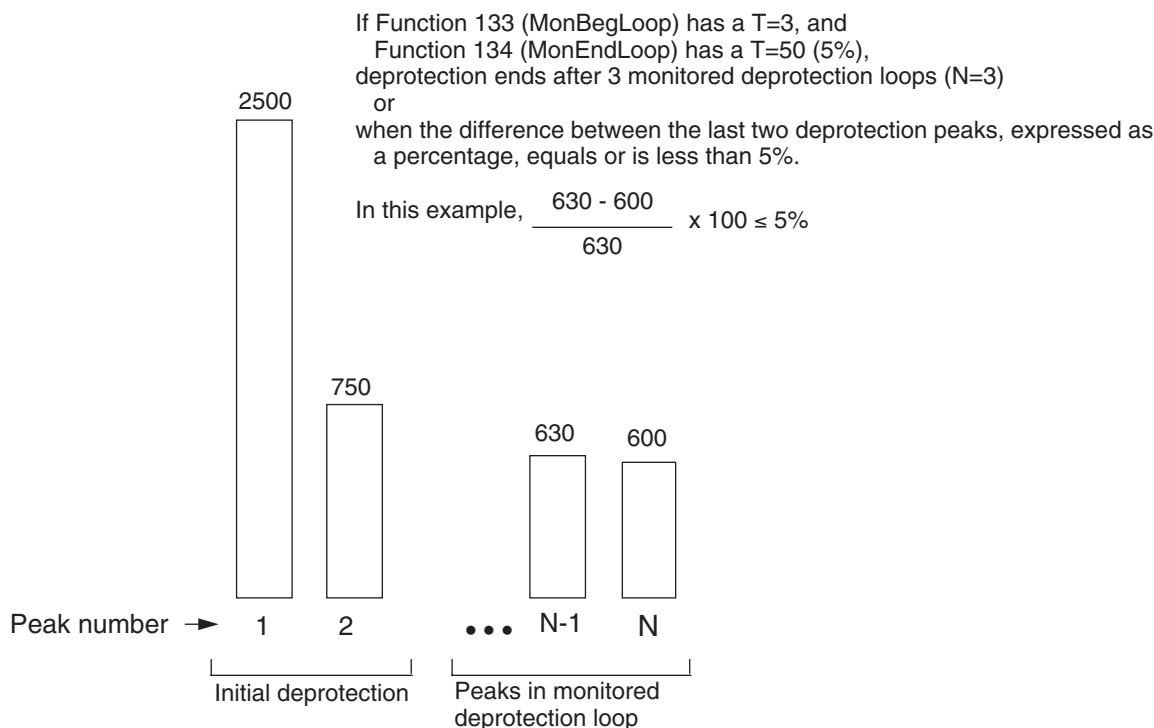


Figure 5-1. Example of Function 130, monitoring previous peak

When you use the Function 130 (MonPrevPeak) monitoring option, you do not need to know the baseline conductivity of the piperidine and NMP reagents. The conductivity baseline varies from instrument to instrument and may change when you change reagent lot numbers. With Function 130, a minimum of 3 deprotections always occurs in each cycle. Deprotection is explained in greater detail on page 5-10. As a result, cycles that use Function 130 use slightly more piperidine than those that use Function 128.

Function 128: Monitor First Peak Minus X

The algorithm applied by Function 128, “Monitor First Peak - X,” compares the initial conductivity peak generated in the deprotection module to the last conductivity peak in the deprotection loop. The “X” in the algorithm name refers to the baseline conductivity of the piperidine and NMP that are added to the reaction vessel during deprotection. The value of “X” is determined by running Flow Test 22 (.10 mmol scale) and Flow Test 23 (.25 mmol scale).

Note *The 1.0 mmol scale has no 1-X algorithm; therefore, “X” cannot (and need not) be determined.*

The value of “T” in Function 128 is the conductivity baseline divided by ten. You enter the conductivity baseline, divided by ten, for the value of “T” wherever Function 128 appears in a module.

Conductivity Baseline To determine the conductivity baseline for your instrument, perform Flow Test 22 for *FastMoc* 0.10 mmol Chemistry, or Flow Test 23 for *FastMoc* 0.25 mmol Chemistry (see page 6-52). The conductivity baseline varies from instrument to instrument and may change when you change reagent lot numbers.

Data Analysis with Function 128 The Function 128 algorithm, First Peak Minus X, performs four steps when it analyzes conductivity data:

1. Subtracts the baseline conductivity value from each deprotection peak.
2. Divides the corrected value of the last deprotection peak by the corrected value of the first deprotection peak.
3. Converts the results to a percentage.
4. Compares this percentage to the user-defined percentage determined by the value of “T” in Function 134 (MonEndLoop). If the calculated percentage is equal to or less than the user-defined percentage, deprotection ends.

You can influence the algorithm by changing the value of “T” for Functions 128, 133, and 134 in Module “B.”

Function 128, Monitor 1st Peak-X

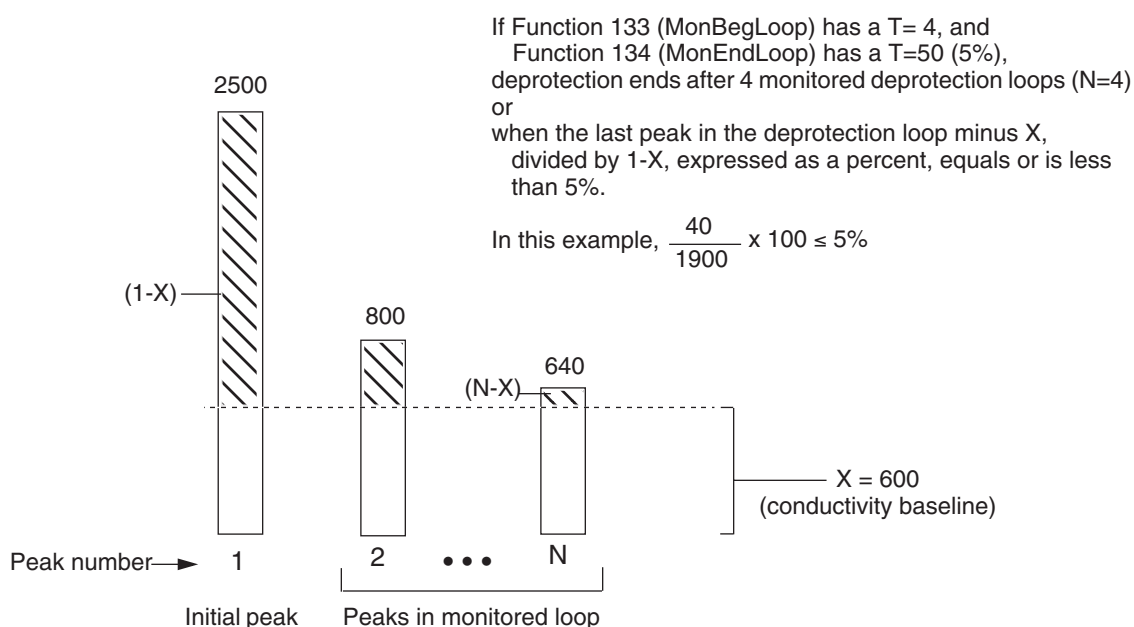


Figure 5-2. Example of Function 128, monitoring 1st Peak-X

A minimum of two deprotections occur with Function 128. Cycles that use Function 128 may use less piperidine and more NMP than those with Function 130, depending on the values you choose for Functions 133 (MonBegLoop) and 134 (MonEndLoop).

Characteristics of Basic Monitoring Module “B” (Deprotection)

Each Module “B” contains a function that assigns an algorithm for analyzing the conductivity data. The algorithm function occurs in two places in the deprotection module: once during initial deprotection and again in the monitored deprotection loop.

Initial deprotection

The first occurrence of the algorithm function generates the first deprotection peak for that cycle. With the **Function 128 (Mon 1stPk-X)** algorithm, the initial peak results from one deprotection only. After baseline correction, the value of this initial peak is compared to the subsequent peaks in the cycle. The subsequent deprotection peaks are generated by the second occurrence of Function 128 (Mon 1stPk-X), during the monitored loop.

The **Function 130 (MonPrevPk)** algorithm first occurs in a deprotection loop that is not monitored but is performed twice. During the first deprotection, most of the Fmoc protecting group is removed, so the first peak is much larger than the second peak. The second deprotection peak in the initial deprotection loop is much closer in value to the subsequent deprotection peaks in the monitored loop.

Subsequent deprotections in the monitored loop

In the *FastMoc* modules without monitoring, deprotection occurs with a set number of piperidine deliveries per cycle, usually two. The Basic Monitoring deprotection modules allow additional piperidine delivery steps as needed for difficult deprotections. To accomplish this, piperidine delivery steps are placed within “loops” (see page 8-32 for a discussion of loop functions). Monitored deprotection loops are introduced with Function 133 (MonBegLoop) and completed with Function 134 (MonEndLoop).

The differences between Function 128 and Function 130

With Function 128 (Mon 1stPk-X), there is *one* initial deprotection peak, followed by at least *one* deprotection in a monitored loop. With Function 130 (MonPrevPk), there are always *two* deprotections in the initial deprotection loop, followed by at least *one* deprotection in a monitored loop. It follows that there are minimally two deprotections completed with Function 128, but a minimum of three deprotection completed with Function 130. So, Function 128 (Mon 1stPk-X) requires less piperidine than Function 130 (MonPrevPk).

When you use Function 128 (Mon 1stPk-X), you must determine the conductivity baseline, and insert that number, divided by ten, wherever Function 128 occurs in every module. The conductivity baseline varies from

one instrument to the next and can change when you change reagent lot numbers. You do not have to know the conductivity baseline when you use Function 130 (MonPrevPk).

Two conditions that end the monitored deprotection loop

The value of “T” in two different monitoring functions determines the conditions that end the monitoring loop. In Function 133 (MonBegLoop), the value of “T” determines the maximum number of times the monitored loop is repeated. In Function 134 (MonEndLoop), the value of “T” represents a percentage multiplied by ten. The percentage is applied to the data to compare two deprotection peaks.

Table 5-6 shows the values of “T” that have been written into the function in the pre-defined deprotection modules. You may change the values of “T” in Functions 128, 133, and 134 to customize the deprotection module.

Table 5-6. Values of “T” in Basic Monitoring Deprotection Modules

Module B	Fxn 128*	Fxn 130	Fxn 133	Fxn 134
Deprotection/ Mon 1st Pk - X, 0.10 mmol	110	—	4	100
Deprotection/ Mon 1st Pk - X, 0.25 mmol	110	—	4	100
Deprotection/ MonPrvPk, 0.10 mmol	—	1	3	100
Deprotection/ MonPrvPk, 0.25 mmol	—	1	3	100
Deprotection/ MonPrvPk, 1.0 mmol	—	1	3	100

*The value of “T” in Function 128 varies with reagents. Determine “T” in Flow Test 22 or Flow Test 23.

IMPORTANT *In the monitoring functions, which include Function 128 through Function 149, “T” does not represent time. Instead, “T” may represent a conductivity baseline, the maximum number of monitoring loops, a percentage, or a monitoring channel.*

Function 133 (MonBegLoop) The value of “T” in Function 133 represents the maximum number of deprotection loops in module “B.” This value varies depending on:

- The algorithm used
- The anticipated difficulty of a deprotection
- The value of “T” in Function 134 (MonEndLoop)

In general, when you use the Function 128 (Mon 1stPk-X) algorithm, the value of “T” in Function 133 is higher than that of Function 130 (MonPrevPk) algorithm. With Function 130, the initial deprotection loop generates two deprotection peaks. As a result, fewer deprotections are necessary in the subsequent deprotection monitoring loop. As a guideline, Applied Biosystems suggests you set the value of “T” in Function 133 (MonBegLoop) between 2 and 6 with either algorithm.

<i>Note</i>	<i>The total number of deprotections possible in any cycle equals the maximum number of deprotections in the monitored deprotection loop—defined by Function 133—plus the number of initial deprotections.</i> <i>With Function 128, Mon 1st Pk-X, there is only one initial deprotection.</i> <i>With Function 130, Mon PrevPk, there are two deprotections in the initial deprotection loop.</i>

Function 134 (MonEndLoop) The value of “T” in Function 134 represents a percentage, multiplied by 10. With the Function 128 (Mon 1stPk-X) algorithm, the percentage in Function 134 represents the relation of the last deprotection peak generated to the initial deprotection peak. With the Function 130 (MonPrevPk) algorithm, the percentage is used to compare two sequential deprotection peaks. As a guideline, Applied Biosystems suggests you set the value of “T” in Function 134 (MonEndLoop) between 50 and 150.

IMPORTANT *The value of “T” for Function 134 represents a percentage, multiplied by ten.*

Example:

- With Function 128 (Mon 1stPk-X), if you set the value of “T” in Function 134 (MonEndLoop) at 60, the algorithm compares the last deprotection peak to the first deprotection peak until the last peak is $\leq 6\%$ of the first peak **or** until the maximum number of monitored deprotection loops—the value of “T” in Function 133 (MonBegLoop)—have occurred.
- With Function 130 (MonPrevPk), if you set the value of “T” in Function 134 (MonEndLoop) at 85, the algorithm compares the last two sequential peaks until the difference between the two peaks is $\leq 8.5\%$ **or** until the maximum number of deprotection loops—the value of “T” in Function 133 (MonBegLoop)—have occurred.

FastMoc Module “B” (Deprotection/MonPrevPeak)

Two Basic Monitoring files contain module “B” (Deprotection/MonPrevPeak), which applies the Function 130 algorithm to the conductivity data:

- *FastMoc 0.10* MonPrevPk
- *FastMoc 0.25* MonPrevPk

Each time module “B” (Deprotection/ Ω MonPrevPeak) occurs in these Basic Monitoring files, it contains the same monitoring functions, in the same order and in the same steps, regardless of scale.

This example describes how you can use the monitoring functions to define your criteria for ending deprotection.

	Step#	Fxn#	Fxn Name	“T”	
	3	135	Mon Reset	1	← T= Channel 1
	<i>(Resin solvation, first delivery of piperidine and NMP)</i>				
Initial deprotection	27	130	MonPrevPk	1	← T=1 tells controller to perform the function
	<i>(Two deprotection loops)</i>				
	29	131	Mon Stop	1	
	30	132	Save MonPk	1	
Monitored deprotection loops	32	133	MonBegLoop	3	← T=maximum # loops
	<i>(Loop deliveries of piperidine and NMP)</i>				
	55	130	MonPrevPk	1	← T=1 tells controller to perform the function
	57	131	Mon Stop	1	
	58	132	Save MonPk	1	
	59	134	MonEndLoop	50	← T=% x 10
	<i>(Rinse valve blocks and delivery lines with NMP, flush with gas)</i>				

Figure 5-3. Using monitoring functions for ending deprotection

Step 3: Function 135 (Mon Reset) eliminates any conductivity values that may be stored in the memory. In this function, “T” =1 tells the controller which channel is collecting conductivity data.

Step 27: Function 130 (MonPrevPk) is in a deprotection loop that is performed twice and then stops. This first deprotection loop does not contain Functions 133 and 134, so it is not a monitored loop. During the first deprotection, most of the Fmoc protecting group is removed, so the first peak is much larger than the second peak. The second peak in the initial

deprotection loop is much closer in value to the subsequent deprotection peaks in the monitored loop. The value of “T” =1, which tells the controller to perform the function.

Step 29: Function 131 (Mon Stop) stops conductivity data collection. The value of “T” = 1 tells the controller to perform the function.

Step 30: Function 132 (Save MonPk) saves the largest data point collected as the peak. The value “T” = 1 tells the controller to perform the function. This function is in the initial deprotection loop that is only repeated twice.

Step 32: Function 133 (MonBegLoop) begins the monitored deprotection loops. The user-defined value of “T” in this example is 3. Here, “T” defines the maximum number of monitored deprotection loops that can be performed in this module. When this maximum number of loops have been performed, the deprotection module ends.

Step 55: Function 130 (MonPrevPk) is inside a monitored loop that generates deprotection peaks. The value “T” =1 tells the controller to perform the function.

Step 57: Function 131 stops conductivity data collection. The value “T” =1 tells the controller to perform the function.

Step 58: Function 132 saves the largest data point collected as the peak. the value “T” =1 tells the controller to perform the function.

Step 59: Function 134 (MonEndLoop) ends the monitored loop when the difference between two sequential peaks is equal to or less than a user-defined percentage. The value of “T” is this percentage multiplied by 10.

In this example, deprotection ends when the difference between the last two sequential peaks is equal to or less than 5%. If the percentage difference is **not** equal to or less than 5% before 3 monitored deprotection loops have occurred, the deprotection loop ends anyway. The number of monitored loops counted “feeds back” to module “F” to determine the number of coupling loops in the cycle.

IMPORTANT *In the Basic Monitoring Chemistry files, the value of “T” in Function 133 (MonBegLoop) defines the maximum number of monitored loops that can be performed in any cycle. If the percentage difference defined by Function 134 (MonEndLoop) does not occur, deprotection ends when the maximum number of loops have been performed.*

Conditional Monitoring Overview

The conditional modules provide alternatives to the Basic Monitoring modules for difficult deprotections during synthesis. Conditional modules contain Functions 136 and 137, which act like switches, turning conditional modules “off” or “on” when a pre-defined set of conditions exist. Conditional modules can direct the ABI 433A Peptide Synthesizer to extend both deprotection and coupling beyond the time allowed for those modules in the Basic Monitoring Chemistry files. Additional conditional modules are available for optional capping after a difficult deprotection, and for conditional double coupling.

Five Conditional Monitoring Chemistry files use conditional modules:

- *FastMoc* 0.10 CondMon1-X
- *FastMoc* 0.10 CondMonPrevPk
- *FastMoc* 0.25 CondMon1-X
- *FastMoc* 0.25 CondMonPrevPk
- *FastMoc* 1.0 CondMonPrevPeak

Module “B” (Deprotection) in the Conditional Monitoring files acts like module “B” in the Basic Monitoring files. In both modules, the conductivity of the deprotection solution is monitored and the deprotection loop is repeated until either a maximum number of loops has been performed or a pre-defined percentage difference between two peaks is detected. But unlike Basic Monitoring files, when module “B” ends in Conditional Monitoring, the ABI 433A instrument controller compares the maximum number of monitored loops allowed for the cycle (defined in Function 133 (MonBegLoop)) to the actual number of loops completed. The results of this comparison determine whether or not the conditional modules become active.

Capping

Sometimes in an area of difficult deprotection peptide chains fail to couple to the activated amino acid. Chains that fail to couple at one or more cycles become deletion peptides after cleavage of the peptide from the resin. These deletion peptides can be difficult to separate from the desired peptide product. The conditional capping module covalently blocks, or caps, the free amino terminuses of chains that fail to couple. During purification after cleavage, the capped deletion peptides are easier to separate from the desired peptide.

Double Coupling

In addition to extending deprotection time during a difficult deprotection, you may choose to increase the concentration of activated amino acid available. When you increase the ratio of activated amino acid to N-terminal amino acid sites, you improve the coupling efficiency.

Cycles for conditional double coupling contain both conditional modules “i” and “a.” When you use the conditional double couple cycle, you place two cartridges for the same amino acid on the guideway. If deprotection is not difficult, conditional module “i” ejects the first amino acid cartridge and advances the second amino acid cartridge in the middle of the cycle. When the subsequent cycle begins, the second amino acid cartridge is ejected, without being punctured or used.

<i>Note</i>	<i>With module “i,” when the duplicate amino acid cartridge is not used for a double couple, it is ejected intact. Retrieve this unpunctured cartridge and use it in another synthesis.</i>

If deprotection is difficult, conditional module “i” is switched off and, in conditional module “a” the needle punctures the duplicate amino acid cartridge. The cartridge’s contents are activated, DIEA is added, and the solution is transferred to the reaction vessel just before coupling (conditional module “f”) begins.

Additional NMP Washes

When reduced diffusion rates through the peptide-resin matrix necessitate extended deprotection and coupling, additional NMP washes at the end of the cycle help wash the coupling mixture out of the reaction vessel and the valve blocks. Washing minimizes carryover into the deprotection in the next cycle by minimizing the presence of conductive hexafluorophosphate, amino acid, HBTU, and DIEA. In general, these additional washes are only needed when the synthesized peptide contains more than thirty amino acids.

When Are Conditions Met?

Conditions are met when less than the maximum number of deprotection loops, defined by "T" in Function 133, are performed in module "B." Figure 5-4 illustrates the cycle possibilities available with the Conditional Monitoring cycles in SynthAssist Software. In any Conditional Monitoring cycle, if the conditions are met, the cycle ends when the coupling module "F" ends.

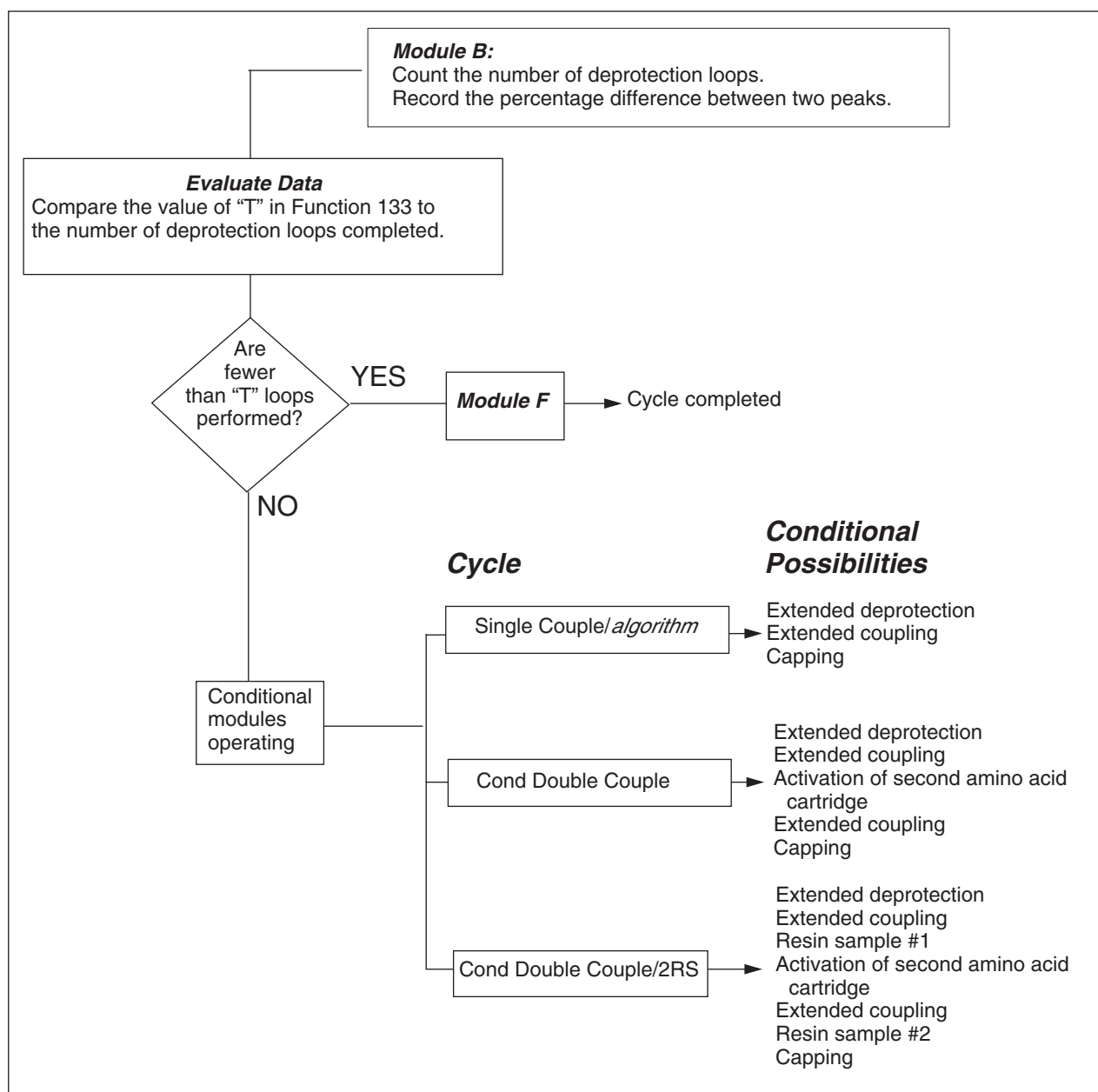
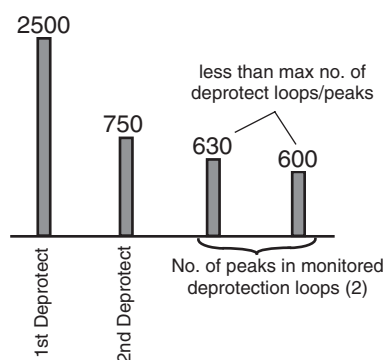


Figure 5-4. Flow chart of possibilities with Conditional Monitoring cycles

After deprotection module “B” has been completed, the controller compares the value of “T” for Function 133 (MonBegLoop) to the actual number of monitored deprotection loops that occurred in the cycle.

- If the maximum number of deprotection loops was not performed, conditions are met.
- When the value of “T” for Function 133 (MonBegLoop) equals the actual number of monitored deprotection loops, the controller interprets this as conditions not met.

Monitor Previous Peak: “Conditions Met”



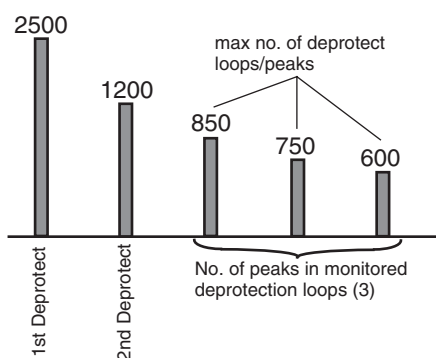
Max Deprotection Loops = 3 (Func 133 T=3)
User-Defined Percentage = 5% (Func 134 T=50)

$$\frac{\text{next to last peak} - \text{last peak}}{\text{next to last peak}} =$$

$$\frac{630 - 600}{630} = \frac{30}{630} = 4.8\%$$

4.8% is less than 5% and the number of deprotection loops (2) were less than the maximum (3). Therefore, Conditions are Met.

Monitor Previous Peak: “Conditions Not Met”



Max Deprotection Loops = 3 (Func 133 T=3)
User-Defined Percentage = 5% (Func 134 T=50)

$$\frac{\text{next to last peak} - \text{last peak}}{\text{next to last peak}} =$$

$$\frac{750 - 600}{750} = \frac{150}{750} = 20\%$$

The number of deprotection loops reached maximum of 3 (Func 133 T value). Therefore, Conditions are Not Met.

Figure 5-5. Examples of conditions met and conditions not met

Functions 136 and 137

Functions 136 and 137 occur at the beginning of conditional modules. These functions act like switches that turn the module off or on, depending on the deprotection conditions.

Function Number	Function Name	Description
136	SkipModMon	Skips module steps if “conditions not met”
137	Do ModMon	Performs modules steps if “conditions not met”

Function 136 (SkipModMon) tells the controller to skip the rest of the steps in the module only when the conditions are **not** met. Function 136 occurs at the beginning of the coupling module “F.” The value of “T” in Function 136 is either 1, 2, or 3, depending on the channel you are monitoring. With conductivity monitoring, “T” in Function 136 always equals one (T=1).

Function 137 (Do ModMon) tells the controller to perform the rest of the steps in the module when the conditions are **not** met. Function 137 occurs at the beginning of modules that extend deprotection, activate additional amino acid for double coupling, extend coupling, take an extra resin sample, or add capping steps. The value of “T” is either 1, 2, or 3, depending on the channel you are monitoring. With conductivity monitoring, “T” always equals 1.

Table 5-7. Functions 136 and 137 in Conditional Modules

Function	Conditions Not Met?	Module Performed?	Occurs in Module:
136	No	Yes	i Cart (eject/advance)/SkipMod
136	Yes	No	F Coupling etc/SkipMod
137	No	No	a MonDoMod (activation/transfer)
137	Yes	Yes	b MonDoMod (deprotection)
			d MonDoMod (capping/wash)
			f MonDoMod (coupling)
			g MonDoMod (resin sampling)

Modules in the Conditional Monitoring Chemistry Files

Table 5-8 shows the pre-defined modules in the Conditional Monitoring Chemistry files. Seven modules—"C," "c," "D," "E," "G," "H," and "I"—are identical to those modules with the same letter names in the Basic Monitoring Chemistry files.

Module "B" contains the same monitoring functions used in the Basic Monitoring modules "B." Module "A" is almost identical to the Basic Monitoring module "A," but with an NMP wash at the beginning.

Six modules—"a," "b," "F," "f," "g," and "i"—are conditional modules. Conditional modules begin with either Function 136 or Function 137 at Step 1.

Table 5-8. Pre-defined modules in the Conditional Monitoring Chemistry file

Module letter	Module Name	Description
A	Activation*	Wash with NMP and dissolve amino acid
B	Deprotection/ <i>algorithm</i> *	Monitored deprotection with specific algorithm
C	Capping with Ac ₂ O	Ac ₂ O capping
D	NMP washes	Wash with NMP
E	Transfer	Add DIEA and transfer to RV
F	Coupling etc/SkipMod*	Clean cartridge, coupling, drain, and NMP wash, with Fxn 136 at Step 1
G	Resin Sampling	Take resin sample
H	Load and cap	Load resin with amino acid and cap
I	Wait (10 min)	10-minute wait
a	MonDoMod (activation/transfer)	Read cart, wash with NMP, dissolve amino acid, add DIEA and transfer to RV, Fxn 137 at Step 1
b	MonDoMod (deprotection/ <i>algorithm</i>)	30 min. extended deprotection, Fxn 137 at Step 1
c	DCM Washes	Wash with DCM
d	MonDoMod (capping/wash)	Ac ₂ O capping, Fxn 137 at Step 1
f	MonDoMod (coupling)	50 min coupling, Fxn 137 at Step 1
g	MonDoMod (resin sampling)	Take resin sample, Fxn 137 at Step 1
h	Module h	Monitored NMP wash
i	Cart (eject/advance)/SkipMod	Eject one cartridge and advance second, Fxn 136 at Step 1

* The modules with an asterisk (*) in their name have been slightly modified and do not have the same number of steps as the modules with the same letter name in the other *FastMoc* Chemistry files.

Conditional Monitoring Cycles

Table 5-9 displays the cycles in the pre-defined Conditional Monitoring files. The third column in Table 5-9 describes what occurs when deprotection is difficult (conditions not met) and the conditional modules are “switched on.”

Table 5-9. Conditional Monitoring Cycles

Cycle	Use for	Conditional possibilities
Single Couple (<i>algorithm</i>)	Single couple	Extended deprotection, extended coupling, capping
Cycle 1-amide/ <i>algorithm</i>	First cycle, with amide resin, single couple	Extended deprotection, extended coupling, capping
SC/Mon <i>algorithm</i> RS	Single couple, with resin sample	Extended deprotection, extended coupling, capping
Cycle 1-SC/ <i>algorithm</i> RS	First cycle, with amide resin, single couple, and resin sample	Extended deprotection, extended coupling, capping
Cond Double Couple	Single couple, with conditional double couple	Extended deprotection, extended coupling, activation of second cartridge, extended coupling, capping
Cond Double Couple/2RS	Single couple, with resin sample, and conditional double couple with a second resin sample	Extended deprotection, extended coupling, activation of second cartridge, extended coupling, capping, extra resin sample
Cycle 1-CDC	First cycle, with amide resin, single couple and conditional double couple	Extended deprotection, extended coupling, activation of second cartridge, extended coupling, capping
Cycle 1-CDC/2RS	First cycle, with amide resin, single couple and resin sample, plus conditional double couple and a second resin sample	Extended deprotection, extended coupling, activation of second cartridge, extended coupling, capping, an extra resin sample
Final Deprotection/ <i>algorithm</i>	Last cycle, remove final Fmoc	No conditional modules
Loading & Benzoic Anhydride Capping	Load HMP resin with initial amino acid and cap	No conditional modules
Final acetylation/ <i>algorithm</i>	Last cycle, acetylate N terminal	Extended deprotection
Complete Wash	Wash preloaded resin	No conditional modules
NMP Wash	Wash with NMP	No conditional modules
DCM Wash	Wash with DCM	No conditional modules

- Use the cycles labeled “Cycle 1-” when beginning a synthesis with an amide resin.
- Use the Complete Wash cycle when beginning a synthesis with a preloaded resin.
- Use the Loading & Benzoic Anhydride Capping cycle when beginning a synthesis with HMP resin.

Table 5-10 displays the modules in the Conditional Monitoring cycles.

Table 5-10. Modules in Conditional Monitoring Cycles

Cycle	CondMon Prev Pk	CondMon 1st-X
Single Couple (SC)	BbADEFfd	BbADEFfDd
Cycle 1-amide	cDBbADEFfd	cDBbADEFfDd
SC/Resin Sample (RS)	BbADEFfDGd	BbADEFfDGd
Cycle 1-SC/RS	cDBbADEFfDGd	cDBbADEFfDGd
Cond Double Couple (CDC)	BbADEFifDafd	BbADEFifDafd
Cond Double Couple /2RS	BbADEFifDGafgd	BbADEFifDGafgd
Cycle 1-CDC	cDBbADEFifDafd	cDBbADEFifDafd
Cycle 1-CDC/2RS	cDBbADEFifDGafgd	cDBbADEFifDGafgd
Final Deprotection	BbDc	BbDc
Loading & Benzoic Anhydride Capping	HF	HF
Final Acetylation	BbDCDc	BbDCDc
Complete Wash	cD	cD
NMP Wash	D	D
DCM Wash	c	c

Table 5-11 displays the cycles and modules in the Conditional Monitoring Default Set.

Table 5-11. Default Set for the Conditional Monitoring Files

AA	Cycle	CondMon Prev Pk	CondMon 1st-X
Default	Single Couple (<i>algorithm</i>)	BbADEFfd	BbADEFfDd
Preloaded	Complete Wash	cD	cD
Loading	Loading & Benzoic Anhydride Capping	HF	HF
Amide	Cycle1-amide	cDBbADEFfd	cDBbADEFfDd
Other	<<None>>		
End	Final Deprotection	BbDc	BbDc

Setting Criteria in the Conditional Monitoring Modules

Table 5-12 shows the monitoring functions in the CondMon 1-X modules with the values of “T” assigned to them. When using these modules, change the values of “T” for Functions 128 (Mon 1st-X), 133 (MonBegLoop), and 134 (MonEndLoop) to set criteria for monitoring.

Table 5-12. Values of “T” in the CondMon 1-X Modules

Module	Fxn 128	Fxn 131	Fxn 132	Fxn 133	Fxn 134	Fxn 136	Fxn 137
a	—	—	—	—	—	—	1
B	100	1	1	2	50	—	—
b	100	1	1	—	—	—	1
d	—	—	—	—	—	—	1
F	—	—	—	—	—	1	—
f	—	—	—	—	—	—	1
g	—	—	—	—	—	—	1
i	—	—	—	—	—	1	—

Table 5-12 shows the monitoring functions in the CondMonPrevPk modules with the values of “T” assigned to them. When using these modules, change the values of “T” for Functions 133 (MonBegLoop), and 134 (MonEndLoop) to set criteria for monitoring.

Table 5-13. Values of “T” in the CondMonPrevPk Modules

Module	Fxn 130	Fxn 131	Fxn 132	Fxn 133	Fxn 134	Fxn 136	Fxn 137
a	—	—	—	—	—	—	1
B	1	1	1	2	100	—	—
b	1	1	1	—	—	—	1
d	—	—	—	—	—	—	1
F	—	—	—	—	—	1	—
f	—	—	—	—	—	—	1
g	—	—	—	—	—	—	1
i	—	—	—	—	—	1	—

As these tables illustrate, with conductivity monitoring, the value of “T” for most monitoring functions is 1. Only Functions 128, 133, and 134 have values that you may wish to modify.

To set the conditional monitoring criteria:

1. First choose a Chemistry file based on the scale and algorithm function you prefer.
 - **CondMon 1-X files** use the Function 128 (Mon 1stPk-X) algorithm. Determine the value of X, the conductivity baseline, by running Flow Test 22 for the 0.10 mmol scale, or Flow Test 23 for the 0.25 mmol scale.

-
- **CondMonPrevPk files** use the Function 130 (MonPrevPk) algorithm.
2. Set criteria in the Deprotection Module “B.”
- In module “B” (Deprotection/Mon1stPeak-X*),** assign a value for “T”:
- At Steps 22 and 49, enter the conductivity baseline value, divided by ten, for the value of “T” in Function 128.
 - At Step 25, Function 133 (MonBegLoop), the value of “T” represents the maximum number of deprotection loops.
 - At Step 52, Function 134 (MonEndLoop), “T”/10 equals the percentage used to compare the last deprotection peak generated in the monitored loop to the initial deprotection peak, after baseline subtraction. As a guideline, Applied Biosystems suggests you set the value of “T” in Function 134 (MonEndLoop) between 50 and 150.
- In module “B” (Deprotection/MonPrevPeak*),** assign a value for “T”:
- At Steps 27 and 55, Function 130 (MonPrevPk), “T” represents the channel collecting monitoring data. With conductivity data, the value of “T” = 1.
 - At Step 32, Function 133 (MonBegLoop), “T” represents the maximum number of monitored deprotection loops.
 - At Step 59, Function 134 (MonEndLoop), “T” /10 equals the percentage used to compare two sequential deprotection peaks. As a guideline, we suggest you set the value of “T” in Function 134 (MonEndLoop) between 50 and 150
3. Set criteria in module “b,” (conditional extended deprotection).
- If you are using **Function 128 (Mon 1stPk-X)**, at Step 16, (“T” x 10) equals the conductivity baseline.
 - If you are using **Function 130 (MonPrevPk)**, at Step 16, “T” represents the channel collecting monitoring data. With conductivity data, the value of “T” = 1.

Module “b”: Conditional Extended Deprotection

Conditional module “b” extends deprotection time only when conditions are not met in module “B.” Additional NMP and piperidine are added to the peptide-resin in the reaction vessel and allowed to react for thirty more minutes.

The conditional module “b” for *FastMoc* 0.10 mmol CondMon1-X, has Function 128 (Mon 1stPk-X) at Step 16. Conditional module “b” in the *FastMoc* CondMonPrevPk files uses Function 130 (MonPrevPk) at Step 16. In *FastMoc* 0.25 mmol monitoring files, delivery times increase at Steps 3, 4, and 6.

Module “F”: Conditional Coupling

Module “F” begins with Function 136 (SkipModMon). If conditions are met in deprotection module “B,” module “F” provides a minimum of 9 minutes of coupling at the 0.10 mmol scale, or 20 minutes of coupling at the 0.25 mmol scale. If conditions are met, module “F” (Coupling/SkipMod*) is used in a cycle, then modules “f” and “d”—the modules that begin with Function 137 (Do ModMon)—are **not** included in the cycle.

Module “f”: Conditional Extended Coupling

Module “f” begins with Function 137 (Do ModMon). If conditions are **not** met in deprotection module “B,” conditional module “f” extends coupling to 50 minutes. It is an alternative to module “F.”

Module “d”: Conditional Capping & NMP Wash

Module “d” begins with Function 137 (Do ModMon). If conditions are not met, conditional module “d” covalently bonds, or caps, the free amino acid terminuses on the peptide chains that failed to couple. During the capping module the peptide-resin is treated with acetic anhydride solution. At the end of the module, the reaction vessel is washed three times with NMP.

To prepare the acetic anhydride solution, see *Capping with Acetic Anhydride* on page 7-14. Place the acetic anhydride solution in bottle position 4. If you are loading the initial amino acid onto HMP resin, you must place DMAP in bottle position 4 and then replace it with acetic anhydride at the end of cycle HF. See *Loading on HMP resin, followed by Capping with Acetic Anhydride* on page 7-16 for this procedure.

If you do not want conditional capping, remove module “d” from the cycle.

Module “g”: Conditional Resin Sampling

Module “g” begins with a Function 137 (DoModMon). With the exception of this added first step, it is exactly like module “G” (Resin Sampling). Use module “g” after coupling module “f,” but before conditional capping module “d.”

<i>Note</i>	<i>When using either module “G” or “g,” answer YES to the Resin Sampling prompt in the Cycle Monitor menu.</i>

Module “a”: Conditional Activation of Additional Amino Acid

If conditions are not met in deprotection module “B,” conditional module “a” activates the contents of the next amino acid on the ABI 433A instrument guideway. At Step 9, before activation begins, Function 150 (MATCH CART) compares the bar code label of the amino acid cartridge to the label of the last amino acid cartridge that was activated. The two cartridges should contain the same amino acid if the instrument was set up for double couple cycles. If the two cartridge labels do not match, the ABI 433A instrument interrupts synthesis (the ***pause** soft key becomes active).

If the cartridge labels do match, module “a” continues. The amino acid is dissolved with HBTU, DIEA is delivered to the cartridge and the contents of the cartridge are delivered to the reaction vessel.

Module “i”: Cartridge eject/advance

Module “i” begins with Function 136 (SkipModMon). Module “i” is only used in conditional double couple cycles, in conjunction with module “a.” Module “i” is performed only when conditions are met, that is, after module “F” is performed. If in any cycle, module “F” and module “a” are performed instead of module “F,” module “i” is skipped.

When conditions are met in deprotection module “B,” a double couple is not performed. The last cartridge used is ejected, and the next cartridge in the guideway, the duplicate, advances. However, with module “i,” the sampling needle does not puncture the duplicate cartridge, and the contents of the cartridge are not activated. The duplicate cartridge is ejected, intact, at the beginning of the next cycle and can be used in another synthesis.

6 Troubleshooting and Maintenance

This chapter shows troubleshooting examples on how to resolve instrument, software, or chemistry problems. Use the maintenance schedule and flow tests described here to ensure that your instrument system runs successfully.

Contents

ABI 433A Instrument Troubleshooting Guide	6-2
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Maintenance Procedures	6-13
Flow Test Descriptions	6-27

ABI 433A Instrument Troubleshooting Guide

Refer to this table for assistance for instrument operation irregularities. When necessary, call Applied Biosystems Service Department.

Symptom	Possible Cause	Possible Remedy	Preventive Action
No DCC or HOBT delivery.	DCU formed in 0.5 mL loop.	Dissolve DCU with NMP or with 60/40 DCM/MeOH.	Remove DCC and HOBT bottles and replace with NMP if the instrument is not used for 2 weeks. Alternatively, run Flow Tests 7 and 8 weekly.
Instrument beeps, and *pause key becomes active.	Controller reads a user function that activates valve 12 or 22 but needle is in up position.	Quit the synthesis (see page 2-43) and rewrite the user function.	Needle must not be in up position when valves 12 or 22 are opened, whether during a synthesis or while operating in the Manual Control menu (see page 2-45).
	Function 58 is active.	Press *pause to continue synthesis.	
Ejector or needle fails.	Gas lines clogged with oil. Gas tank near empty; regulator not set at 65 psi.	Clean lines and restrictors with MeOH. Replace nitrogen gas tank.	
Flow test volume is low on Flow Tests 11 and 12.	AA cartridge in-line filter is clogged.	Change AA cartridge in-line filter.	
Leak in RV in-line filter.	Flared tubing is worn.	Replace tubing.	Do not overtighten the tube connection when changing the in-line filters.
	In-line filter is not making a tight seal.	Replace in-line filter.	
Printer not working outside of synthesis; does not print modules.	Printer assigned by Cycle Monitor to a synthesis.	Restart a synthesis with printing, then quickly terminate the synthesis (see).	Press begin soft key after responding yes to "Print run events?"
NMP bottle cap strips when cap is tightened.	NMP has swollen the cap.	Replace bottle cap assembly.	Avoid splashing NMP on cap.

Symptom	Possible Cause	Possible Remedy	Preventive Action
Vortexer is noisy.	Ball bearings in vortexer bearing assembly are worn.	Call Service Engineer to replace vortexer bearing assembly.	Take care not to spill solvents on vortexer bearing. This usually happens when RV leaks.
Vortexer is not working.	Vortexer belt is loose or has fallen off.	Call Service Engineer.	
	Vortexer motor faulty.	Call Service Engineer.	
	Vortexer bearings completely frozen.	Call Service Engineer.	
Pressure is not maintained in bottles 1, 4, 5, 6, 7, and 8.	Bottle seal is cracked.	Check and replace defective seals.	
	Bottle rim is chipped.	Check bottle rims and replace if necessary.	
Bottle #2 is pressurized when it should not be pressurized.	TFA pressure release valve is not working.	Call Service Engineer to check Angar Valve 26, valve blocks and vacuum ballast.	If not using ABI 433A instrument at least once a week, remove the TFA bottle and run Bottle 2 change procedure.
Bar code reader is misreading.	Spill on barcode reader.	Check calibration and call Service with information.	
	Barcode reader is out of alignment.		
	Label is damaged. Potentiometers need adjustment.	Use a cartridge with a new label.	Avoid splashing solvents on cartridge label.

Troubleshooting Monitoring Traces

The following example shows the high level of conductivity that can occur with Flow Test 22 when the synthesizer has been used for a period of time.

Unexpected high conductivity values for Flow Test 22

Figure 6-1 illustrates what can happen to the conductivity values after the 433A instrument has been idle for several days. Running Flow Test 22 (Module d in Flow Tests 19-23) generated the first set of conductivity values. The Flow Test was re-run without any adjustments to the 433A instrument or the reagents. The second set of conductivity measurements were within an expected range of values.

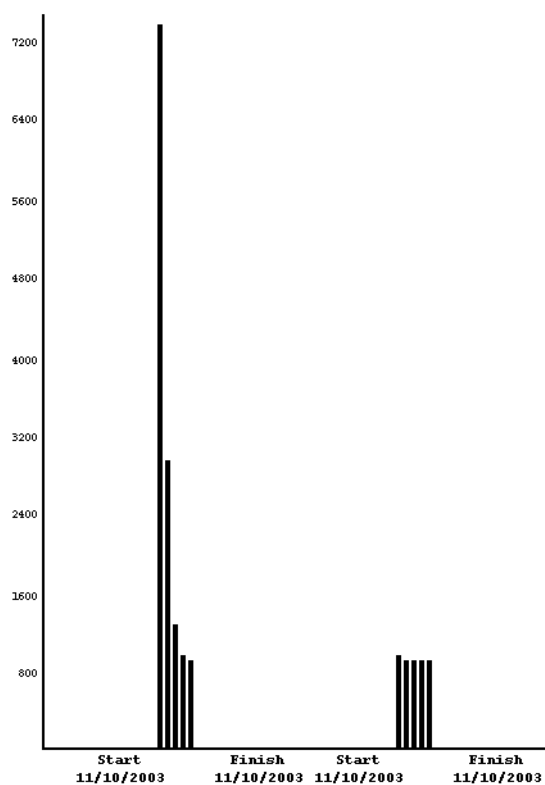


Figure 6-1. Effect of ABI 433A instrument sitting idle

Unexpected high conductivity values on Flow Tests 20 and 22

Figure 6-2 illustrates the inadvisability of using non-Aldrich-supplied piperidine. Flow Tests 20 (Module b) and 22 (Module d) were run using piperidine from a vendor other than Aldrich. Although the resulting conductivity values (A) are reasonable when the piperidine is used alone, the values generated when using a piperidine/NMP mixture (B) are too high to be used for conductivity monitoring of a synthesis. When piperidine supplied by Aldrich is used, the values for the piperidine/NMP mixture (C) are in the expected range and can be used in a conductivity-monitored synthesis.

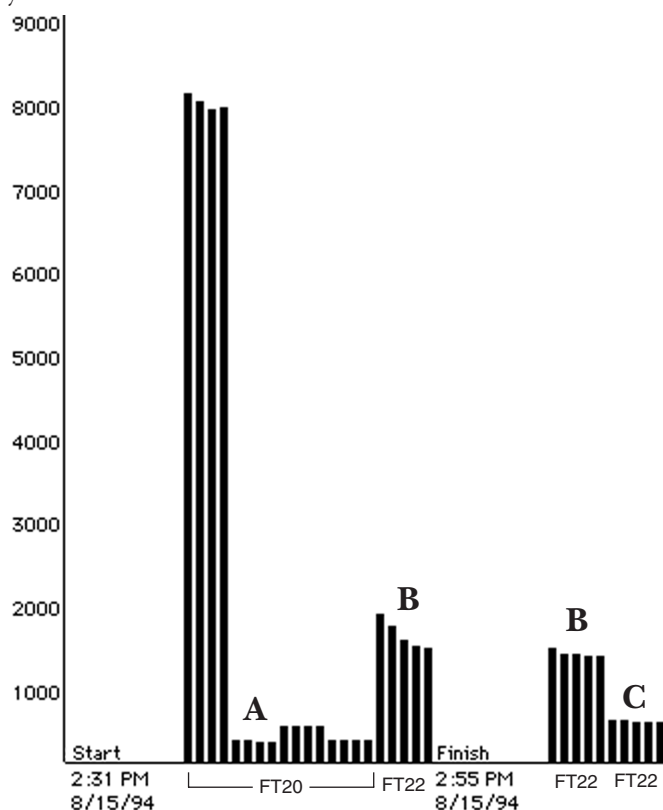


Figure 6-2. Effect of piperidine quality on Flow Tests 20 and 22

Note

If the conductivity value is greater than 800, then your NMP may be bad. Consider using a different lot of NMP.

Extremely high initial monitoring value

Figure 6-3 on page 6-7 illustrates the incorrect (out of expected range) deprotection values obtained when an empty cartridge becomes stuck in the autosampler during a synthesis.

The first sequence of three deprotections (A) are in the expected ranges. The first amino acid cartridge has been correctly read by the barcode reader, but an empty cartridge from a previous run has become stuck in the autosampler, preventing the cartridge that was just read from advancing into the autosampler.

The normal activation reagents are added to the stuck empty cartridge, then transferred to the reaction vessel. Because there was no coupling to the free amine on the resin, the next sequence of deprotection values (B) represent washout of the coupling reagents rather than removal of an Fmoc group. Because the stuck cartridge is read for a second time, the instrument sees a cartridge that is not the correct one in the sequence and pauses, requiring the run to be ended.

IMPORTANT *Cartridges swell after extended contact with solvents such as NMP and DCM. After only a single synthesis cycle, a cartridge can swell enough to exceed the recommended cartridge size. As a result, reusing a cartridge can result in the cartridge becoming stuck in the autosampler and shutting down your synthesis.*

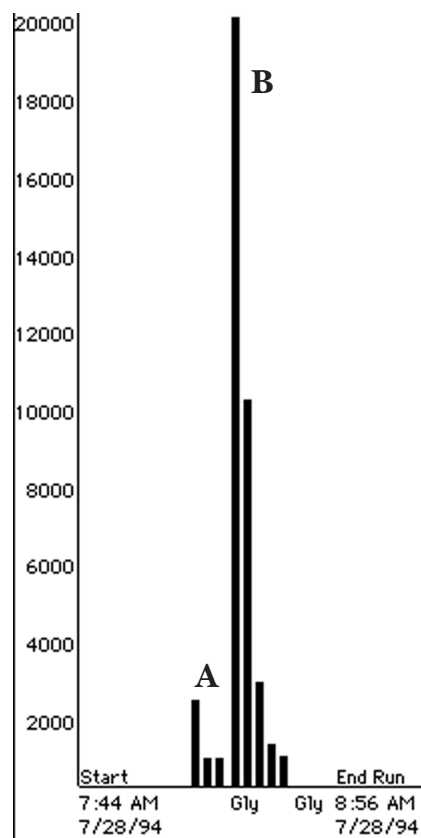


Figure 6-3. Effect of swollen amino acid cartridge stuck in autosampler

Extended deprotections of starting resin

In Figure 6-4, the initial deprotection (A) has five peaks, an unnecessarily extended deprotection. The resin used in this synthesis releases conductive species, especially during the initial deprotection. This problem is a result of the resin's manufacture and cannot be corrected by the user.

Figure 6-4 also illustrates the larger-than-normal first deprotection value of Fmoc-Arg(PMC)-OH, the 11th and 19th residues coupled (B and C). This symptom is specific to Arginine and is not entirely understood from a chemical point of view.

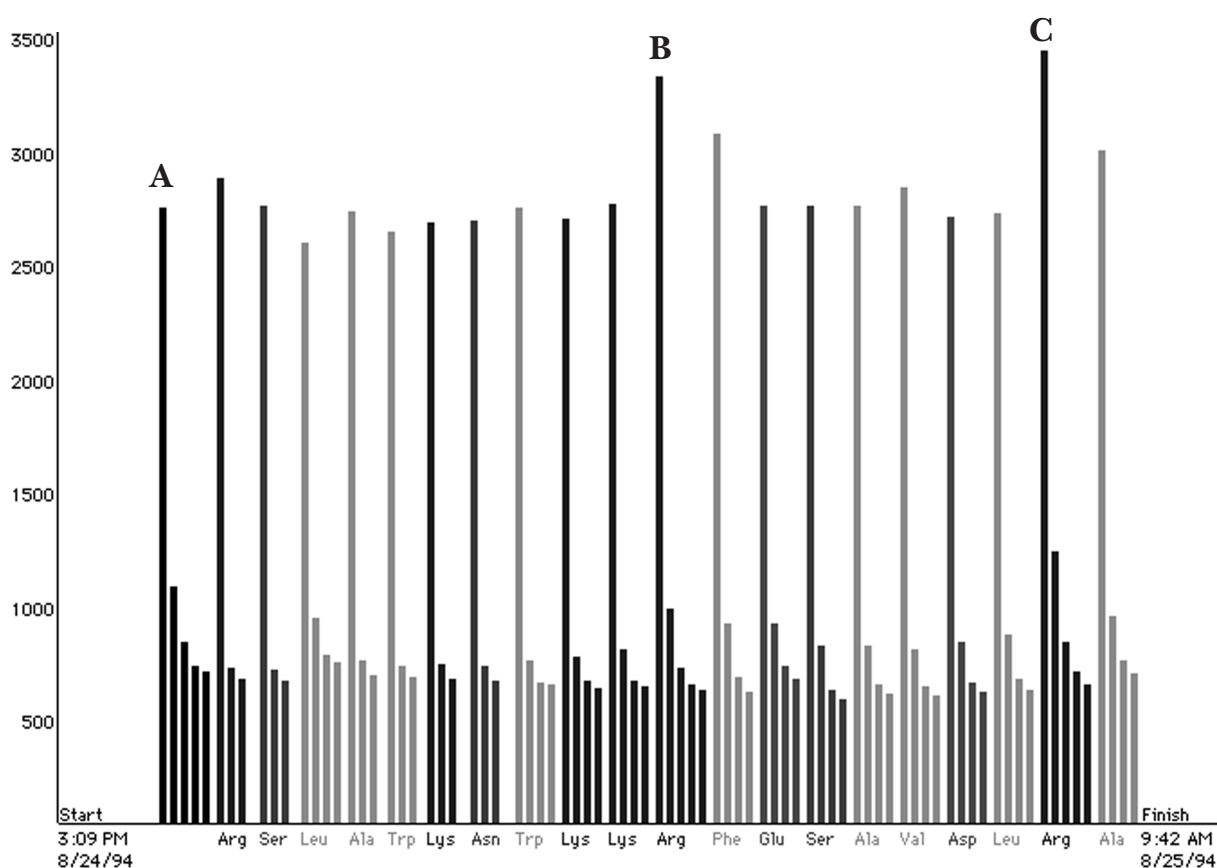


Figure 6-4. Extended deprotection with starting resin

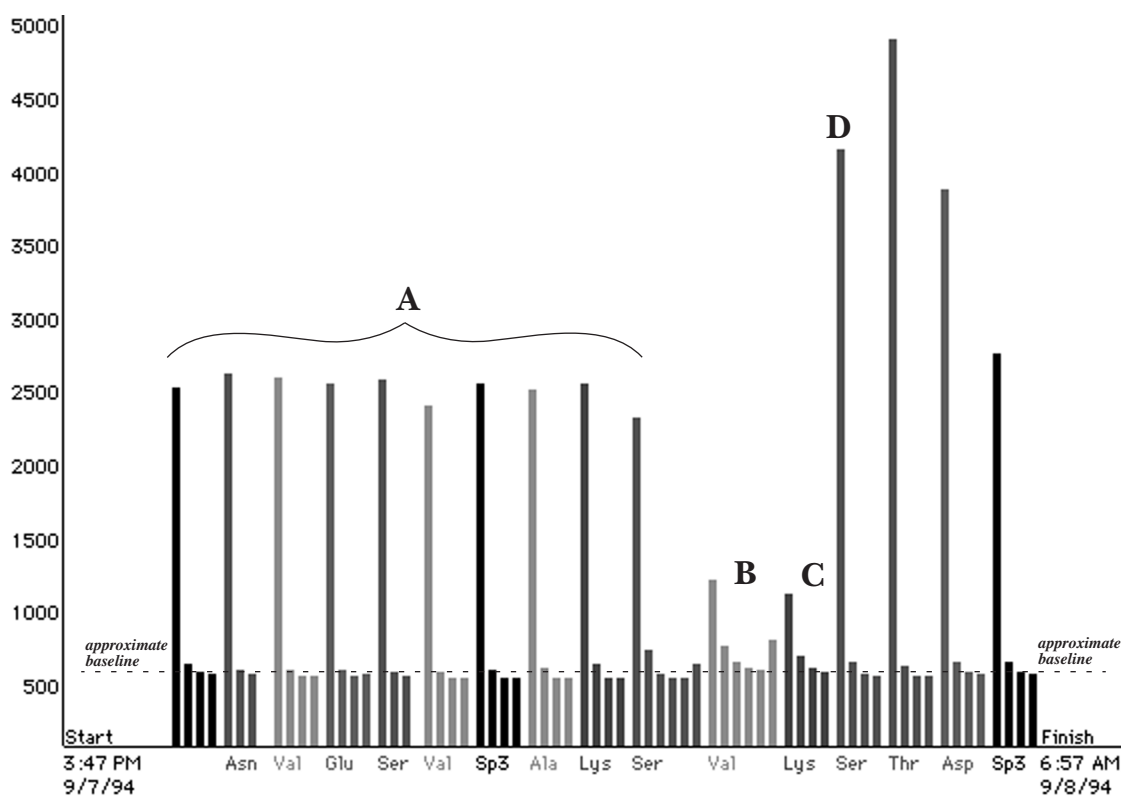
High monitoring values after difficult regions

Figure 6-5 illustrates a synthesis that uses ConditionalMonPrevPeak chemistry with conditional capping.

During the cycles (illustration point A) before the difficult region, the washes after each coupling are sufficient to remove the conductive materials before the next cycle's deprotection.

When the conditional capping and washing modules are added and the coupling reagents washed out (B), the deprotection values accurately indicate the amount of Fmoc group removed.

After the difficult region (C), increased values of the initial deprotections (D) result from an incomplete washout of the previous coupling because the conditional capping and wash modules are not used.



Initial deprotection peak of starting resin extremely high when compared to subsequent peaks

Figure 6-6 illustrates a conductivity trace generated when using MBHA resin in place of Fmoc-amide resin. The extremely high initial deprotection value is caused by the HCl salt in the MBHA resin being washed out by the piperidine/NMP solution.

Note the presence of difficult regions in this synthesis (four deprotections instead of three). These difficult regions are generally identical to those in a similar synthesis using an Fmoc-amide resin.

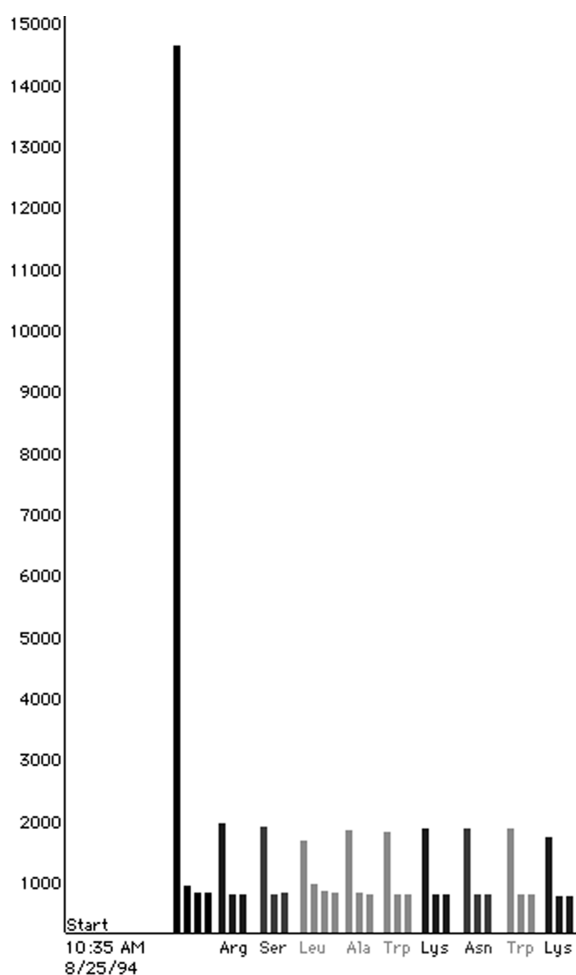


Figure 6-6. Initially high deprotection peak

Monitoring traces suddenly become extremely high

Figure 6-7 illustrates the effect on the monitoring traces when the supply of NMP is exhausted, in this case, before the last cycle. In the last cycle, the deprotection traces show sudden extreme high values. In addition, the synthesizer performed the maximum number of deprotection loops. Both phenomena are the result of conductive species not being washed out.

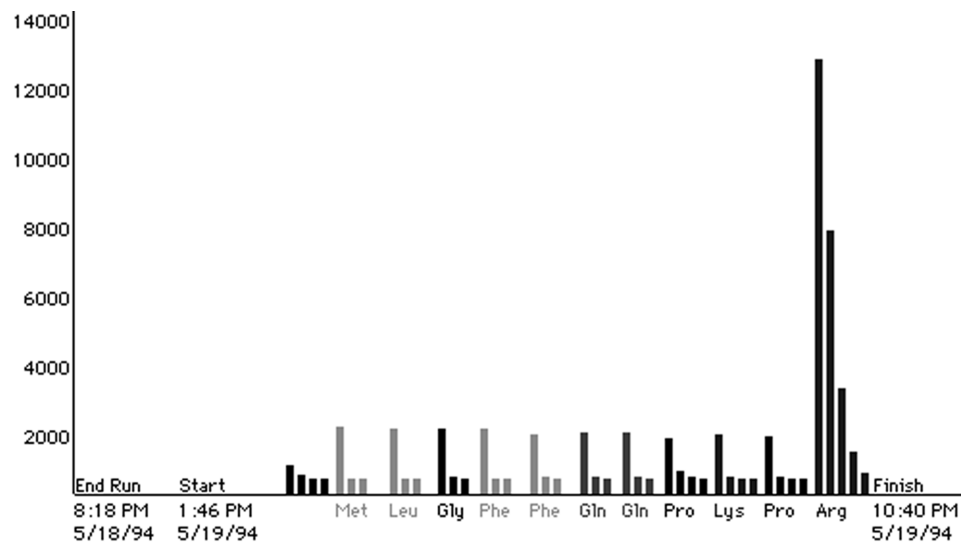


Figure 6-7. Monitoring trace goes suddenly extremely high

Initial deprotection values look erratic or inconsistent

Figure 6-8 shows a somewhat typical conductivity trace in which the initial deprotection values are erratic or inconsistent.

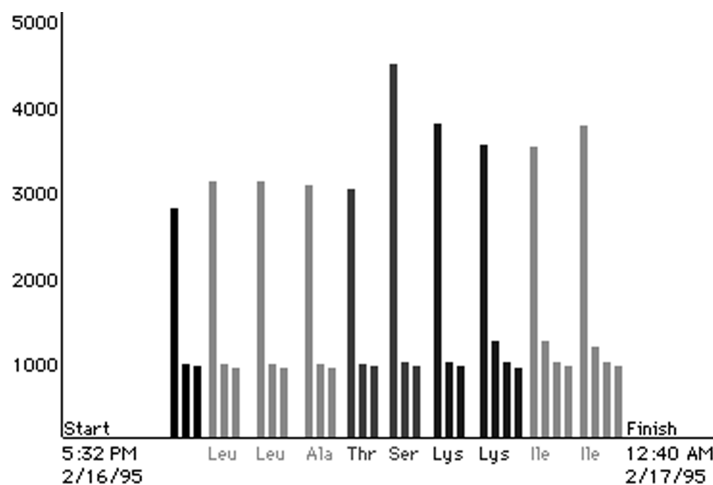


Figure 6-8. Erratic initial deprotection values

This erratic initial value is normal when using the standard “Monitor Previous Peak” chemistries because these chemistries do not contain a module “D” (NMP wash) at the end of each cycle. There is no need for this extra wash because the conductivity monitoring is based upon the previous peak value rather than the initial baseline value.

Conductivity values get progressively higher

Figure 6-9 illustrates the accumulation of conductive species during a long synthesis (usually greater than 25mer) using a “Monitor Previous Peak” chemistry. This phenomenon is normal and, as in Figure 6-8, results from the absence of a module “D” at the end of each cycle. In any “Monitor Previous Peak” chemistry, the height of the initial deprotection peak is not important.

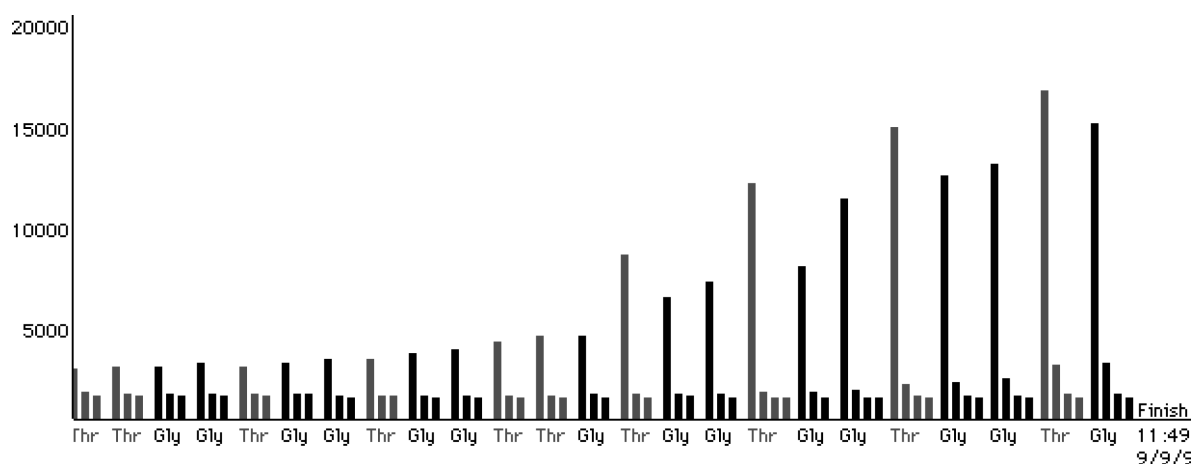


Figure 6-9. Conductivity values get progressively higher

Maintenance Procedures

Maintenance Schedule

Use the maintenance sheet on page 2-9 to log your information.

Table 6-1 lists the ABI 433A Peptide Synthesizer parts that need regular maintenance to assure proper instrument operation.

Table 6-1. ABI 433A Instrument Maintenance Schedule

Instrument Part	Maintenance
In-line filters	Replace needle position at least every 25 cycles. Replace bottom RV filter at least every 50 cycles. Replace top RV filter at least every 75 cycles.
Seals: Bottles 9 & 10	Examine monthly. Change as needed, or annually.
Vent line	Examine monthly for waste build-up.
Fan filter	Clean monthly. Change as needed, or annually.
Nitrogen cylinder	Check before each run. Change when primary gauge falls below 300 psi.
Waste bottle	Check before each run. Empty when necessary.
Waste port	Replace annually. See page 6-20. Check every time waste is emptied.

Adjusting Regulators

Run Flow Test 2 (see page 6-30) to check the delivery of TFA in Bottle 2 and Flow Test 10 (see page 6-39) to check the delivery of NMP in Bottle 10. If the volume of delivered TFA is outside the range shown in Table 6-2, adjust the upper gas regulator. If the volume of delivered NMP is outside the range shown in Table 6-2, adjust the lower gas regulator.

Table 6-2. Regulator Calibration Volume

Flow Test	Reg.	Volume (mL) in Metering Vessel	Reagent	Delivery Time (sec)	Typical Pressure (psi)
10	lower	2.45 -2.55	NMP	5	9.0–11.0
2	upper	2.0 ± 0.05	TFA	18	2.0–3.0

To adjust the lower regulator:

1. Place the metering vessel in the RV holder and run Flow Test 10 (Flow Tests 1-18, module A). The volume of NMP should be 2.5 ± 0.1 mL.

If the delivery volume of NMP is greater than 2.55 mL, vent Bottles 9 and 10 for several seconds and then turn the lower-regulator control knob counterclockwise.

When Bottle 10 is repressurized, a new pressure reading appears on the regulator.

Note *If the pressure decreases more than necessary, turn the control knob clockwise to increase the pressure. Let the pressure equilibrate for about 30 seconds and repeat the flow test.*

If the delivery volume of NMP is less than 2.45 mL, increase the pressure by turning the control knob clockwise. Let the pressure equilibrate for about 30 seconds before repeating the flow test.

2. Run Flow Test 11 (Flow Tests 1-18, module B), placing a pre-weighed, empty cartridge in the autosampler. The weight of NMP delivered to the cartridge should be 1.95–2.35 g. If the weight of NMP is less than 1.95 g with a new cartridge in-line filter, increase the pressure to the lower regulator to increase the weight of delivered NMP to 2.35 g.

Note *If you must increase the pressure on the lower regulator to increase NMP delivery in Flow Test 11, the delivery of NMP in Flow Test 10 may increase to as much as 2.75 mL.*

To adjust the upper regulator:

WARNING **CHEMICAL HAZARD** *To prevent contact with TFA, do not remove the metering vessel until Flow Test 2 is completely finished.*

1. Place the metering vessel in the RV holder and begin Flow Test 2 (Flow Tests 1-18, module b).
2. Pause at step 29 (a 60-second wait period).
3. Adjust the top regulator knob:
 - If the volume of TFA is less than 1.95 mL, turn the top regulator knob clockwise at least half a turn, to increase the pressure.
 - If the volume of TFA is greater than 2.05 mL, turn the top regulator knob counter-clockwise to decrease the pressure. (Notice that the regulator gauge will not register a change in the pressure reading at this time.)
4. Jump to step 1 of Flow Test 2, select pause again (to continue Flow Test 2), and repeat the measurement.

The pressure gauge reading should drop at step 2 of Flow Test 2 because function 75 (GAS-VENT #2) releases the gas. If the pressure drops excessively, you may increase it at step 9 (PRS #2).

Flushing the Measuring Loop

If the measuring loop does not fill completely during Flow Tests 7 and 8, or if there is inadequate delivery during Flow Tests 17 and 18, perform the following procedure to flush the measuring lines with NMP or a solution of DCM/MeOH (60/40) and dissolve the crystals.

WARNING **CHEMICAL HAZARD.** *N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *Dichloromethane (DCM) may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING **CHEMICAL HAZARD.** *Methanol is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

To clear the HOBt and DCC lines of crystals:

1. Fill an empty 200-mL bottle with 20 mL NMP, or DCM/MeOH (60/40), and place it in position number 7.
2. Run Flow Test 7 (Flow Tests 1-18, module g). When the test reaches step 2 (Meas #7), press the **hold** soft key and wait until all the contents of the bottle have been drained. Press **hold*** and finish Flow Test 7.
3. Wipe the delivery line with a lint-free tissue and replace the 1M HOBt/NMP bottle.
4. Repeat the first 2 steps of Flow Test 8 (Flow Tests 1-18, module h), with the bottle of 20 mL NMP, or DCM/MeOH (60/40), in position number 8. After the line is clear, wipe with a lint-free tissue and replace the 1M DCC/NMP bottle.

-
5. Check to see if the procedure worked by repeating Flow Tests 7 and 8, or Flow Test 17 and 18. You may have to repeat the NMP flushes more than once.

If flushing the lines with NMP does not improve reagent flow, check the lines for crimps.

If reagent flow is still insufficient, check that Bottles 5, 6, 7, and 8 are pressurized. Open one of the bottles and listen for the hiss of escaping air. If there is no hiss when a bottle is vented after Flow Test 7 or 8 is run, the system is not pressurized. Call Technical Support for assistance.

Barcode Calibration

Barcode calibration standardizes the channel readings so that all channels accurately detect the black and white bands on the amino acid cartridge labels. (For an illustration of the barcode system, see page 8-16.) Calibrate the barcode reader before your first synthesis. Once you calibrate the barcode reader, you do not have to repeat the calibration except after a memory cartridge replacement.

How to calibrate the barcode reader:

Note *If you need to perform calibration during a synthesis, you must first press the **pause** soft key.*

1. In the Main Menu, press the **barcode reader** soft key.
2. Press **calib** to begin the barcode calibration.

Interrupt when barcode incorrect:NO

calib	YES/NO				

↓

Place calibrator cartridge at reader

					enter

↓

Reading barcode

Turn calibrator cartridge around...

2200	600	2300	586	2170	enter

Barcode reader is calibrated

1500	1660	1700	1575	1300

3. When prompted, place the calibrating cartridge (P/N 400269) in the guideway. Place the pressure block against the cartridge and lower the retaining rod. Press **enter**.

Complementary barcodes appear on opposite sides of the calibrating cartridge. The label on this cartridge must be straight and have an unmarred surface.

<i>Note</i>	<i>If you do not have the calibrating cartridge, you can use two cartridges with complementary barcodes (the black and white bars of one cartridge are opposite or complimentary to the bars on the other cartridge as with the threonine and glutamine cartridges).</i>

The barcode calibration values range from 0 to 4096.

4. When prompted, turn the calibrating cartridge around so the reader can scan the opposite side. Place the pusher block against the cartridge and lower the retaining rod. Press **enter**.

The LCD displays the message **Barcode reader is calibrated** when calibration is completed. If the LCD displays the message **Barcode reader needs service**, repeat the calibration procedure.

If the barcode does not calibrate after repeating the calibration procedure several times, turn the ABI 433A instrument off and on again, then re-calibrate. If the barcode reader still does not calibrate, call the Applied Biosystems Technical Support Department.

Use either Flow Test 3 or the following procedure to check the barcode reader calibration.

To check the barcode reader calibration:

1. From the Manual Control Menu (see *The Manual Control Menu* on page 9-9), activate Function 7 (EJECT CART).
2. Place an amino acid cartridge in the guideway and slide the pusher block in place.
3. Activate Function 4 (SAVE CART).

When you activate Function 4 from the Manual Control Menu, the name of the cartridge appears on the screen. For a printout of the cartridge names, see the directions for using Flow Test 3 (see page 6-32).

Check Monitoring Values

A/D Reading (Conduct):					Press Main to go to the Main menu
Conduct	Chnl 2	Chnl 3	V Ref	Ground	

Figure 6-10. Monitor Check menu

Run Flow Test 20 (page 6-49) to check the conductivity of each of four reagents in the conductivity flow cell. You do not need to perform this Flow Test regularly.

Use the Monitor Check menu (Figure 6-10) to obtain the reference voltage (VRef), the ground voltage (Ground), and the voltage currently produced by the solution in either the conductivity cell (Conduct) or the spectro-photometric flow cell (Chnl 2). Compare your readings to the typical readings shown in Table 6-3.

Table 6-3. Typical Monitoring Values

Menu Selection	Value
Conduct	200-300
Chnl 2/Chnl 3	dependent on input
VRef	16328
Ground	0

Keep a record of your conductivity readings to track fluctuations.

Monitoring Voltages

Date	Conductivity	Ground	Date	Conductivity	Ground

Inspecting and Replacing the Waste Port

WARNING *A leaky waste port can cause damage to the instrument and is both a fire and personnel hazard. If you find that the waste port has been leaking, immediately switch off the instrument power and carefully inspect all electronic cables for damage. Damaged electrical cables present a shock hazard.*

Waste Port Inspection Guidelines

As a safety precaution, Applied Biosystems recommends that the waste port be replaced annually. Order part number 604226.

Check the waste port for damage every time you empty the waste bottle. Replace the port if it shows any indication of breaking, leaking, or is heavily discolored.

Make sure the waste line is long enough and positioned so that it is *not* under tension or severely pulled by routine handling of the waste bottle or instrument.

Be sure the line is not so long that dips are created where liquid could collect.

IMPORTANT *Do not use the installation instructions contained within the waste port assembly packaging because they are incomplete. Use these instructions instead.*

Before You Replace the Waste Port

You need the following equipment:

- 13/16-inch open-end wrench or adjustable wrench
- Utility knife or new single-edged razor blade
- 2 inches of Teflon® tape

WARNING **CHEMICAL HAZARD.** *The tubes in and around the waste port contain dangerous liquids that can damage your eyes and skin. Always wear protective lab coat, gloves, and safety goggles when handling tubes that may contain even small amounts of reagents such as N-methylpyrrolidone (NMP) or trifluoroacetic acid (TFA).*

Preparing the ABI 433A Instrument

Before you remove the existing waste port, make sure the ABI 433A instrument is idle.

Removing the Existing Waste Port

To remove the existing waste port assembly:

1. Loosen the nut nearest the waste tube by turning the nut with your fingers or with the 13/16-inch wrench (Figure 6-11).
2. Remove the nut from the fitting body.

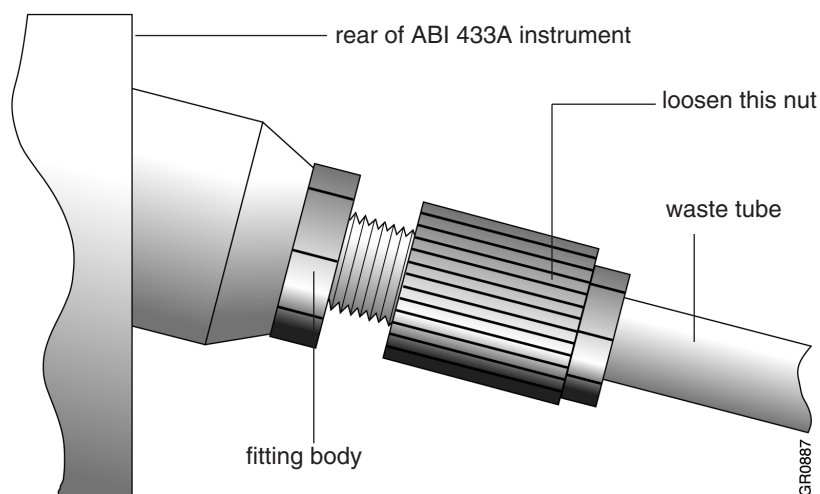


Figure 6-11. Waste port view at rear of ABI 433A instrument

3. Remove the nut from the waste tube.
4. Set the nut aside and lay the waste tube on the table top.
5. Look inside the fitting body still attached to the ABI 433A instrument.

Note that you can see seven small feeder tubes that drain into the waste tube (Figure 6-12). It is important to install the new fitting body so that these seven feeder tubes are not crimped, bent, or otherwise displaced from their present position.

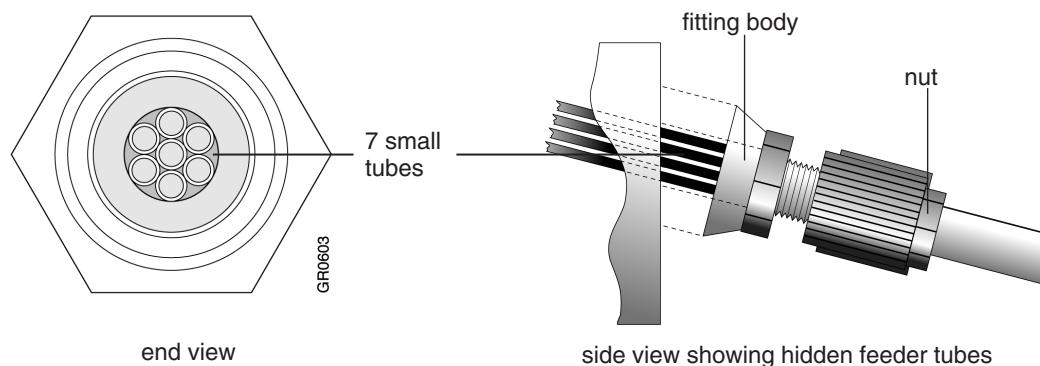


Figure 6-12. Waste port feeder tubes

6. Using the 13/16-inch wrench, remove the fitting body from the ABI 433A instrument.
7. Discard the old fitting body in accordance with all local, state, and federal hazardous waste regulations.

Installing the New Waste Port Fitting Body

IMPORTANT *Do not use the installation instructions contained within the waste port assembly packaging because they are incomplete. Use these instructions instead.*

To install the new waste port fitting body:

1. Remove the new waste port fitting assembly from the plastic packaging.
2. Carefully separate the fitting body from the nut. The ferrule and gripper are located inside the assembly and can be easily lost if dropped.
3. Wrap the Teflon tape around the threads of the wider end of the fitting body (Figure 6-13). Make sure you wrap the tape in the direction shown in the figure.

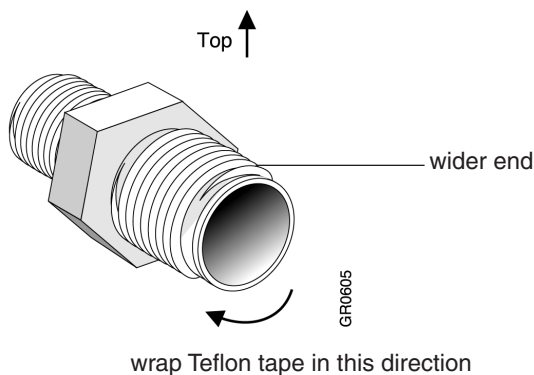


Figure 6-13. Fitting body showing direction to wrap Teflon tape

Caution *Read the entire next step before proceeding.*

4. Thread the wide end of the fitting body (the end with the Teflon tape) into the waste port aperture on the back of the ABI 433A instrument by hand, taking care not to bend or crimp any of the seven small feeder tubes. As you thread the fitting body, look inside to make sure you see all seven of the small tubes you observed in step 5. Thread the fitting body into the aperture by hand until fully seated.
5. After the fitting body is fully seated by hand, use the wrench to tighten the fitting body 1/2 turn more.

Installing the new Waste Port Nut/Ferrule Assembly

To install the new waste port nut/ferrule assembly:

1. Using the utility knife or razor blade, cut off a short piece of waste tube to remove any indentation, discoloration, or degradation. Discard the short piece.

IMPORTANT *You must use a perpendicular cut when you remove the piece from the end of the waste tube. If the end cut is not perpendicular, a leak may develop at the fitting body.*

2. The new waste port is one of two different assemblies that have two varieties of ferrule and gripper. Identify which assembly you have received by comparing the ferrule and gripper with the illustrations shown in Figure 6-14.
3. Remove the gripper from the waste port nut.

If you have a new style A waste port, use a pair of needle-nose pliers to push or pull the gripper from inside the nut.

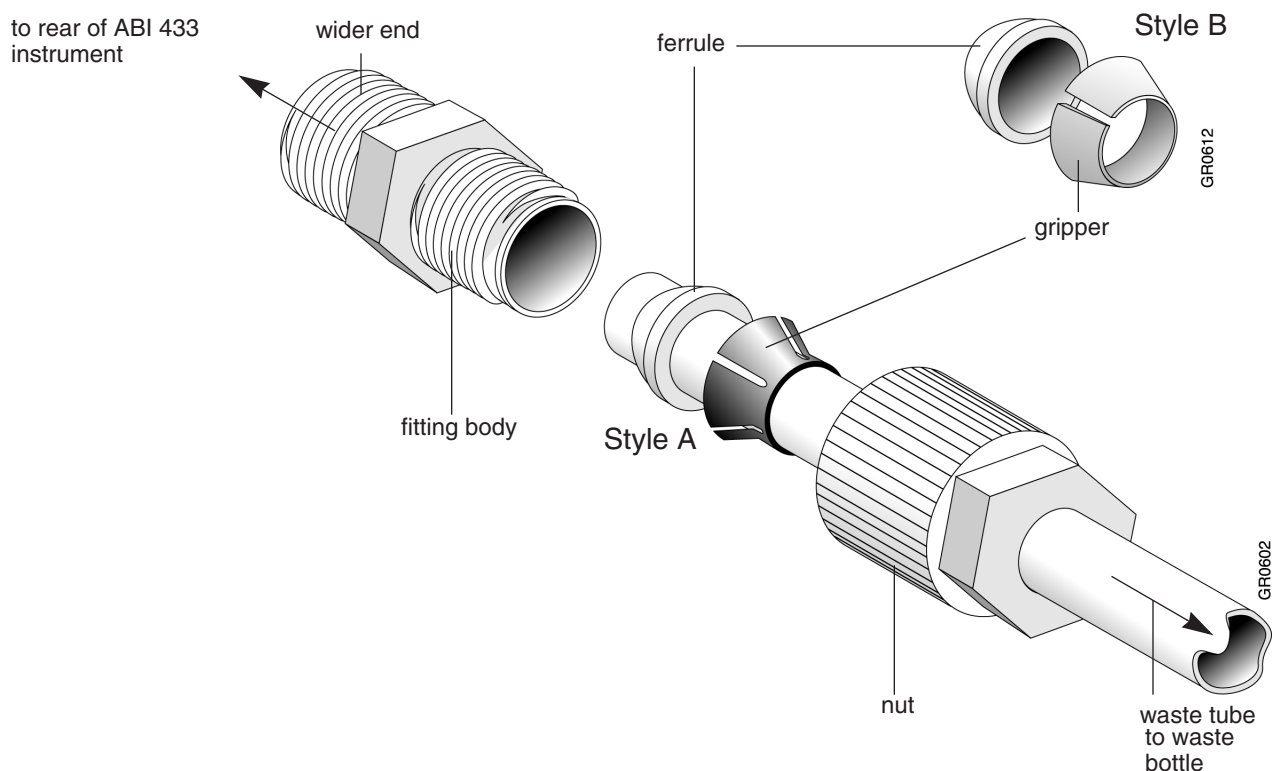


Figure 6-14. Waste port fitting assembly

4. Insert the newly-cut end of the waste tube through the non-threaded end of the nut (Style A has a nut on that end) so that several inches of waste tube protrudes through the nut.
5. Slide the gripper onto the waste tube (Figure 6-14).
6. Slide the ferrule onto the waste tube with the wide shoulder of the ferrule going on first. The shoulder of the ferrule should rest evenly against the gripper.
7. Insert the waste tube into the fitting body until the internal tube stop is reached (Figure 6-15).
8. Slide the ferrule and gripper up against the fitting body.

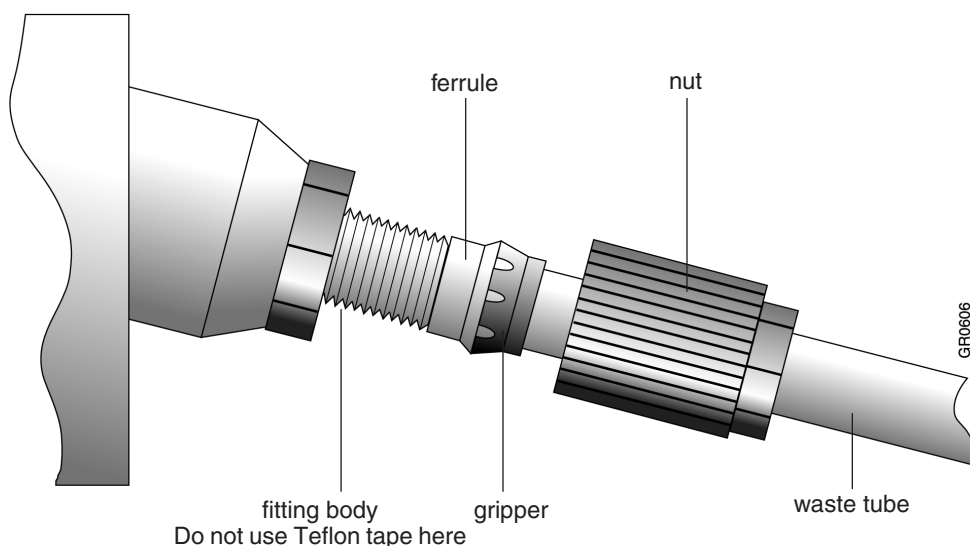


Figure 6-15. Fitting body with ferrule and gripper (style A)

9. Start threading the nut to the fitting by hand. Ensure that you completely insert the waste tube so it can travel no further into the fitting.

Caution *Do not use Teflon tape between the nut and the fitting. This can cause the ferrule to seat incorrectly and leak.*

10. Thread the nut onto the fitting by hand until you cannot turn the nut any further.
11. If you are installing a style A waste port, use the 13/16-inch wrench to tighten the nut 1/2 turn more.

<i>Note</i>	<i>Do not tighten the style B waste port any further for now. If the waste port leaks, tighten the waste port 1/2 turn using channel-lock pliers.</i>

12. Check the waste system:

- Make sure the waste line is long enough and positioned so that it is *not* under tension or severely pulled by routine handling of the waste bottle or instrument.
- Be sure the line is not so long that dips are created where liquid could collect.
- Check the waste port for damage every time you empty the waste bottle. Replace the port if it shows any indication of breaking, leaking, or is heavily discolored.

Flow Test Descriptions

Each flow test consists of a sequence of timed steps, written as a short module in SynthAssist® Software. Explanations and printouts of each flow test are found in this section. Flow tests are used to

- Adjust the gas regulators
- Flush chemicals through the lines
- Check for proper chemical flows
- Troubleshoot the instrument

There are 22 flow tests (see Figure 2-5 on page 2-20 and Figure 2-6 on page 2-21), in two SynthAssist files.

When performing many flow tests, you must place a metering vessel (P/N 400256) in the RV holder. Six flow tests (11, 12, 13, 14, 17 and 18) require an empty, septum-sealed cartridge placed in the autosampler.

IMPORTANT *To prevent accidental chemical spills, put an empty, septum-sealed cartridge in the guideway and place a pressure block against it before starting flow tests 11, 12, 13, 14, 17 and 18.*

Flow test 19 requires an RV with the resin sample (RS) tube attached to the resin sample bulkhead, and a test tube to collect the resin sample.

Flow Test 22 requires a 0.10 mmol reaction vessel with filters in place. Flow Test 23 requires a 0.25 mmol reaction vessel with filters in place.

Table 6-4. Flow Tests 19-23

Flow Test Number	SynthAssist Module	Description
19	a	Resin sample
20	b	Conductivity, Bottles 1, 6, 9, 10
22	d	Conductivity baseline, 0.10 mmol
23	e	Conductivity baseline, 0.25 mmol

Some flow tests should be performed before every synthesis. Table 6-5 lists these flow tests, according to the chemistry option you choose for your synthesis, in the order they should be performed.

Table 6-5. Flow tests performed before each synthesis

Chemistry Option	Flow Test
<i>FastMoc</i>	10, 11, 1, 13, 17, 20
Fmoc/HOBt/DCC	10, 11, 1, 17, 18, 20
Fmoc/ <i>FastMoc</i> TM Loading	4, 18

Boc/HOBt/DCC

10, 11, 2, 1, 17, 18

Use Flow Tests 11, 10, and 2 to adjust the lower and upper regulators. Use Flow Tests 1, 4, 5, 6, and 9 to check the reagent flows. Run Flow Tests 7 and 8 before a synthesis when the instrument has not been used for one or more weeks.

Flow test 20 tests the conductivity of reagents. If reagents have been sitting around or you suspect that the reagents could be in poor condition, run flow test 20. Check the conductivity data generated and compare with Figure 6-16 on page 6-50.

Flow Tests with a Pause

If you want to use a version of the flow test in which the synthesizer pauses when a visual measurement must be taken, then use “FT1-18 Alternative” and “FT 19-23 Alternative.” These flow tests include a beep that alerts you when the flow test is paused.

To run a flow test:

1. In SynthAssist Software, find the module that corresponds to the flow test you want to run. Flow test modules are grouped together in SynthAssist files called “Flow Tests 1-18” or “Flow Tests 19-23.”
2. Transfer the appropriate set of flow test modules to the ABI 433A instrument.
3. In the Main Menu, press the **module test** soft key.

Press **cancel** to
return to Main Menu

Select test MOD: <i>H</i> (99 steps)				
cancel		prev		next start
↓				
running test Mod H				
		end run		more
↓				
S: 2 / 99 Fxn 55: #9 B RVT: 8/60				
hold		jmp stp		pause nxt stp more

4. Press the **next** or **prev** soft key until the flow test module appears on the top line after the words “Select test MOD:”
5. Press the **start** soft key to run the flow test.

Press the **cancel** soft key to return to the Main Menu without running the flow test.

Flow Test 1 (Flow Tests 1-18, module a)

Purpose: To check for proper flow of Bottle 1 and to flush the delivery line.

Delivery: 5-second delivery of the contents of Bottle 1 to the metering vessel in the RV holder.

Expected Results: *FastMoc* or Fmoc/HOBt/DCC: Piperidine, 0.80–1.30 mL
Boc/HOBt/DCC: DIEA, 1.9–2.4 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note *After delivery and measurement, wash the metering vessel with NMP. Do not wash it with DCM, because a solution of piperidine and DCM slowly reacts to form crystals of piperidine hydrochloride.*

Flow Test 1

Step#	Fxn	Fxn Name	Time	Add
1	13	#10 T VB	1	0
2	14	#10 B VB	1	0
3	79	PRS #1	15	0
4	16	#1 B VB	3	0
5	9	GAS T VB	2	0
6	42	DRAIN RV	5	0
7	10	GAS B VB	2	0
8	51	#1 B RV	5	0
9	40	MIX RV	3	0
10	1	WAIT	10	0
11	42	DRAIN RV	10	0
12	10	GAS B VB	2	0
13	56	#10 B RV	10	0
14	40	MIX RV	3	0
15	42	DRAIN RV	5	0
16	50	#10 RV-DRN	5	0
17	42	DRAIN RV	10	0
18	13	#10 T VB	1	0
19	14	#10 B VB	1	0
20	9	GAS T VB	2	0
21	10	GAS B VB	2	0
22	98	BEGIN LOOP	2	0
23	2	VORTEX ON	1	0
24	56	#10 B RV	20	0
25	40	MIX RV	2	0
26	3	VORTEX OFF	1	0
27	42	DRAIN RV	20	0
28	99	END LOOP	1	0
29	13	#10 T VB	1	0
30	14	#10 B VB	1	0
31	9	GAS T VB	10	0
32	10	GAS B VB	10	0

Flow Test 2 (Flow Tests 1-18, module b)

Note *This flow test is not necessary when the ABI 433A Peptide Synthesizer is using FastMoc or Fmoc/HOBt/DCC chemistry.*

Purpose: To set the upper regulator pressure, to check Bottle 2 delivery, and to check for leaks in the TFA seal.

Flow test 2 only measures TFA once. To readjust the regulator or to repeat a delivery measurement, jump back to step 1 when the flow test reaches step 29 (wait 60 seconds).

To avoid physical contact with TFA, run the neutralization and washing steps (steps 30 through 68) before removing the metering vessel from the RV holder.

Delivery: 18-second delivery of the contents of Bottle 2 to the metering vessel in the RV holder.

Expected Results: Boc/HOBt/DCC: TFA, 1.95–2.05 mL

FastMoc or Fmoc/HOBt/DCC: no reagent

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Caution *To prevent over-pressurization of the TFA bottle, the pressure reading on the upper regulator should never rise above 3.5 psi. Typical readings on this regulator, which controls the gas pressure to Bottle 2, range between 2.3 to 3.0 psi.*

Flow Test 2

Step#	Fxn #	Fxn Name	Time	Add
1	73	VENT #2	2	0
2	75	GAS-VENT #2	2	0
3	73	VENT #2	2	0
4	2	VORTEX ON	1	0
5	55	#9 B RV	5	0
6	40	MIX RV	2	0
7	3	VORTEX OFF	1	0
8	42	DRAIN RV	10	0
9	76	PRS #2	25	0
10	71	#2 B VB	2	0
11	10	GAS B VB	2	0
12	12	#9 B VB	2	0
13	10	GAS B VB	3	0
14	72	#2 B RV	18	0
15	40	MIX RV	2	0
16	41	VENT RV	2	0
17	10	GAS B VB	3	0

Flow Test 2, continued

Step#	Fxn #	Fxn Name	Time	Add
18	73	VENT #2	3	0
19	74	FLUSH #2	3	0
20	73	VENT #2	3	0
21	75	GAS-VENT #2	3	0
22	73	VENT #2	3	0
23	74	FLUSH #2	3	0
24	73	VENT #2	3	0
25	41	VENT RV	2	0
26	42	DRAIN RV	5	0
27	49	#9 RV-DRAIN	10	0
28	42	DRAIN RV	10	0
29	1	WAIT	60	0
30	2	VORTEX ON	1	0
31	55	#9 B RV	5	0
32	40	MIX RV	2	0
33	3	VORTEX OFF	1	0
34	42	DRAIN RV	8	0
35	11	#9 T VB	2	0
36	12	#9 B VB	2	0
37	9	GAS T VB	2	0
38	10	GAS B VB	2	0
39	2	VORTEX ON	1	0
40	55	#9 B RV	15	0
41	40	MIX RV	1	0
42	79	PRS #1	10	0
43	41	VENT RV	2	0
44	51	#1 B RV	6	0
45	55	#9 B RV	10	0
46	15	#1 T VB	1	0
47	16	#1 B VB	1	0
48	11	#9 T VB	2	0
49	12	#9 B VB	2	0
50	40	MIX RV	10	0
51	10	GAS B VB	3	0
52	3	VORTEX OFF	1	0
53	42	DRAIN RV	20	0
54	11	#9 T VB	2	0
55	12	#9 B VB	2	0
56	9	GAS T VB	2	0
57	10	GAS B VB	2	0
58	2	VORTEX ON	1	0
59	55	#9 B RV	20	0
60	40	MIX RV	5	0
61	9	GAS T VB	2	0
62	10	GAS B VB	2	0
63	3	VORTEX OFF	1	0
64	42	DRAIN RV	20	0
65	11	#9 T VB	2	0
66	12	#9 B VB	2	0
67	9	GAS T VB	10	0
68	10	GAS B VB	10	0

Flow Test 3 (Flow Tests 1-18, module c)

Purpose: To check barcode reader accuracy.

Expected Results: A printed list with each amino acid cartridge properly identified.

Requirements: A series of cartridges with legible, unmarred barcode labels and a printer.

Procedure to run module c:

1. Load the amino acid cartridges.
2. Transfer Flow Tests 1-18 from SynthAssist Software and run module c (See "To run a flow test:" on page 6-28).

Flow Test 3

Step#	Fxn #	Fxn Name	Time	Add
1	98	BEGIN LOOP	99	0
2	1	WAIT	1	0
3	4	SAVE CART	10	0
4	7	EJECT CART	10	0
5	8	ADVAN CART	10	0
6	99	END LOOP	1	0

Note *If the flow test pauses at step 3, the cartridge is being compared to a previously run sequence. To inactivate this comparison, either turn off the barcode check or send the desired sequence.*

Flow Test 4 (Flow Tests 1-18, module d)

Purpose: To check for proper flow of Bottle 4 and to flush the delivery line.

Delivery: Five-second delivery of the contents of Bottle 4 to the metering vessel in the RV holder.

Expected Results: *FastMoc* or Fmoc/HOBt/DCC: DMAP, 2.0–2.3 mL
Boc/HOBt/DCC: Ac₂O, 1.70–2.30 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Flow Test 4

Step #	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	42	DRAIN RV	3	0
3	10	GAS B VB	2	0
4	77	PRS #4	15	0
5	52	#4 B RV	5	0
6	40	MIX RV	2	0
7	1	WAIT	10	0
8	42	DRAIN RV	7	0
9	10	GAS B VB	5	0
10	49	#9 RV-DRN	5	0
11	42	DRAIN RV	10	0
12	11	#9 T VB	1	0
13	12	#9 B VB	1	0
14	9	GAS T VB	2	0
15	10	GAS B VB	2	0
16	2	VORTEX ON	1	0
17	55	#9 B RV	15	0
18	40	MIX RV	3	0
19	3	VORTEX OFF	1	0
20	42	DRAIN RV	20	0
21	11	#9 T VB	1	0
22	12	#9 B VB	1	0
23	9	GAS T VB	10	0
24	10	GAS B VB	10	0

Flow Test 5 (Flow Tests 1-18, module e)

Purpose: To check for proper flow of Bottle 5 with Boc chemistry and to flush the delivery line.

Delivery: 5-second delivery of contents of Bottle 5 to the metering vessel in the RV holder.

Expected Results: Boc/HOBt/DCC: DMSO, 0.8–1.3 mL

FastMoc or Fmoc/HOBt/DCC: Use Flow Test 13

IMPORTANT When using HBTU solution, use Flow Test 13, not Flow Test 5.

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Flow Test 5

Step#	Fxn#	Fxn Name	Time	Add
1	42	DRAIN RV	3	0
2	10	GAS B VB	2	0
3	78	PRS #M	15	0
4	53	#5 B RV	5	0
5	40	MIX RV	2	0
6	1	WAIT	10	0
7	42	DRAIN RV	10	0
8	50	#10 RV-DRN	15	0
9	42	DRAIN RV	10	0
10	49	#9 RV-DRN	20	0
11	42	DRAIN RV	12	0
12	10	GAS B VB	3	0

Flow Test 6 (Flow Tests 1-18, module f)

Purpose: To check for proper flow of Bottle 6 and to flush lines.

Delivery: 5-second delivery of contents of Bottle 6 to the metering vessel in the RV holder.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
Methanol, 1.95-2.55 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Flow Test 6

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	42	DRAIN RV	3	0
3	10	GAS B VB	2	0
4	78	PRS #M	15	0
5	54	#6 B RV	5	0
6	40	MIX RV	2	0
7	9	GAS T VB	10	0
8	42	DRAIN RV	15	0
9	10	GAS B VB	10	0

Flow Test 7 (Flow Tests 1-18, module g)

Purpose: To flush the delivery line of Bottle 7.

Delivery: Four-second delivery of contents of Bottle 7 to waste.

Expected Results: Boc/HOBt/DCC and Fmoc/HOBt/DCC: 1M HOBt, no measurements are taken. *FastMoc*: 2 M DIEA, no measurements are taken.

Requirements: Place a metering vessel in the RV holder. No reagent is delivered to the metering vessel during Flow Test 7.

To run Flow Test 7:

1. Run the flow test before a synthesis if the instrument has not been used for several days. The 0.5 mL loop should fill up during the first 3 seconds of step 2.
2. Remove the right side panel to watch the 0.5 mL loop fill up during the flow test. If the 0.5 mL loop does not fill in 3 seconds, there may be precipitate blocking the line to Bottle 7.
3. To dissolve the crystals, connect a bottle of DCM/MeOH (60/40) in place of Bottle 7 and run Flow Test 7 again.
4. At step 2, press **hold** until DCM/MeOH flows easily through the loop.
5. Remove the DCM/MeOH bottle and replace it with an empty bottle.
6. Repeat Flow Test 7 once more to let nitrogen flow through the loop.
7. Clean the outside of the tubing with a lint-free tissue. Replace Bottle 7.
8. If rinsing with DCM/MeOH does not improve flow, check the measuring loop and the attached tubing for crimps or leaks. Also check for leaks in the tubing associated with Bottles 5 through 8. For a more detailed flushing procedure, see page 6-16.

Flow Test 7

Step #	Fxn #	Fxn Name	Time	Add
1	78	PRS #M	15	0
2	68	MEAS #7	4	0
3	70	PURGE ML	5	0
4	10	GAS B VB	2	0
5	14	#10 B VB	2	0
6	10	GAS B VB	10	0

Flow Test 8 (Flow Tests 1-18, module h)

Purpose: To flush the delivery line for Bottle 8.

Delivery: 4-second delivery of contents of Bottle 8 to waste.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: 1M DCC, no measurements are taken.

Requirements: See Flow Test 7, page 6-36.

This flow test should be run before a synthesis if the instrument has not been used for several days. The 0.5 mL loop should fill up during the first 3 seconds of step 2.

1. Remove the right side panel to watch the 0.5 mL loop fill up during the flow test. If the 0.5 mL loop does not fill in three seconds, there may be precipitate blocking the line to Bottle 8.
2. To dissolve the crystals, connect a bottle of DCM/MeOH (60/40) in place of Bottle 8 and run Flow Test 8 again.
3. At step 2, press **hold** until DCM/MeOH flows easily through the loop.
4. Remove the DCM/MeOH bottle and replace it with an empty bottle.
5. Repeat Flow Test 8 once to let nitrogen flow through the loop.
6. Clean the outside of the tubing with a lint-free tissue. Replace Bottle 8.
7. If rinsing with DCM/MeOH does not improve flow, check the measuring loop and the attached tubing for crimps or leaks. Also check for leaks in the tubing associated with Bottles 5 through 8. For a more detailed washing procedure, see page 6-16.

Flow Test 8

Step #	Fxn #	Fxn Name	Time	Add
1	78	PRS #M	15	0
2	69	MEAS #8	4	0
3	70	PURGE ML	5	0
4	10	GAS B VB	2	0
5	14	#10 B VB	2	0
6	10	GAS B VB	10	0

Flow Test 9 (Flow Tests 1-18, module i)

Purpose: To check for proper flow of Bottle 9 and to flush the delivery line.

Delivery: 5-second delivery of contents of Bottle 9 to the metering vessel in the RV holder.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
DCM, 2.90–3.50 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Flow Test 9

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	42	DRAIN RV	3	0
3	10	GAS B VB	2	0
4	55	#9 B RV	5	0
5	40	MIX RV	3	0
6	9	GAS T VB	10	0
7	42	DRAIN RV	10	0
8	10	GAS B VB	10	0

Flow Test 10 (Flow Tests 1-18, module A)

Purpose: To set lower regulator pressure and to flush line.

Delivery: 5-second delivery of contents of Bottle 10 to the metering vessel in the RV holder.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
NMP, 2.45–2.55 mL*

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

<i>Note</i>	<i>The lower regulator monitors gas to the valve blocks and pressure to all the chemical bottles, except bottle 2. The pressure on the lower regulator should never be permitted to go above 11 psi.</i>

Flow Test 10

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	42	DRAIN RV	3	0
3	10	GAS B VB	2	0
4	56	#10 B RV	5	0
5	40	MIX RV	3	0
6	9	GAS T VB	10	0
7	42	DRAIN RV	10	0
8	10	GAS B VB	10	0

* If the pressure on the lower regulator has been increased to assure a 2.0 g delivery of Bottle 10 in Flow Test 11, the delivery in Flow Test 10 may be as much as 2.75 mL. However, before increasing the pressure on the lower regulator, first change the cartridge in-line filter and check the needle for blockage. (See "Changing Disposable In-Line Filters" on page 2-14)

Flow Test 11 (Flow Tests 1-18, module B)

Purpose: To check for proper flow from Bottle 10 and from the in-line filter to cartridge.

Delivery: 5-second delivery of contents of Bottle 10 to cartridge.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
NMP, 2.0– 2.3 grams

Requirements: Pre-weighed cartridge

Procedure to run Flow Test 11:

1. Place pre-weighed cartridge in autosampler.
2. Run Flow Test 11.
3. Re-weigh ejected cartridge to verify expected weight of reagent delivered to cartridge.

<i>Note</i>	<i>If the flow to the cartridge is low, and Flow Test 10 is correct, replace the in-line filter to the cartridge and check the needle for blockage (See "Changing Disposable In-Line Filters" on page 2-14). If the delivery is still inadequate, you may increase the pressure on the lower regulator. However, the pressure on the lower regulator should not exceed 11 psi.</i>

Flow Test 11

Step#	Fxn#	Fxn Name	Time	Add
1	8	ADVANCE CART	10	0
2	5	NEEDLE DOWN	10	0
3	10	GAS B VB	2	0
4	65	#10 CART	5	0
5	60	MIX CART	5	0
6	6	NEEDLE UP	10	0
7	7	EJECT CART	10	0
8	8	ADVANCE CART	10	0
9	10	GAS B VB	10	0

Flow Test 12 (Flow Tests 1-18, module C)

Purpose: To check for proper flow from Bottle 9 and from the in-line filter to cartridge.

Delivery: 5-second delivery of contents of Bottle 9 to cartridge.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
DCM, 3.40–4.10 grams

Requirements: Place a pre-weighed cartridge in the autosampler. Measure the volume of reagents delivered to the cartridge.

<i>Note</i>	<i>If the flow to the cartridge is low and Flow Test 9 is correct, the in-line filter to the needle may need replacing.</i>

Flow Test 12

Step#	Fxn#	Fxn Name	Time	Add
1	8	ADVAN CART	10	0
2	5	NEEDLE DWN	10	0
3	10	GAS B VB	2	0
4	64	#9 CART	5	0
5	60	MIX CART	5	0
6	6	NEEDLE UP	10	0
7	7	EJECT CART	10	0
8	8	ADVAN CART	10	0
9	10	GAS B VB	10	0

Flow Test 13 (Flow Tests 1-18, module D): HBTU/HOBt Delivery

IMPORTANT Always perform Flow Test 11 **before and after** performing each Flow Test 13, to rinse the in-line filter and assure accurate Bottle 5 delivery.

Purpose: To check delivery of the HBTU/HOBt solution to the cartridge. Perform this flow test each time you change either the in-line filter to the amino acid cartridge or the filter on the end of the Bottle 5 delivery line.

Delivery: 8-second delivery of contents of Bottle 5 to the cartridge.

Expected Range: *FastMoc*: 1.90–2.10 g of 0.45 M HBTU/HOBt solution.

Requirement: Previously used cartridge.

Procedure to run module D:

1. Place a tared cartridge at the needle position.
2. Run Flow Test 13.
3. Re-weigh the ejected cartridge to verify the expected weight of reagent delivered to the cartridge.

Note If the weight of 0.45 M HBTU delivered to the cartridge is not in the 1.9-2.1 g range, use the Module Editor menu to adjust the time in step 4, Fxn 94 (#5 TO CART) so that the delivery weight falls within the expected range.

Use SynthAssist Software to enter the correct time for Fxn 94 in the activation module A, in the *FastMoc* chemistry that will be used for the synthesis.

Flow Test 13

Step#	Fxn#	Fxn Name	Time	Add
1	5	NEEDLE DWN	10	0
2	10	GAS B VB	2	0
3	78	PRS #M	15	0
4	94	#5 TO CART	8	0
5	60	MIX CART	5	0
6	61	VENT CART	3	0
7	6	NEEDLE UP	10	0
8	7	EJECT CART	10	0
9	8	ADVAN CART	10	0
10	10	GAS B VB	10	0

Flow Test 14 (Flow Tests 1-18, module E)

Purpose: Use for instrument test and troubleshooting.

Delivery: 5-second delivery of contents of Bottle 10 to the ACT, then to the metering vessel. This step is followed by a 5-second delivery of the contents of Bottle 10 to the cartridge, which is transferred to the ACT, and finally to the metering vessel.

Expected Results: At step # 10, *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, 2.00–2.80 mL

At step #28, *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, minimum 1.00 mL

Requirements: Place a metering vessel in the RV holder. Place a cartridge in the autosampler. Measure the volume of reagents delivered to the metering vessel.

Note *This test is not performed routinely before a synthesis.*

Flow Test 14

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	22	DRAIN ACT	3	0
3	42	DRAIN RV	3	0
4	10	GAS B VB	2	0
5	36	#10 B ACT	5	0
6	20	MIX ACT	2	0
7	28	GAS T ACT	3	0
8	38	ACT TO RV _o	5	0
9	40	MIX RV	2	0
10	1	WAIT	10	0
11	22	DRAIN ACT	15	0
12	42	DRAIN RV	15	0
13	5	NEEDLE DWN	10	0
14	10	GAS B VB	2	0
15	65	#10 CART	5	0
16	60	MIX CART	3	0
17	24	CART TO AC	10	0
18	61	VENT CART	2	0
19	6	NEEDLE UP	10	0
20	7	EJECT CART	10	0
21	8	ADVAN CART	10	0
22	9	GAS T VB	2	0
23	42	DRAIN RV	3	0
24	10	GAS B VB	2	0
25	28	GAS T ACT	3	0
26	38	ACT TO RV _o	4	0
27	40	MIX RV	2	0
28	1	WAIT	10	0
29	22	DRAIN ACT	15	0
30	42	DRAIN RV	15	0
31	9	GAS T VB	10	0
32	10	GAS B VB	10	0

Flow Test 15 (Flow Tests 1-18, module F)

Purpose: Use for instrument quality control, for troubleshooting, and for testing the sheeting action of the activator.

Delivery: Contents of Bottle 9 to top of activator with drain

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: DCM, no measurements are taken. Check for proper sheeting action.

Requirements: Place a metering vessel in the RV holder. No reagent is delivered to the metering vessel during Flow Test 15.

Note *This test is not performed routinely before a synthesis.*

Flow Test 15

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	29	#9 ACT-DRN	10	0
3	22	DRAIN ACT	25	0
4	9	GAS T VB	10	0
5	10	GAS B VB	10	0

Flow Test 16 (Flow Tests 1-18, module G)

Purpose: Use for instrument Q. C., and for troubleshooting.

Delivery: Contents of Bottle 10 to top of metering vessel.

Expected Results: Boc/HOBt/DCC, Fmoc/HOBt/DCC, and *FastMoc*: NMP, 0.90–1.30 mL.

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note *This test is not performed routinely before a synthesis.*

Flow Test 16

Step#	Fxn#	Fxn Name	Time	Add
1	9	GAS T VB	2	0
2	42	DRAIN RV	3	0
3	41	VENT RV	5	0
4	46	#10 T RV	5	0
5	48	GAS T RV	3	0
6	41	VENT RV	2	0
7	1	WAIT	10	0
8	42	DRAIN RV	15	0
9	9	GAS T VB	10	0
10	10	GAS B VB	10	0

Flow Test 17 (Flow Tests 1-18, module H)

Purpose: Use to calibrate delivery of Bottle 7 through 0.5 mL loop.

Delivery: 0.5 mL metering loop is filled from Bottle 7 and delivered to cartridge.

Expected Results: Instrument calibration: NMP, 0.515-0.554 g

FastMoc: 2M DIEA, 0.46–0.50 g

Fmoc/HOBt/DCC: 1M HOBt/NMP, 0.52-0.55 g

Boc/HOBt/DCC: 1M HOBt/NMP, 0.52-0.55 g

Requirements: Pre-weighed cartridge.

Procedure: See Flow Test 11, page 6-40.

<i>Note</i>	<i>This test is not performed routinely before a synthesis. However, if the instrument has not been used for several days, this flow test or Flow Test 7 should be performed to ensure proper delivery of reagents.</i>

Flow Test 17

Step#	Fxn#	Fxn Name	Time	Add
1	8	ADVANCE CART	10	0
2	5	NEEDLE DOWN	10	0
3	78	PRS #M	15	0
4	68	MEAS #7	3	0
5	10	GAS B VB	2	0
6	63	ML TO CART	5	0
7	61	VENT CART	2	0
8	6	NEEDLE UP	10	0
9	7	EJECT CART	10	0
10	8	ADVANCE CART	10	0

Flow Test 18 (Flow Tests 1-18, module I)

WARNING **CHEMICAL HAZARD** *DCC can cause allergic reactions in sensitive persons. When running this flow test with 1M DCC in NMP, use appropriate safety precautions. Wear gloves and clean the used cartridge in a well-ventilated hood.*

Purpose: Use to calibrate delivery of Bottle 8 through 0.5 mL loop.

Delivery: 0.5 mL metering loop is filled from bottle 8 and delivered to cartridge.

Expected Results: *FastMoc*, Fmoc/HOBt/DCC, and Boc/HOBt/DCC:
1 M DCC/NMP, 0.515–0.554 g

Requirements: Pre-weighed cartridge.

Procedure: See Flow Test 11, page 6-40.

Note *This test is not performed routinely before a synthesis. However, if the instrument has not been used for several days, this flow test or Flow Test 8 should be performed to ensure proper delivery of DCC.*

Flow Test 18

Step#	Fxn#	Fxn Name	Time	Add
1	8	ADVAN CART	10	0
2	5	NEEDLE DWN	10	0
3	78	PRS #M	15	0
4	69	MEAS #8	3	0
5	10	GAS B VB	2	0
6	63	ML TO CART	5	0
7	61	VENT CART	2	0
8	6	NEEDLE UP	10	0
9	7	EJECT CART	10	0
10	8	ADVAN CART	10	0

Flow Test 19 (Flow Tests 19-23, module a)

Purpose: Use to troubleshoot resin-sampler valves, switches, and the resin-sampler line with Bottle 10.

Delivery: A solution for resin sampling is delivered to a test tube.

Requirements: Resin sample test tube and reaction vessel with resin-sampling line connected to a bulkhead. If you want to check resin sample delivery, charge the RV with resin.

Procedure: To run this flow test, you must first define at least one cycle in the Run Editor menu. Then, in the Cycle Monitor menu, answer **Yes** to the resin sampling option (see page 2-40).

IMPORTANT *Do not use the metering vessel with this flow test. Use a reaction vessel with the resin-sampling line connected to a bulkhead.*

Flow Test 19

Step#	Fxn#	Fxn Name	Time	Add
1	56	#10 B RV	14	0
2	41	VENT RV	1	0
3	88	RS TO RV	1	0
4	91	#10 TO RS	4	0
5	89	RS TO FC	1	0
6	2	VORTEX ON	1	0
7	40	MIX RV	2	0
8	91	#10 TO RS	4	0
9	88	RS TO RV	1	0
10	93	GAS TO RS	2	0
11	91	#10 TO RS	4	0
12	41	VENT RV	2	0
13	91	#10 TO RS	1	0
14	48	GAS T RV	2	0
15	87	TAKE SAMPL	2	0
16	89	RS TO FC	1	0
17	90	#9 TO RS	1	0
18	93	GAS TO RS	2	0
19	90	#9 TO RS	1	0
20	93	GAS TO RS	5	0
21	41	VENT RV	2	0
22	88	RS TO RV	1	0
23	90	#9 TO RS	4	0
24	89	RS TO FC	1	0
25	39	RELAY 0	1	0
26	3	VORTEX OFF	1	0
27	42	DRAIN RV	10	0
28	41	VENT RV	2	0
29	88	RS TO RV	1	0
30	93	GAS TO RS	3	0
31	41	VENT RV	2	0
32	89	RS TO FC	1	0
33	42	DRAIN RV	15	0
34	9	GAS T VB	10	0
35	10	GAS B VB	10	0

Flow Test 20 (Flow Tests 19-23, module b)

<i>Note</i>	<i>If the instrument has been sitting idle for a while, do Flow Tests 20, 22, and 23 at least twice to get a consistent baseline value.</i>

Purpose: Use to test the conductivity cell

Delivery: Four deliveries to conductivity cell, from Bottle 6 (MeOH), Bottle 1 (piperidine), Bottle 10 (NMP) and Bottle 9 (DCM).

Requirements: Conductivity cell and metering vessel, reagents for *FastMoc* or Fmoc/HOBt/DCC chemistry

Procedure: (Refer to the SynthAssist user guide for procedures related to SynthAssist Software).

To run module b:

1. Send Flow Tests 19-23 from SynthAssist Software to the ABI 433A instrument.
2. In SynthAssist Software, open a new Run.
3. From the Chemistry pop-up menu, select **Choose**.
4. From the dialog box, select **Flow Tests 19-23**, then click **OK**.
5. From the Sequence pop-up menu, select **None**.
6. From the pop-up menu showing Calculations, select **Cycles**.
7. Under the Heading called Modules, click in the empty space just below the heading to highlight the space.
8. Type the letter “b” and press **Return**.
9. Select **File > Save**.
10. Send this Run File to the ABI 433A instrument.
11. Open the monitoring window.
12. On the ABI 433A instrument, run module b in the Module Test menu.

The monitoring window should display four peaks for each solvent delivery, as shown in Figure 6-16 on page 6-50.

For a complete module printout of Flow Test 20, see page 6-50.

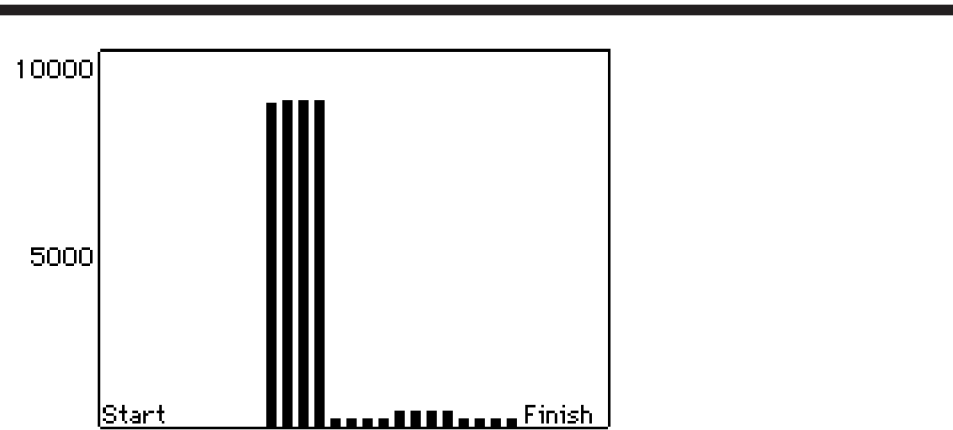


Figure 6-16. Monitoring window display of Flow Test 20

Flow Test 20

Step#	Fxn#	Fxn Name	Time	Add
1	1	WAIT	2	
2	78	PRS #M	10	
3	19	#6 B VB	5	
4	98	BEGIN LOOP	4	
5	135	Mon Reset	1	
6	54	#6 B RV	5	
7	130	Mon PrevPk	1	
8	1	Wait	3	
9	131	Mon Stop	1	
10	132	Save MonPk	1	
11	42	DRAIN RV	10	
12	99	END LOOP	1	
13	79	PRS #1	10	
14	16	#1 B VB	5	
15	98	BEGIN LOOP	4	
16	135	Mon Reset	1	
17	51	#1 B RV	5	
18	130	Mon PrevPk	1	
19	1	Wait	3	
20	131	Mon Stop	1	
21	132	Save MonPk	1	
22	42	DRAIN RV	10	
23	99	END LOOP	1	
24	14	#10 B VB	5	
25	98	BEGIN LOOP	4	
26	135	Mon Reset	1	
27	56	#10 B RV	5	
28	130	Mon PrevPk	1	
29	1	Wait	3	
30	131	Mon Stop	1	
31	132	Save MonPk	1	
32	42	DRAIN RV	10	
33	99	END LOOP	1	
34	12	#9 B VB	5	
35	98	BEGIN LOOP	4	
36	135	Mon Reset	1	
37	55	#9 B VB	5	

Flow Test 20, continued

Step#	Fxn#	Fxn Name	Time	Add
38	130	Mon PrevPk	1	
39	1	Wait	3	
40	131	Mon Stop	1	
41	132	Save MonPk	1	
42	42	DRAIN RV	10	
43	99	END LOOP	1	
44	9	GAS T VB	5	
45	10	GAS B VB	5	
46	41	VENT RV	10	

Flow Test 22 (Flow Tests 19-23, module d)

Note *If the instrument has been sitting idle for a week, do Flow Tests 20, 22, and 23 at least twice to get a consistent baseline value.*

Purpose: Use to determine the conductivity baseline of a solution of NMP and piperidine for *FastMoc* 0.10 mmol modules.

Delivery: Five samples are taken from RV and sent to conductivity flow cell.

Requirements: Reaction vessel (8 mL), and reagents for *FastMoc* chemistry.

Procedure to run module d:

1. Send Flow Tests 19-23 from SynthAssist Software to the ABI 433A instrument.
 2. In SynthAssist Software, open a new Run.
 3. From the Chemistry pop-up menu, select **Choose**.
 4. From the dialog box, select **Flow Tests 19-23**, then click **OK**.
 5. From the Sequence pop-up menu, select **None**.
 6. From the pop-up menu showing Calculations, select **Cycles**.
 7. Under the Heading called Modules, click in the empty space just below the heading to highlight the space.
 8. Type the letter “d” and press **Return**.
 9. Select **File > Save**.
 10. Send this Run File to the ABI 433A instrument.
 11. Open the monitoring window.
 12. Place the 8-mL reaction vessel, with filters, on the ABI 433A instrument.
 13. On the ABI 433A instrument, run module d in the Module Test menu.
The Log window displays the conductivity of each of five samples of the NMP/piperidine solution.
 14. Use the value of the last peak for the conductivity baseline. Enter this value, divided by ten, for “T” in Function 128 when it appears in the *FastMoc* 0.10 mmol module B-”Deprotection/ Mon 1st Peak-X.”
- For a complete module printout of Flow Test 22, see page 6-53.

Flow Test 22

Step#	Fxn#	Fxn Name	Time	Add
1	1	WAIT	1	
2	58	INTERRUPT		
3	135	Mon Reset	1	
4	110	begin loop	5	
5	42	DRAIN RV	10	
6	98	BEGIN LOOP	1	
7	56	#10 TO RV	5	
8	40	MIX RV	2	
9	2	VORTEX ON	5	
10	3	VORTEX OFF	1	
11	42	DRAIN RV	8	
12	99	END LOOP	1	
13	56	#10 TO RV	3	
14	79	PRS #1	10	
15	51	#1 TO RV	5	
16	56	#10 TO RV	4	
17	40	MIX RV	2	
18	2	VORTEX ON	10	
19	3	VORTEX OFF	1	
20	40	MIX RV	1	
21	130	MonPrevPk	1	
22	42	DRAIN RV	3	
23	1	WAIT	3	
24	131	MonStop	1	
25	132	Save Mon	1	
26	111	MonEndLoop	1	
27	42	DRAIN RV	10	
28	98	BEGIN LOOP	3	
29	56	#10 TO RV	5	
30	40	MIX RV	2	
31	2	VORTEX ON	1	
32	1	WAIT	5	
33	3	VORTEX OFF	1	
34	42	DRAIN RV	8	
35	41	VENT RV	2	
36	50	#10 RV-DRN	3	
37	42	DRAIN RV	5	
38	99	END LOOP	1	
39	41	VENT RV	5	
40	98	BEGIN LOOP	2	
41	13	#10 T VB	3	
42	14	#10 B VB	3	
43	9	GAS T VB	5	
44	10	GAS B VB	5	
45	99	END LOOP	1	

Flow Test 23 (Flow Tests 19-23, module e)

<i>Note</i>	<i>If the instrument has been sitting idle for a week, do Flow Tests 20, 22, and 23 at least twice to get a consistent baseline value.</i>

Purpose: Use to determine the conductivity baseline of a solution of NMP and piperidine for *FastMoc* 0.25 mmol modules.

Delivery: Five samples are taken from RV and sent to conductivity flow cell.

Requirements: Reaction vessel (41 mL), and reagents for *FastMoc* chemistry.

Procedure: to run module e:

1. Send Flow Tests 19-23 from SynthAssist Software to the ABI 433A instrument.
2. In SynthAssist Software, open a new Run.
3. From the Chemistry pop-up menu, select **Choose**.
4. From the dialog box, select **Flow Tests 19-23**, then click **OK**.
5. From the Sequence pop-up menu, select **None**.
6. From the pop-up menu showing Calculations, select **Cycles**.
7. Under the Heading called Modules, click in the empty space just below the heading to highlight the space.
8. Type the letter “e” and press Return.
9. Select **File > Save**.
10. Send this Run File to the ABI 433A instrument.
11. Open the monitoring window.
12. Place the 41-mL reaction vessel, with filters, on the ABI 433A instrument.
13. On the ABI 433A instrument, run module e in the Module Test menu.
The Log window displays the conductivity of each of five samples of the NMP/piperidine solution.
14. Use the value of the last peak for the conductivity baseline. Enter this value, divided by ten, for “T” in Function 128 when it appears in the *FastMoc* 0.25 mmol module “B-Deprotection/ Mon 1st Peak-X.”

For a complete module printout of Flow Test 23, see page 6-55.

Flow Test 23

Step#	Fxn#	Fxn Name	Time	Add
1	1	WAIT	1	
2	58	INTERRUPT		
3	135	Mon Reset	1	
4	110	begin loop	5	
5	42	DRAIN RV	18	
6	98	BEGIN LOOP	1	
7	56	#10 TO RV	13	
8	40	MIX RV	2	
9	2	VORTEX ON	5	
10	3	VORTEX OFF	1	
11	42	DRAIN RV	18	
12	99	END LOOP	1	
13	56	#10 TO RV	12	
14	79	PRS #1	10	
15	51	#1 TO RV	10	
16	56	#10 TO RV	4	
17	40	MIX RV	2	
18	2	VORTEX ON	10	
19	3	VORTEX OFF	1	
20	40	MIX RV	1	
21	130	MonPrevPk	1	
22	42	DRAIN RV	3	
23	1	WAIT	3	
24	131	MonStop	1	
25	132	Save Mon	1	
26	111	MonEndLoop	1	
27	42	DRAIN RV	18	
28	98	BEGIN LOOP	3	
29	56	#10 TO RV	10	
30	40	MIX RV	2	
31	2	VORTEX ON	1	
32	1	WAIT	5	
33	3	VORTEX OFF	1	
34	42	DRAIN RV	12	
35	41	VENT RV	2	
36	50	#10 RV-DRN	3	
37	42	DRAIN RV	5	
38	99	END LOOP	1	
39	41	VENT RV	5	
40	98	BEGIN LOOP	2	
41	13	#10 T VB	3	
42	14	#10 B VB	3	
43	9	GAS T VB	5	
44	10	GAS B VB	5	
45	99	END LOOP	1	

Note

The 1.0 mmol scale has no 1-X algorithm; therefore, "X" cannot (and need not) be determined.



7 Advanced Operations

This chapter gives examples of how Sequence and Chemistry files in SynthAssist® Software interact to generate a Run. The chapter presents the cycles in the pre-defined Chemistry file and examples of Chemistry cycles or modules you may create or modify to help you work with specific conditions that can occur in a synthesis.

The following pre-defined Sequence files in SynthAssist® Software are used throughout this section as examples:

Angiotensin 1, Human: H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH

Substance P: H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂

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Components of a Run

Switches include solenoid valves and other devices that can be activated or deactivated on the ABI 433A Peptide Synthesizer to perform a specific task.

Valve A valve is a mechanical device that opens to provide passage. The ABI 433A instrument has two types of valves: delivery valves and Angar valves. Delivery valves are solenoid valves located on the valve blocks. Most Angar valves permit gas flow to the chemical bottles for pressurization and subsequent delivery of the chemical.

Function A function is a switch or set of switches that activate simultaneously to accomplish a chemical flow or to perform a task. There are 152 functions, each designated by a number and a name.

Step A step is a function that has been programmed to occur for a specified amount of time.

Module A module is a group of steps that accomplish a specific chemical task. Letters are used to name modules ('a–i' and 'A–I'). For example, module "a" accomplishes activation. Pre-defined modules, supplied by Applied Biosystems, are stored in the SynthAssist® Software. You can edit existing modules or create new ones.

Cycle Pre-defined cycles in SynthAssist Software contain all the modules necessary to perform a particular task in a synthesis. Most cycles contain more than one module and add 1 amino acid to the peptide. A few pre-defined cycles in SynthAssist Software perform other tasks, such as a final deprotection, a DCM wash (in Boc chemistry), or an NMP wash; these cycles may contain only one module. You can edit existing cycles or create new ones.

A pre-defined **Chemistry file** in SynthAssist Software contains a series of cycles for performing a synthesis with one of the chemistry options.

A **Sequence file** in SynthAssist Software contains the sequence of amino acids in a particular peptide.

A **Default Set** in SynthAssist Software contains a list of preferred cycles for a particular Chemistry file.

A **Run file** in SynthAssist Software combines a Sequence file and a Chemistry file for a particular peptide synthesis.

Run A run on the ABI 433A instrument consists of repetitions of the cycles needed to synthesize the peptide. A typical run may start with a first cycle, then perform many repetitions of a standard cycle, and end with the last cycle.

FastMoc 0.25 mmol and 0.10 mmol Cycles

Table 7-1 displays the cycles and modules that compose the pre-defined SynthAssist Software *FastMoc*™ 0.25 and 0.10 mmol Chemistry files.

Table 7-1. Cycles in Pre-defined *FastMoc* (0.25 and 0.10 mmol) Chemistry Files

Cycle	Modules
Cycle 1-amide	cDBADEF
Single Couple	BADEF
Single Couple with RS (resin sample)	BADEFG
Single Couple/Ac ₂ O Capping	BADEFCD
Double Couple	BADEIADEF
Double Couple /Ac ₂ O Capping	BADEIADEFCD
Final Deprotection	BIDc
Loading & Benzoic Anhydride Capping	HF
NMP Wash	D
DCM Wash	C

Table 7-2 displays the cycles that define the Default Set for 0.25 and 0.10 mmol *FastMoc* Chemistry files in SynthAssist Software. You may change the default cycles for any of the items listed in the AA column, or you may add new AA items and cycles to the Default Set. Cycles in the Default Set for a specific Chemistry file are automatically applied to any SynthAssist Run file that uses that Chemistry.

Table 7-2. Default Set of Cycles for *FastMoc* (0.25 and 0.10 mmol) Chemistry Files

AA	Cycle	Modules
Default	Single Couple	BADEF
Preloaded	NMP Wash	D
Load	Loading and Benzoic Anhydride Capping	HF
Amide	Cycle 1, Amide	cDBADEF
Other	<<None>>	
End	Final Deprotection	BIDc

The following pages give examples that illustrate how to apply these cycles to various synthesis conditions. Although all the examples use the *FastMoc* 0.25 mmol Chemistry cycles, you can create the same Run files with the *FastMoc* 0.10 mmol Chemistry file. The following sequences were entered in SynthAssist Software as Sequence file examples:

Angiotensin 1, Human: H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH

Substance P: H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂

When necessary, refer to the SynthAssist user guide for assistance.

FastMoc 0.25 mmol Cycles for Preloaded Resins with final Fmoc removal

To synthesize Angiotensin starting with Fmoc-Leu-resin and removal of the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the “FastMoc 0.25 mmol” Chemistry file.
3. Choose the “Angiotensin” Sequence file.
4. Choose Preloaded resin. Enter the resin substitution and resin weight and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	NMP Wash	D
2	His	Single Couple	BADEF
3	Phe	Single Couple	BADEF
4	Pro	Single Couple	BADEF
5	His	Single Couple	BADEF
6	Ile	Single Couple	BADEF
7	Tyr	Single Couple	BADEF
8	Val	Single Couple	BADEF
9	Arg	Single Couple	BADEF
10	Asp	Single Couple	BADEF
11		Final Deprotection	BIDc

6. Send the “FastMoc 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: D
 Cy: 2 Rpt: 9 M: BADEF
 Cy: 11 Rpt: 1 M: BIDc

FastMoc 0.25 mmol Cycles for synthesis on an Fmoc-Amide resin and final Fmoc removal

To synthesize Substance P, starting with an Fmoc-amide resin, and remove the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the “FastMoc 0.25 mmol” Chemistry file.
3. Choose the “Substance P” Sequence file.
4. Choose Amide resin. Enter the resin substitution and resin weight and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Cycle 1-Amide	cDBADEF
2	Leu	Single Couple	BADEF
3	Gly	Single Couple	BADEF
4	Phe	Single Couple	BADEF
5	Phe	Single Couple	BADEF
6	Gln	Single Couple	BADEF
7	Gln	Single Couple	BADEF
8	Pro	Single Couple	BADEF
9	Lys	Single Couple	BADEF
10	Pro	Single Couple	BADEF
11	Arg	Single Couple	BADEF
12		Final Deprotection	BIDc

6. Send the “FastMoc 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: cDBADEF
 Cy: 2 Rpt: 10 M: BADEF
 Cy:12 Rpt: 1 M: BIDc

FastMoc Cycles Starting with Unloaded Resins

To load an Fmoc-amino acid on to a p-alkoxybenzyl alcohol (HMP) resin during a *FastMoc* chemistry synthesis, use the *FastMoc* cycle that contains modules HF. This cycle uses DCC activation, with 0.1 eq. of DMAP as the catalyst, to load the C-terminal amino acid onto the resin. Because this loading is not one hundred per cent complete, it is followed by a capping step with benzoic anhydride, again using DMAP as the catalyst. The capping step prevents unreacted hydroxyl groups from reacting with the HBTU-activated Fmoc-amino acids.

To accommodate the capping step, the initial Fmoc-amino acid cartridge must be followed by a cartridge containing approximately 3 mmol (0.60-0.70 g) benzoic anhydride.

When the initial Fmoc-amino acid cartridge contains Fmoc-Arg(Mtr), Fmoc-Arg(Pmc), Fmoc-Gln(Trt), Fmoc-Asn(Trt), or Fmoc-His(Bum), you must modify the following steps in module “H”:

	Fmoc-His(Bum)	Fmoc-Arg(Mtr) Fmoc-Arg(Pmc)	Fmoc-Gln(Trt) Fmoc-Asn(Trt)
Step 14 (#9 CART)	0 sec	0 sec	4 sec
Step 15 (#10 CART)	8 sec	7 sec	4 sec

IMPORTANT Do not load with unprotected Fmoc-Asn or Fmoc-Gln. Loading with His(Trt) can cause racemization.

FastMoc (0.25 mmol) Cycles for synthesis with unloaded resin and final Fmoc removal

To synthesize Angiotensin on HMP resin with removal of the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the “*FastMoc* 0.25 mmol” Chemistry file.
3. Choose the “Angiotensin” Sequence file.
4. Choose HMP resin. Enter the resin substitution and resin weight, and save the Run file.

5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	Loading & Benzoic An	HF
2	His	Single Couple	BADEF
3	Phe	Single Couple	BADEF
4	Pro	Single Couple	BADEF
5	His	Single Couple	BADEF
6	Ile	Single Couple	BADEF
7	Tyr	Single Couple	BADEF
8	Val	Single Couple	BADEF
9	Arg	Single Couple	BADEF
10	Asp	Single Couple	BADEF
11		Final Deprotection	BIDc

6. Send the “*FastMoc* 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: HF
 Cy: 2 Rpt: 9 M: BADEF
 Cy: 11 Rpt: 1 M: BIDc

Starting at a Cycle Other Than Cycle Number One

You might end a *FastMoc* chemistry Run before the entire peptide is synthesized and then decide to continue the synthesis. If you have **not** washed the peptide-resin with DCM to dry it, you may continue the synthesis.

To re-start synthesis at a cycle other than cycle number one:

In this example, synthesis of Substance P was stopped after 6 cycles and you now want to complete the synthesis.

1. In the SynthAssist Dictionary, add “residue” to the list of amino acids. Check the Palette box for this entry, but do not give it a code name.
2. Open the Sequence file for the peptide you have partially synthesized, in this case Substance P.
3. Delete the part of the peptide that has already been synthesized and replace them with the amino acid entry “residue.”

In this example, the first 6 amino acids are deleted. The Sequence file now appears as follows:

H-Arg-Pro-Lys-Pro-Gln-residue-NH₂

4. Open a new Run file.
5. Choose *FastMoc* 0.25 mmol chemistry.
6. Choose the modified Sequence file you created in step 3.

Because the peptide is already attached to the resin, choose Preloaded resin.

7. Choose Cycles in the pop-up menu. The first entry should be “residue” followed by the remaining amino acids in the sequence. For our example, the Run appears as follows:

AA	Cycle	Modules
1 residue	NMP wash	D
2 Gln	Single Couple	BADEF
3 Pro	Single Couple	BADEF
4 Lys	Single Couple	BADEF
5 Pro	Single Couple	BADEF
6 Arg	Single Couple	BADEF
7	Final Deprotection	BIDc

8. Send the “*FastMoc* 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send the Run to the ABI 433A instrument.

-
9. Go to the 433A Run Editor menu. Use the **next** and **prev** soft keys to look at all the cycles. For our example, the Run Editor shows the following cycles:

Cycle: 1 Rpt: 1 M: D

Cycle: 2 Rpt: 5 M: BADEF

Cycle: 7 Rpt: 1 M: BIDc

10. Now edit Cycle 1 in the 433A Run Editor.

- a. Delete all the modules in Cycle 1.
- b. After **Rpt:**, enter the number of amino acids that have already been coupled.

For our example, the modified Run Editor appears as follows:

Cycle: 1 Rpt: 6 M:

Cycle: 7 Rpt: 5 M: BADEF

Cycle: 12 Rpt: 1 M: BIDc

Double Couple

Note *Use two amino acid cartridges for each amino acid that is double-coupled in the synthesis.*

If you want to double couple all the amino acids in a sequence, in SynthAssist Software change the Default in the Default Set to **Double Couple**.

If one particular amino acid—for example, Gln—should always be double coupled, you can modify the Default Set.

If some amino acids are sometimes double-coupled, then leave the Default at Single Couple, but change the appropriate cycles to Double Couple.

FastMoc 0.25 mmol Cycles with a double couple every cycle

To synthesize Substance P, starting with Fmoc-amide resin, double couple every cycle after Met and remove the final Fmoc group:

1. Open the “FastMoc 0.25 mmol” Chemistry file. Save as **FastMoc 0.25 unlocked**. When a dialog box asks if you want to “Lock chemistry File”, answer No. This will unlock the file so you can make modifications.
2. Click **Default Set**. For the Default cycle, select Double Couple in the pop-up menu. Send the chemistry to the 433A instrument.
3. Open a new Run file in SynthAssist Software.
4. Choose the modified Chemistry file and the “Substance P” Sequence file.
5. Choose Amide resin. Enter the resin substitution and resin weight, and save the Run file.
6. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Cycle 1- amide	cDBADEF
2	Leu	Double Couple	BADEIADEF
3	Gly	Double Couple	BADEIADEF
4	Phe	Double Couple	BADEIADEF
5	Phe	Double Couple	BADEIADEF
6	Gln	Double Couple	BADEIADEF
7	Gln	Double Couple	BADEIADEF
8	Pro	Double Couple	BADEIADEF
9	Lys	Double Couple	BADEIADEF
10	Pro	Double Couple	BADEIADEF
11	Arg	Double Couple	BADEIADEF

AA	Cycle	Modules
12	Final Deprotection	BIDc

- Send the “FastMoc 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: cDBADEF

Cy: 2 Rpt: 10 M: BADEIADEF

Cy:12 Rpt:1 M: BIDc

FastMoc 0.25 mmol Cycles with double couple of all Gln

To synthesize Substance P, starting with Fmoc-amide resin, double couple all Gln, and remove the final Fmoc group:

- Open the “FastMoc 0.25 mmol unlocked” file created on the previous page. Click **Default Set** and set the Default cycle to **Single Couple**.
- Select **End** in the AA column and choose the **Insert** command in the Edit menu. Click the new entry and choose Gln from the pop-up menu.
- Choose **Double Couple** as the default Cycle for Gln. You may save this modified Chemistry file with a new name.
- Open a new Run file in SynthAssist Software.
- Choose the modified Chemistry file and the “Substance P” Sequence file.
- Choose **Amide resin**. Enter the resin substitution and resin weight, and save the Run file.

Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Cycle 1- Amide	cDBADEF
2	Leu	Single Couple	BADEF
3	Gly	Single Couple	BADEF
4	Phe	Single Couple	BADEF
5	Phe	Single Couple	BADEF
6	Gln	Double Couple	BADEIADEF
7	Gln	Double Couple	BADEIADEF
8	Pro	Single Couple	BADEF
9	Lys	Single Couple	BADEF
10	Pro	Single Couple	BADEF
11	Arg	Single Couple	BADEF
12		Final Deprotection	BIDc

7. Send the Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

```
Cy: 1    Rpt: 1    M: cDBADEF
Cy: 2    Rpt: 4    M: BADEF
Cy: 6    Rpt: 2    M: BADEIADEF
Cy: 8    Rpt: 4    M: BADEF
Cy: 12   Rpt: 1    M: BIDc
```

FastMoc 0.25 mmol Cycles, with only one double couple

To synthesize Substance P, starting with Fmoc-amide resin, with only one double couple at Gln⁵, and remove the final Fmoc group:

1. Open the “FastMoc 0.25 mmol” Chemistry file and click the Default Set button. Verify that the Default cycle is Single Couple.
2. Open a new Run file in SynthAssist Software.
3. Choose the “FastMoc 0.25 mmol” Chemistry file and the “Substance P” Sequence file.
4. Choose **Amide resin**. Enter the resin substitution and resin weight, and save the Run file.
5. Choose **Cycles** from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Cycle 1-Amide	cDBADEF
2	Leu	Single Couple	BADEF
3	Gly	Single Couple	BADEF
4	Phe	Single Couple	BADEF
5	Phe	Single Couple	BADEF
6	Gln	Single Couple	BADEF
7	Gln	Single Couple	BADEF
8	Pro	Single Couple	BADEF
9	Lys	Single Couple	BADEF
10	Pro	Single Couple	BADEF
11	Arg	Single Couple	BADEF
12		Final Deprotection	BIDc

6. Select the words “Single Couple” on line 7, and click again to make the pop-up entry field appear. Choose “Double Couple” and press **Return**.

The modified Run file looks like this:

	AA	Cycle	Modules
1	Met	Cycle 1-Amide	cDBADEF
2	Leu	Single Couple	BADEF
3	Gly	Single Couple	BADEF
4	Phe	Single Couple	BADEF
5	Phe	Single Couple	BADEF
6	Gln	Single Couple	BADEF
7	Gln	Double Couple	BADEIADEF
8	Pro	Single Couple	BADEF
9	Lys	Single Couple	BADEF
10	Pro	Single Couple	BADEF
11	Arg	Single Couple	BADEF
12		Final Deprotection	BIDc

- Send the “*FastMoc* 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument. The 433A Run Editor menu contains the following information:

Cy: 1	Rpt: 1	M: cDBADEF
Cy: 2	Rpt: 5	M:BADEF
Cy: 7	Rpt: 1	M: BADEIADEF
Cy: 8	Rpt: 4	M: BADEF
Cy: 12	Rpt: 1	M: BIDc

Capping with Acetic Anhydride

To cap with *FastMoc* chemistry, use module C with an acetic anhydride mixture (0.5 M acetic anhydride, 0.125 M DIEA, and 0.015 M HOBt in NMP) in Bottle 4. Preloaded resins provide maximum ease of use when capping with acetic anhydride. However, if only HMP resin is available, you may either switch bottles at position 4 after the initial loading on HMP, or preload the HMP resin on the ABI 433A Peptide Synthesizer and dry it before continuing synthesis.

Prepare Acetic Anhydride Mixture for Bottle 4

WARNING	CHEMICAL HAZARD. <i>Acetic Anhydride is a combustible liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled and may cause allergic reactions. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING	CHEMICAL HAZARD. <i>Diisopropylethylamine (DIEA) is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING	CHEMICAL HAZARD. <i>1-Hydroxybenzotriazole hydrate (HOBt) has a risk of explosion if heated under confinement. Keep away from heat and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

WARNING	CHEMICAL HAZARD. <i>N-Methylpyrrolidone (NMP) may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</i>

To make 400 mL of acetic anhydride capping solution (0.5 M acetic anhydride, 0.125 M DIEA, and 0.015 M HOBt in NMP):

1. Place in a clean, dry, 100 mL graduated cylinder:
19 mL acetic anhydride (P/N 400660)
9 mL DIEA (P/N 400136)
6 mL 1 M HOBt/NMP (P/N 400662) **or** 0.8 g solid HOBt
2. Add NMP (P/N 400580) to a volume of 100 mL.
3. Pour this solution into a clean, dry, 500 mL bottle and add another 300 mL NMP. Mix this solution.
4. Place a gasket on the bottle and screw the bottle into the ratchet cap assembly at bottle position 4.
5. Run Flow Test 4. Approximately 1.7 ± 0.2 mL solution should be delivered.

The capping solution turns slightly yellow after a couple of weeks, due to the presence of HOBt. Although the effectiveness of the capping solution is not reduced, it is a good practice to make fresh capping solution at least every two weeks.

FastMoc 0.25 mmol Cycles for Capping, Starting with Preloaded Resin

Synthesize Angiotensin, starting with pre-loaded resin, with acetic anhydride capping at each cycle and final Fmoc removal.

To synthesize Angiotensin:

1. Open the “FastMoc 0.25 unlocked” file created on page 7-10. Click Default Set. Set the Default to **Single Coupling/Ac₂O Capping**.
2. Open a new Run file in SynthAssist Software.
3. Choose the **modified** “FastMoc 0.25” Chemistry file and the “Angiotensin” Sequence file.
4. Choose **Pre-loaded resin**. Enter the resin substitution and resin weight and save the Run file.

5. Choose **Cycles** from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	NMP Wash	D
2	His	Single Coupling/Ac2O	BADEFCD
3	Phe	Single Coupling/Ac2O	BADEFCD
4	Pro	Single Coupling/Ac2O	BADEFCD
5	His	Single Coupling/Ac2O	BADEFCD
6	Ile	Single Coupling/Ac2O	BADEFCD
7	Tyr	Single Coupling/Ac2O	BADEFCD
8	Val	Single Coupling/Ac2O	BADEFCD
9	Arg	Single Coupling/Ac2O	BADEFCD
10	Asp	Single Coupling/Ac2O	BADEFCD
11		Final Deprotection	BIDc

6. Send the “*FastMoc* 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: D
 Cy: 2 Rpt: 9 M: BADEFCDD
 Cy: 11 Rpt: 1 M: BIDc

Loading on HMP resin, followed by Capping with Acetic Anhydride

To load the initial amino acid to HMP resin, bottle 4 should contain 0.1 M DMAP/DMF solution. In this situation, you must interrupt synthesis after the loading and change the bottle at position 4.

To change bottle #4 after initial loading on HMP resin:

- Press the **pause** soft key after the loading cycle (HF) is complete.
 As an alternative, press the **set int** key (see page 7-35) in the Cycle Monitor menu and set an interruption at the end of the loading cycle.
- Remove the 0.1 M DMAP/DMF solution. Wipe the delivery tubing for bottle 4 with a lint-free tissue.
- Place the bottle of acetic anhydride mixture in bottle position 4.
- In the Manual Control menu, activate Fxn 17 (#4 B VB) for 5 seconds to flush the #4 line. Then clean the valve block by activating for 5 seconds each, Fxn 10 (GAS B VB), Fxn 14 (#10 B VB), and again, Fxn 10 (GAS B VB).

-
5. Return to the Main menu, go to the Cycle Monitor menu and press the ***pause** soft key to continue synthesis.

Before the next synthesis, if you plan to use DMAP again for loading, thoroughly clean the #4 bottle position.

Cleaning #4 position after capping with acetic anhydride

To clean position #4:

1. Remove the acetic anhydride solution in bottle position #4. Wipe the bottle delivery line with a lint-free tissue.
2. Place a bottle containing about 20 mL NMP on bottle position #4.
3. In the Manual Control menu, activate Fxn 17 (#4 B VB) until the bottle at position #4 is empty.
4. Remove the empty bottle in position #4. Wipe the bottle delivery line with a lint-free tissue.
5. Place the bottle of 0.1 M DMAP/DMF in bottle position #4. Run Flow Test #4. The delivery volume should be approximately 2.0-2.3 mL.

N-terminal acetylation using amino acid cartridge

The following procedure is an easy way to acetylate the N terminal of a peptide-resin using an amino acid cartridge instead of a reagent bottle.

The procedure uses 1 mmol glacial acetic acid (60.05 FW, d 1.049, 57 μ L/mmol), pipetted into an empty amino acid cartridge, which is used in a normal coupling cycle. The acetyl group resulting from the coupling of the acetic acid acts as an N-terminal group. This procedure can be used for *FastMoc* or Fmoc and all scales (in 1 mmol scale, you must use 3 amino acid cartridges).

IMPORTANT *Applied Biosystems recommends that you use a capping solution in bottle position 4 and use preloaded resin. See FastMoc 0.25 mmol Cycles for Capping, Starting with Preloaded Resin on page 7-15.*

To acetylate the N terminal of a peptide-resin:

1. Open the Chemistry folder.
2. Select the scale and chemistry you wish to use. A dialog box will appear, informing you that the document is locked. Click **OK** to go on.
3. Open the SynthAssist Software normal activation module A by double-clicking the word Activation.
4. Select all the steps (z-A) and copy the steps (z-C).
5. Close the Activation module window.
6. Open an empty module by double-clicking on the word module.
7. Paste (z-V) the steps you copied previously into the empty module window.
8. Click the step containing function “4,” then delete the step (z-K).
9. Close the Module window.
10. Name the module you just modified by typing in a name. Use a descriptive name, such as Special Activation. Note the letter designation of this module for use in step 20.
11. Click the space under the last cycle name so that the space becomes highlighted.
12. Select **Edit > Insert** (or press z-J) to add a new cycle to the end of the list of cycles.
13. Name the new cycle Final N-terminal acetylation.
14. Open the single-couple cycle for your selected chemistry by double-clicking the cycle name.
15. Select all the modules (z-A) and copy the modules (z-C).
16. Close the Cycle window.
17. Open the newly-created cycle (step 13) by double-clicking the name.
18. From the pop-up menu showing Procedure, select **Single Couple**.
19. Paste (z-V) the modules you copied previously into the empty cycle window.
20. Click the letter A to select it and type the letter of the newly-created module (the letter you noted in step 10. For example, if Special Activation was module “e,” then type “e”). Press **Return** to enter this information.
21. Replace any modules following a coupling module(s) (for example F or f) with a D module followed by a c module.
22. Close the Cycle window.

23. Save the new chemistry file in *your* chemistry folder under a new name, then close the chemistry file.
24. Open or create a new (z-N) synthesis Run File.
25. Select the chemistry file you modified previously and select a sequence.
26. Use the pop-up menu to change from Calculations to Cycles.
27. Click the final cycle to select it, then click it again to bring up the pop-up menu. Select the cycle you just created, then press **Return**.
28. Save the Run File, then send it to the ABI 433A instrument.

Additional *FastMoc* Chemistry Cycles

Table 7-3 shows more module combinations that you may use to create additional cycles for *FastMoc* (0.25 mmol and 0.10 mmol) chemistry.

Table 7-3. Additional *FastMoc* Chemistry Cycles

<i>FastMoc</i> Chemistry Cycle	Cycle Application	Requires
BADEFGCD	Single couple with Ac ₂ O capping and resin samples after coupling	1 amino acid cartridge
BADEIADEFG	Double couple with a resin sample after second coupling	2 amino acid cartridges
BADEIADGEFG	Double couple with resin samples after both couplings	2 amino acid cartridges
BADEIADGEFGCD	Double couple with Ac ₂ O capping after the second coupling and resin samples after both couplings	2 amino acid cartridges

Fmoc/HOBt/DCC Cycles

Fmoc/HOBt/DCC 0.25 mmol Cycles

Table 7-4 displays the cycles and modules that compose the pre-defined SynthAssist Software Fmoc/HOBt/DCC 0.25 mmol Chemistry files.

Table 7-4. Cycles in Pre-defined Fmoc/HOBt/DCC 0.25 mmol Chemistry Files

Cycle	Modules*
Single Couple	a - bdef / fg
Double Couple	a - bdeaffgef / fg
Final Deprotection	f - bdc
Load	hef / fg
NMP wash	d
DCM Wash	c

* A hyphen (-) indicates "finish previous cycle," a virgule or slash (/) indicates "start next cycle"

Table 7-5 displays the cycles that define the Default Set for Fmoc/HOBt/DCC 0.25 mmol Chemistry files in SynthAssist Software. You may change the default cycles for any of the items listed in the AA column, or you may add new AA items and cycles to the Default Set. Cycles in the Default Set for a specific Chemistry file are automatically applied to any SynthAssist Run file that uses that Chemistry.

Table 7-5. Default Set of Cycles for Fmoc/HOBt/DCC 0.25 mmol Chemistry Files

AA	Cycle	Modules
Default	Single Couple	a - bdef / fg
Preloaded	NMP wash	d
Load	Load	hef / fg
End	Final Deprotection	f - bdc

* A hyphen (-) indicates "finish previous cycle," a virgule or slash (/) indicates "start next cycle."

Fmoc/HOBt/DCC 0.10 mmol Cycles

Table 7-6 displays the cycles and modules that compose the pre-defined SynthAssist Software Fmoc/HOBt/DCC 0.10 mmol Chemistry files.

Table 7-6. Cycles in Pre-defined Fmoc/HOBt/DCC 0.10 mmol Chemistry Files

Cycle	Modules*
Single Couple	a - bde / fg
Double Couple	a - bdeafffgef / fg
Final Deprotection	fff - bdc
Load	hefff / fg
NMP wash	d
DCM Wash	c

* A hyphen (-) indicates “finish previous cycle,” a virgule, or slash, (/) indicates “start next cycle.”

Table 7-7 displays the cycles that define the Default Set for Fmoc/HOBt/DCC 0.10 mmol Chemistry files in SynthAssist Software. You may change the default cycles for any of the items listed in the AA column, or you may add new AA items and cycles to the Default Set. Cycles in the Default Set for a specific Chemistry file are automatically applied to any SynthAssist Run file that uses that Chemistry.

Table 7-7. Default Set of Cycles for Fmoc/HOBt/DCC (0.10 mmol) Chemistry Files

AA	Cycle	Modules
Default	Single Couple	a - bdef / fg
Preloaded	NMP wash	d
Load	Load	hef / fg
End	Final Deprotection	f - bdc

* A hyphen (-) indicates “finish previous cycle,” a virgule, or slash, (/) indicates “start next cycle.”

Hyphen and Virgule Symbols in the Fmoc/HOBt/DCC Cycles

Because the Fmoc/HOBt/DCC Chemistry cycles use HOBt/DCC activation, they require 30-40 minutes of pre-activation for the formation of the Fmoc-amino acid-OBt ester. So, with any two cycles in a synthesis, module “a” (activation) begins on the second cycle before the previous cycle is finished. As a consequence, the initial cycle in a synthesis must be different than subsequent cycles.

The hyphen (-) and the virgule, or slash, (/) symbols in the Default Set of Fmoc/HOBt/DCC Chemistry cycles are actually signals. The hyphen means “finish the previous cycle”; by default, module “i” is inserted when a hyphen appears if the previous cycle did not contain a virgule. The virgule means “start the next cycle now.” The modules that follow the virgule are inserted in place of the next available hyphen.

To illustrate how the hyphen and virgule symbols operate, look at a Run composed of only Single Couple cycles and a Final deprotection. The Default cycle definitions in the pre-defined Fmoc/HOBt/DCC 0.25 mmol Chemistry file show the following modules:

Single couple	a - bdef / fg
Final deprotection	f - bdc

For the first cycle, SynthAssist Software replaces the hyphen with a module “i.” When it reads the virgule symbol (/), SynthAssist Software ends the cycle so that the activation of the next amino acid may begin. The first cycle becomes: a i b d e f.

For subsequent cycles, SynthAssist Software inserts the modules that followed the virgule in the previous cycle, so that the previous coupling can be completed. In this case, the modules “fg” replace the hyphen. As a result, subsequent single couple cycles become: a f g b d e f.

For the final deprotection cycle, SynthAssist Software continues to replace the hyphen with the modules “fg,” so that final deprotection cycle becomes: f f g b d c.

All these changes occur automatically in SynthAssist Software. You only have to open a Run file, choose both the Fmoc/HOBt/DCC Chemistry file and the Sequence file, and then open the Cycle window to see the outcome.

The following examples of Fmoc/HOBt/DCC 0.25 mmol cycles demonstrate the Run files you can create with SynthAssist Software. The Fmoc/HOBt/DCC 0.10 mmol cycles differ slightly from the 0.25mmol cycles; you can create similar Run files with the Fmoc/HOBt/DCC 0.10 mmol Chemistry file.

Fmoc/HOBt/DCC 0.25 mmol Chemistry Cycles for Preloaded Resins with Final Fmoc Removal

To synthesize Angiotensin starting with Fmoc-Leu-resin and removal of the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file.
3. Choose the "Angiotensin" Sequence file.
4. Choose Preloaded resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	NMP Wash	d
2	His	Single Couple	aibdef
3	Phe	Single Couple	afgbdef
4	Pro	Single Couple	afgbdef
5	His	Single Couple	afgbdef
6	Ile	Single Couple	afgbdef
7	Tyr	Single Couple	afgbdef
8	Val	Single Couple	afgbdef
9	Arg	Single Couple	afgbdef
10	Asp	Single Couple	afgbdef
11		Final Deprotection	ffgbdc

6. Send the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

```

Cy: 1      Rpt: 1      M: d
Cy: 2      Rpt: 1      M: aibdef
Cy: 3      Rpt: 8      M: aifgbdef
Cy: 11     Rpt: 1      M: ffgbdc
  
```

Fmoc/HOBt/DCC 0.25 mmol Chemistry Cycles for Fmoc-Amide Resins and Final Fmoc Removal

To synthesize Substance P, starting with an Fmoc-amide resin, and remove the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file.
3. Choose the "Substance P" Sequence file.
4. Choose Amide resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibdef
2	Leu	Single Couple	afgbdef
3	Gly	Single Couple	afgbdef
4	Phe	Single Couple	afgbdef
5	Phe	Single Couple	afgbdef
6	Gln	Single Couple	afgbdef
7	Gln	Single Couple	afgbdef
8	Pro	Single Couple	afgbdef
9	Lys	Single Couple	afgbdef
10	Pro	Single Couple	afgbdef
11	Arg	Single Couple	afgbdef
12		Final Deprotection	ffgbdc

6. Send the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibdef

Cy: 2 Rpt: 10 M: afgbdef

Cy: 12 Rpt: 1 M: ffgbdc

Fmoc-amide resins can also be used to make C-terminal Asn or Gln peptides (see references 31 and 32 in *References* on page 3-22).

Fmoc/HOBt/DCC 0.25 mmol Chemistry Cycles for Unloaded Resins

To load an Fmoc-amino acid on a p-alkoxybenzyl alcohol (HMP) resin during an Fmoc/HOBt/DCC 0.25 mmol synthesis, you may use either the cycle composed of modules “hef” from the Fmoc/HOBt/DCC 0.25 mmol Chemistry file or the cycle “HF” from the *FastMoc* 0.25 mmol Chemistry file.

IMPORTANT Use the HF cycle to load His, Arg, Gln, or Asn on HMP resin. Do not load His, Arg, Gln, or Asn with modules “hef.”

The “hef” cycle uses 0.5 eq DCC to activate 1 eq Fmoc-amino acid, with 0.1 eq DMAP as a catalyst to load the C-terminal amino acid onto the resin. HOBt/DCC-activated amino acids do not appreciably react with the unreacted hydroxymethyl group on the HMP resin, so no capping step with benzoic anhydride is needed. **Do not load His, Arg, Gln, or Asn with modules “hef.”**

The cycle “HF” uses 1 eq DCC to activate 1 eq of Fmoc-amino acid with 0.1 eq DMAP as the catalyst to load the C-terminal amino acid onto the resin. Because this *FastMoc* Chemistry cycle was developed for use with HBTU activation, it does include a capping step with benzoic anhydride. The “HF” loading cycle may be used with all amino acids with the Fmoc/HOBt/DCC Chemistry cycles. See page 7-6 for directions for benzoic anhydride capping with the modification to module “H” for His, Arg, Gln, and Asn.

Fmoc/HOBt/DCC 0.25 mmol Cycles for synthesis with unloaded resin (modules “hef”) and final Fmoc removal

To synthesize Angiotensin on HMP resin with removal of the final Fmoc group:

1. Open a new Run file in SynthAssist Software.
2. Choose the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file.
3. Choose the “Angiotensin” Sequence file.
4. Choose HMP resin. Enter the resin substitution and resin weight, and save the Run file.

5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	Load	hef
2	His	Single Couple	afgbdef
3	Phe	Single Couple	afgbdef
4	Pro	Single Couple	afgbdef
5	His	Single Couple	afgbdef
6	Ile	Single Couple	afgbdef
7	Tyr	Single Couple	afgbdef
8	Val	Single Couple	afgbdef
9	Arg	Single Couple	afgbdef
10	Asp	Single Couple	afgbdef
11		Final Deprotection	ffgbdc

6. Send the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: hef

Cy: 2 Rpt: 9 M: afgbdef

Cy: 11 Rpt: 1 M: ffgbdc

Fmoc/HOBt/DCC 0.25 mmol Cycles for Synthesis with Unloaded Resin, Using *FastMoc* Modules “HF,” and Final Fmoc Removal

Before setting up the SynthAssist Run file, use the following procedure to transfer the modules “HF” from the “*FastMoc* 0.25 mmol” Chemistry file to the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file.

Remember, when using the modules “HF” with the 0.25 mmol cycle, always place a cartridge containing 3 mmol (0.60-0.70 g) benzoic anhydride after the amino acid cartridge that is to be loaded on to the resin.

To transfer a *FastMoc* module to the Fmoc/HOBt/DCC Chemistry file:

1. Open the “*FastMoc* 0.25 mmol” Chemistry file. In the list of Modules, double-click on the name of the module you want to transfer. For example, double-click Module H to open the window that displays all the steps in module H.
2. Choose the Select All command in the Edit menu to select all the steps in the module. Choose the Copy command to put a copy of the module into the Clipboard.

3. Open the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file. In the list of Modules, double-click the name of the module you want to transfer to. The window for the module opens. This window should be empty.
4. Choose the Paste command to transfer a copy of the *FastMoc* module into the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file.

To synthesize Angiotensin on HMP resin, using FastMoc modules HF, with final Fmoc removal:

1. Open the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file that contains the transferred *FastMoc* modules "HF."
2. Create a new cycle called "Loading and Benzoic Anhydride Capping" composed of the transferred *FastMoc* modules "HF." See *Creating a Custom Chemistry File* in the *SynthAssist User's Guide* located on your SynthAssist Software CD.
3. Open a new Run file in SynthAssist Software.
4. Choose the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file.
5. Choose the "Angiotensin" Sequence file.
6. Choose HMP resin. Enter the resin substitution and resin weight.
7. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	DCM Wash	c
2	His	Single Couple	afgbdef
3	Phe	Single Couple	afgbdef
4	Pro	Single Couple	afgbdef
5	His	Single Couple	afgbdef
6	Ile	Single Couple	afgbdef
7	Tyr	Single Couple	afgbdef
8	Val	Single Couple	afgbdef
9	Arg	Single Couple	afgbdef
10	Asp	Single Couple	afgbdef
11		Final Deprotection	ffgbdc

8. Select the "DCM Wash" cycle on line 1. Click again to make the entry field pop-up menu appear. Choose "Loading and Benzoic acid capping" from the pop-up menu and press **Return**.

The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	Loading and Benzoic anhydride capping	HF
2	His	Single Couple	aibdef
3	Phe	Single Couple	afgbdef
4	Pro	Single Couple	afgbdef
5	His	Single Couple	afgbdef
6	Ile	Single Couple	afgbdef
7	Tyr	Single Couple	afgbdef
8	Val	Single Couple	afgbdef
9	Arg	Single Couple	afgbdef
10	Asp	Single Couple	afgbdef
11		Final Deprotection	ffgbdc

- Send the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: HF
 Cy: 2 Rpt: 9 M: aifbdef
 Cy: 11 Rpt: 1 M: ffgbd

Starting at a Cycle Other Than Cycle One

You might end an Fmoc/HOBt/DCC chemistry Run before the entire peptide is synthesized and then decide to continue the synthesis. If you have **not** washed the peptide-resin with DCM to dry it, you may continue synthesis.

To re-start synthesis at a cycle other than cycle number one:

In this example, synthesis of Substance P was stopped after 6 cycles and you now want to complete the synthesis.

1. In the SynthAssist Dictionary, add “residue” to the list of amino acids. Check the Palette box for this entry, but do not give it a code name.
2. Open the Sequence file for the peptide you have partially synthesized, in this case Substance P.
3. Delete the part of the peptide that has already been synthesized and replace them with the amino acid entry “residue.”

In this example, the first 6 amino acids are deleted. The Sequence file now appears as follows:

H-Arg-Pro-Lys-Pro-Gln-residue-NH₂.

4. Open a new Run file.
5. Choose the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry.
6. Choose the modified Sequence file you created in step 3.

Because the peptide is already attached to the resin, use Preloaded resin. Save the Run file.

7. Choose Cycles in the pop-up menu. The first entry should be “residue” followed by the remaining amino acids in the sequence. For our example, the Run appears as follows:

	AA	Cycle	Modules
1	residue	NMP wash	d
2	Gln	Single Couple	aibdef
3	Pro	Single Couple	afgbdef
4	Lys	Single Couple	afgbdef
5	Pro	Single Couple	afgbdef
6	Arg	Single Couple	afgbdef
7		Final Deprotection	ffgbdc

8. Send the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send the Run to the ABI 433A instrument.

-
9. Go to the 433A Run Editor menu. Use the **next** and **prev** soft keys to look at all the cycles. For our example, the Run Editor shows the following cycles:

Cycle: 1	Rpt: 1	M: d
Cycle: 2	Rpt: 1	M: aibdef
Cycle: 3	Rpt: 4	M: afgbdef
Cycle: 7	Rpt: 1	M: ffgbdc

10. Now edit Cycle 1 in the 433A Run Editor.
- Delete all the modules in Cycle 1.
 - After **Rpt:**, enter the number of amino acids that have already been coupled.

For our example, the modified Run Editor appears as follows:

Cycle: 1	Rpt: 6	M:
Cycle: 7	Rpt: 1	M: aibdef
Cycle: 8	Rpt: 4	M: afgbdef
Cycle: 12	Rpt: 1	M: ffgbdc

Double Couple

Note *Use two amino acid cartridges for each amino acid that is double-coupled in the synthesis.*

If you want to double couple all the amino acids in a sequence, in SynthAssist Software change the Default in the Default Set to **Double Couple**.

If one particular amino acid—for example, Gln—should always be double coupled, you can modify the Default Set.

If some amino acids are sometimes double-coupled, then leave the Default at Single Couple, but change the appropriate cycles to Double Couple.

Notice that when an amino acid is double-coupled with two resin samples in the cycle, SynthAssist Software allows extra space in the Calculations for two resin samples.

Fmoc/HOBt/DCC 0.25 mmol Cycles with a double couple every cycle

To synthesize Substance P, starting with Fmoc-amide resin, double couple every cycle and remove the final Fmoc group:

1. Open the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file. Click the Default Set button. For the Default cycle, choose Double Couple in the pop-up menu. You may save this modified Chemistry file with a new name, such as “Fmoc/HOBt/DCC Double Couple.”
2. Open a new Run file in SynthAssist Software.
3. Choose the **modified** Chemistry file and the “Substance P” Sequence file.
4. Choose Amide resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Double Couple	aibdeaffgef
2	Leu	Double Couple	afgbdeaffgef
3	Gly	Double Couple	afgbdeaffgef
4	Phe	Double Couple	afgbdeaffgef
5	Phe	Double Couple	afgbdeaffgef
6	Gln	Double Couple	afgbdeaffgef
7	Gln	Double Couple	afgbdeaffgef
8	Pro	Double Couple	afgbdeaffgef
9	Lys	Double Couple	afgbdeaffgef
10	Pro	Double Couple	afgbdeaffgef
11	Arg	Double Couple	afgbdeaffgef
12		Final Deprotection	ffgbd

AA	Cycle	Modules
	<<None>>	

- Send the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibdeaffgef
 Cy: 2 Rpt: 10 M: afgbdeaffgef
 Cy: 12 Rpt: 1 M: ffgbdc

Fmoc/HOBt/DCC 0.25 mmol Cycles with double couple of all Gln

To synthesize Substance P, starting with Fmoc-amide resin, double couple all Gln, and remove the final Fmoc group:

- Open the "Fmoc/HOBt/DCC 0.25 mmol" Chemistry file. Click the Default Set button. Verify that the Default is Single Couple.
- Select "End" in the AA column and choose the Insert command in the Edit menu. Click the new entry and choose "Gln" from the pop-up menu.
- Choose Double Couple as the default Cycle for Gln. You may save this modified file with a new name.
- Open a new Run file in SynthAssist Software.
- Choose the **modified** Chemistry file and the "Substance P" Sequence file.
- Choose Amide resin. Enter the resin substitution and resin weight, and save the Run file.
- Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibdef
2	Leu	Single Couple	afgbdef
3	Gly	Single Couple	afgbdef
4	Phe	Single Couple	afgbdef
5	Phe	Single Couple	afgbdef
6	Gln	Double Couple	afgbdeaffgef
7	Gln	Double Couple	afgbdeaffgef
8	Pro	Single Couple	afgbdef
9	Lys	Single Couple	afgbdef
10	Pro	Single Couple	afgbdef
11	Arg	Single Couple	afgbdef
12		Final Deprotection	ffgbdc

-
- Send the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument. The 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibdef
 Cy: 2 Rpt: 4 M: afgbdef
 Cy: 6 Rpt: 2 M: afgbdeaffgef
 Cy: 8 Rpt: 4 M: afgbdef
 Cy: 12 Rpt: 1 M: ffgbdc

Fmoc/HOBt/DCC (0.25 mmol) Cycles, with only one double couple

To synthesize Substance P, starting with Fmoc-amide resin, with only one double couple at Gln⁵, and remove the final Fmoc group:

- Open the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file and click the Default Set button. Verify that the Default cycle is Single Couple.
- Open a new Run file in SynthAssist Software.
- Choose the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file and the “Substance P” Sequence file.
- Choose Amide resin. Enter the resin substitution and resin weight, and save the Run file.
- Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibdef
2	Leu	Single Couple	afgbdef
3	Gly	Single Couple	afgbdef
4	Phe	Single Couple	afgbdef
5	Phe	Single Couple	afgbdef
6	Gln	Single Couple	afgbdef
7	Gln	Single Couple	afgbdef
8	Pro	Single Couple	afgbdef
9	Lys	Single Couple	afgbdef
10	Pro	Single Couple	afgbdef
11	Arg	Single Couple	afgbdef
12		Final Deprotection	ffgbdc

- Select the “Single Couple” cycle on line 7. Click the selection to make the pop-up entry field appear. Choose Double Couple in the pop-up menu and press the Return key.

The modified Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibdef
2	Leu	Single Couple	afgbdef
3	Gly	Single Couple	afgbdef
4	Phe	Single Couple	afgbdef
5	Phe	Single Couple	afgbdef
6	Gln	Single Couple	afgbdef
7	Gln	Double Couple	afgbdeaffgef
8	Pro	Single Couple	afgbdef
9	Lys	Single Couple	afgbdef
10	Pro	Single Couple	afgbdef
11	Arg	Single Couple	afgbdef
12		Final Deprotection	ffgbdc

- Send the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument. The 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibdef
 Cy: 2 Rpt: 5 M: afgbdef
 Cy: 7 Rpt: 1 M: afgbdeaffgef
 Cy: 8 Rpt: 4 M: afgbdef
 Cy: 12 Rpt: 1 M: ffgbdc

Fmoc/HOBt/DCC 0.25 mmol Synthesis, Without Removal of the Final Fmoc Group

To synthesize a peptide that has the final Fmoc group still attached to the N-terminal amino acid, you must modify the End cycle in the Default Set:

- Open the Fmoc/HOBt/DCC 0.25 mmol Chemistry file. Create a new cycle, called “Final Wash,” composed of the modules “f - dc.”
- Click the Default Set button. In the Cycle pop-up menu for End, choose the “Final Wash.” You may choose the Save As command to save this modified Default Set with a new file name.
- Open a new Run file.
- Choose the Angiotensin Sequence file and the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry file with the modified Default Set.
- Choose Preloaded resin. Enter the resin substitution and resin weight, and save the Run file.

6. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	NMP Wash	d
2	His	Single Couple	aibdef
3	Phe	Single Couple	afgbdef
4	Pro	Single Couple	afgbdef
5	His	Single Couple	afgbdef
6	Ile	Single Couple	afgbdef
7	Tyr	Single Couple	afgbdef
8	Val	Single Couple	afgbdef
9	Arg	Single Couple	afgbdef
10	Asp	Single Couple	afgbdef
11		Final Wash	ffgdc

7. Send the “Fmoc/HOBt/DCC 0.25 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

The 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: d
 Cy: 2 Rpt: 1 M: aibdef
 Cy: 3 Rpt: 8 M: aifgbdef
 Cy: 11 Rpt: 1 M: ffgdc

The Set Interrupt Key

Use the **set int** key to interrupt a synthesis at any time. For example, after starting an Fmoc/HOBt/DCC 0.25 mmol synthesis of 25 cycles, you may decide to stop synthesis after 20 cycles.

Set Interrupt to occur at:

Cycle 21 Module 1Step 1

The instrument is set to automatically pause when the synthesis reaches Module a, Step 1. This is a safe step for an interruption because it occurs before amino acid activation can begin.

After interrupting a synthesis, press the **pause** key to continue the synthesis.

Boc/HOBt/DCC Cycles

Boc/HOBt/DCC 0.50 mmol Cycles

Table 7-8 displays the cycles and modules that compose the pre-defined SynthAssist Software Boc/HOBt/DCC 0.50 mmol Chemistry files.

Table 7-8. Cycles in Pre-defined Boc/HOBt/DCC 0.50 mmol Chemistry Files

Cycle	Modules*
Single Couple	a - bcdef / gc
Double Couple, 1 RS (resin sample)	a - bcdeafhFdef / gc
Double Couple, 2 RS (resin samples)	a - bcdeafhGdef / gc
Final Wash	h -
Final Deprotection	h - bcdc
N-Terminal Acetylation	h - bcdCc
DCM Wash	c

* A hyphen (-) indicates "finish previous cycle," a virgule, or slash, (/) indicates "start next cycle."

Table 7-9 displays the cycles that define the Default Set for Boc/HOBt/DCC 0.50 mmol Chemistry files in SynthAssist Software. You may change the default cycles for any of the items listed in the AA column, or you may add new AA items and cycles to the Default Set. Cycles in the Default Set for a specific Chemistry file are automatically applied to any SynthAssist Run file that uses that Chemistry.

Table 7-9. Default Set of Cycles for Boc/HOBt/DCC 0.50 mmol Chemistry Files

AA	Cycle	Modules*
Default	Single Couple	a - bcdef / gc
Preloaded	DCM Wash	c
Load**	<<None>>	
End	Final Wash	h -

* A hyphen (-) indicates "finish previous cycle," a virgule, or slash, (/) indicates "start next cycle."

**Although Boc Chemistry does not require a load cycle, a Load cycle is included in the Boc chemistry Default Set because it is available in SynthAssist Software.

Boc/HOBt/DCC 0.10 mmol Cycles

Table 7-10 displays the cycles and modules that compose the pre-defined SynthAssist Software Boc/HOBt/DCC 0.10 mmol Chemistry files.

Table 7-10. Cycles in Pre-defined Boc/HOBt/DCC 0.10 mmol Chemistry Files

Cycle	Modules*
Single Couple	a - bcde / gc
Double Couple, 1 RS (resin sample)	a - bcdeafFdef / gc
Double Couple, 2 RS (resin samples)	a - bcdeafGdef / gc
Final Wash	h -
Final Deprotection	f - bcdc
N-Terminal Acetylation	f - bcdCc
DCM wash	c

* A hyphen (-) indicates "finish previous cycle," a virgule or slash (/) indicates "start next cycle."

Table 7-11 displays the cycles that define the Default Set for Boc/HOBt/DCC 0.10 mmol Chemistry files in SynthAssist Software. You may change the default cycles for any of the items listed in the AA column, or you may add new AA items and cycles to the Default Set. Cycles in the Default Set for a specific Chemistry file are automatically applied to any SynthAssist Run file that uses that Chemistry.

Table 7-11. Default Set of Cycles for Boc/HOBt/DCC 0.10 mmol Chemistry Files

AA	Cycle	Modules*
Default	Single Couple	a - bcde / gc
Preloaded	DCM Wash	c
Load**	<<None>>	
End	Final Wash	h -

* A hyphen (-) indicates "finish previous cycle," a virgule or slash (/) indicates "start next cycle."

**Although Boc Chemistry does not require a load cycle, a Load cycle is included in the Boc chemistry Default Set because it is available in SynthAssist Software.

Hyphen and Virgule Symbols in the Boc/HOBt/DCC Cycles

Because the Boc/HOBt/DCC Chemistry cycles use HOBt/DCC activation, they require 30-40 minutes of pre-activation for the formation of the Boc-amino acid-OBt ester. So, with any two cycles in a synthesis, module "a" (activation) begins on the second cycle before the previous cycle is finished. As a consequence, the initial cycle in a synthesis must be different than subsequent cycles.

The hyphen (-) and the virgule, or slash, (/) symbols in the Default Set of Boc/HOBt/DCC Chemistry cycles are actually signals. The hyphen means "finish the previous cycle"; by default, module "i" is inserted when a hyphen

occurs in a module. The virgule means “start the next cycle now.” The modules that follow the virgule are inserted in place of the next available insertion point.

To illustrate how the hyphen and virgule symbols operate, look at a Run composed of only Single Couple cycles and a Final wash. The Default cycle definitions in the pre-defined Boc/HOBt/DCC 0.50 mmol Chemistry file show the following modules:

Single couple	a - bcdef / gc
Final wash	h -

For the first cycle, SynthAssist Software replaces the hyphen with a module “i.” When it reads the virgule, SynthAssist Software ends the cycle so that the activation of the next amino acid may begin. The first cycle becomes: a i b c d e f.

For subsequent cycles, SynthAssist Software inserts the modules that followed the virgule in the previous cycle, so that the previous coupling can be completed. In this case, the modules “gc” replace the hyphen. As a result, subsequent single couple cycles become: a g c b c d e f.

For the final deprotection cycle, SynthAssist Software continues to replace the hyphen with the modules “gc,” so that final wash cycle becomes: h g c.

All these changes occur automatically in SynthAssist Software. You only have to open a Run file, choose both the Boc/HOBt/DCC Chemistry file and the Sequence file, and then open the Cycle window to see the outcome.

The following examples of Boc/HOBt/DCC 0.50 mmol cycles demonstrate the Run files SynthAssist Software creates. The Boc/HOBt/DCC 0.10 mmol cycles differ slightly from the 0.50 mmol cycles; you can create similar Run files with the Boc/HOBt/DCC 0.10 mmol Chemistry file.

Boc/HOBt/DCC 0.50 mmol Chemistry Cycles

To synthesize Angiotensin starting with Boc-Leu- PAM resin and leave the final Boc group on the peptide-resin:

1. Open a new Run file in SynthAssist Software.
2. Choose the “Boc/HOBt/DCC 0.50 mmol” Chemistry file.
3. Choose the “Angiotensin” Sequence file.
4. Choose PAM resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	DCM Wash	c
2	His	Single Couple	aibcdef
3	Phe	Single Couple	agbcdef
4	Pro	Single Couple	agbcdef
5	His	Single Couple	agbcdef
6	Ile	Single Couple	agbcdef
7	Tyr	Single Couple	agbcdef
8	Val	Single Couple	agbcdef
9	Arg	Single Couple	agbcdef
10	Asp	Single Couple	agbcdef
11		Final Wash	hgc

6. Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

```

Cy: 1      Rpt: 1      M: c
Cy: 2      Rpt: 1      M: aibcdef
Cy: 3      Rpt: 8      M: agbcdef
Cy: 11     Rpt: 1      M: hgc

```

Boc/HOBt/DCC 0.50 mmol Chemistry Cycles for MBHA Resins

To synthesize Substance P, starting with MBHA resin:

1. Open a new Run file in SynthAssist Software.
2. Choose the "Boc/HOBt/DCC 0.50 mmol" Chemistry file.
3. Choose the "Substance P" Sequence file.
4. Choose MBHA resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

AA	Cycle	Modules
1 Met	Single Couple	aibcdef
2 Leu	Single Couple	agbcdef
3 Gly	Single Couple	agbcdef
4 Phe	Single Couple	agbcdef
5 Phe	Single Couple	agbcdef
6 Gln	Single Couple	agbcdef
7 Gln	Single Couple	agbcdef
8 Pro	Single Couple	agbcdef
9 Lys	Single Couple	agbcdef
10 Pro	Single Couple	agbcdef
11 Arg	Single Couple	agbcdef
12	Final Wash	hgc

6. Send the "Boc/HOBt/DCC 0.50 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

```

Cy: 1    Rpt: 1    M: aibcdef
Cy: 2    Rpt: 10   M: agbcdef
Cy: 12   Rpt: 1    M: hgc

```

In the first cycle, TFA treatment is not needed. You may replace module “b” in cycle 1 with a module “i.” If you edit the Module 433A Run to replace module “b” in the first cycle with module “i,” the Run Editor becomes:

Cy: 1 Rpt: 1 M: aiicdef

Cy: 2 Rpt: 10 M: agcbcddef

Cy: 12 Rpt: 1 M: hgc

Starting at a Cycle Other Than Cycle One

You might end a Boc/HOBt/DCC chemistry Run before the entire peptide is synthesized and then decide to continue the synthesis. For this procedure, do **not** remove the Boc protecting group when you interrupt synthesis. When synthesis restarts, the next amino acid is activated (module “a”), followed by TFA treatment during module “b” to remove the Boc group. This critical TFA treatment optimizes resin swelling for maximum accessibility to the N terminal and high yields as the synthesis continues.

To re-start synthesis at a cycle other than cycle number one:

In this example, synthesis of Substance P was stopped after 6 cycles and you now want to complete the synthesis.

1. In the SynthAssist Dictionary, add “residue” to the list of amino acids. Check the Palette box for this entry, but do not give it a code name.
2. Open the Sequence file for the peptide you have partially synthesized, in this case Substance P.
3. Delete the part of the peptide that has already been synthesized and replace them with the amino acid entry “residue.”

In this example, the first 6 amino acids are deleted. The Sequence file now appears as follows:

H-Arg-Pro-Lys-Pro-Gln-residue-NH₂.

4. Open a new Run file.
5. Choose the “Boc/HOBt/DCC 0.50 mmol” Chemistry.
6. Choose the modified Sequence file you created in step 3.

Because peptide is already attached to the resin, use PAM resin.

7. Choose Cycles in the pop-up menu. The first entry should be “residue” followed by the remaining amino acids in the sequence. For our example, the Run appears as follows:

AA	Cycle	Modules
1 residue	DCM wash	c
2 Gln	Single Couple	aibcddef

	AA	Cycle	Modules
3	Pro	Single Couple	agcbcddef
4	Lys	Single Couple	agcbcddef
5	Pro	Single Couple	agcbcddef
6	Arg	Single Couple	agcbcddef
7		Final Wash	hgc

8. Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send the Run to the ABI 433A instrument.

9. Go to the 433A Run Editor menu. Use the **next** and **prev** soft keys to look at all the cycles. For our example, the Run Editor shows the following cycles:

```
Cycle: 1    Rpt: 1    M: c
Cycle: 2    Rpt: 1    M: aibcddef
Cycle: 3    Rpt: 4    M: agcbcddef
Cycle: 7    Rpt: 1    M: hgc
```

10. Now edit Cycle 1 in the 433A Run Editor.

- Delete all the modules in Cycle 1.
- After **Rpt:**, enter the number of amino acids that have already been coupled.

For our example, the modified Run Editor appears as follows:

```
Cycle: 1    Rpt: 6    M:
Cycle: 2    Rpt: 1    M: aibcddef
Cycle: 8    Rpt: 4    M: agcbcddef
Cycle: 12   Rpt: 1    M: hgc
```

Double Couple

<i>Note</i>	<i>Use two amino acid cartridges for each amino acid that is double-coupled in the synthesis.</i>

If you want to double couple all the amino acids in a sequence, in SynthAssist Software change the Default in the Default Set to **Double Couple**.

If one particular amino acid—for example, Gln—should always be double coupled, you can modify the Default Set.

If some amino acids are sometimes double-coupled, then leave the Default at Single Couple, but change the appropriate cycles to Double Couple.

Module G: Double Couple Cycle with Two Resin Samples and Capping

Both resin sampling and acetic anhydride capping occur in module “g” of the Boc/HOBt/DCC Chemistry cycles. Obviously, we do not want to cap between the first and second couplings, so we have modified module “g” to remove capping. This modified module “g” is module “G.” When module “G” replaces the first module “g” in a Boc/HOBt/DCC Chemistry double couple cycle, a resin sample is removed after the first coupling, but no capping occurs until after the second coupling and the second resin sample. The Boc/HOBt/DCC double couple cycle with two resins samples and capping after the second coupling contains these modules:

a - b c d e a f h G d e f / g c

Module F: Double Couples with One Resin Sample and Capping

We have also created another modified “g” module by removing the steps for both capping and resin sampling. This modified “g” module is module “F.” When module “F” replaces the first module “g” in a Boc/HOBt/DCC Chemistry double couple cycle, resin sampling does not occur after the first coupling, but, after the second coupling, both resin sampling and capping occur. The Boc/HOBt/DCC double couple cycle with resin sampling and capping only after the second coupling contains these modules:

a - b c d e a f h F d e f / g c

The resin sample line to the test tube is cleaned more times during a double couple than in a single couple cycle. If you only take one resin sample in a double couple cycle, all of the solvent goes into one tube. When using the double couple cycles with only one resin sample, make sure your test tubes can hold 12 mL of waste solvent. If the test tube is not large enough, add a Fxn 39, Relay 0, to module G and place 2 waste test tubes on your fraction collector for each resin sample tube.

If you want to double couple but do not want resin sampling, use the double couple cycle with module “F” and answer **No** when the message “Are you taking resin samples?” appears in the Cycle Monitor menu (see page 2-40).

The module “g” at the end of the double couple cycle with module “F” provides steps for capping and resin sampling, but when you answer **NO** to the resin sampling option, the controller ignores the steps that direct resin sampling.

The examples that follow describe synthesis of a peptide-resin with the final Boc group attached. If you prefer to remove the final Boc group, see *Acetylation of the N-Terminal Amine* on page 7-48 for the procedure.

Boc/HOBt/DCC (0.50 mmol) cycles with a double couple every cycle

This example uses module F described on the previous page to synthesize Substance P, starting with MBHA resin, with a double couple every cycle, and without removal of the final Boc group.

To synthesize Substance P from resin, double couple every cycle and keeping the final Boc group:

1. Open the “Boc/HOBt/DCC 0.50 mmol” Chemistry file. Click the Default Set button. For the Default cycle, choose Double Couple (1 RS) in the pop-up menu. You may save this modified Chemistry file with a new name, such as “Boc/HOBt/DCC Double Couple.”
2. Open a new Run file in SynthAssist Software.
3. Choose the **modified** Chemistry file and the “Substance P” Sequence file.
4. Choose MBHA. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Double Couple (1 RS)	aibcdeafhFdef
2	Leu	Double Couple (1 RS)	agcbcdeafhFdef
3	Gly	Double Couple (1 RS)	agcbcdeafhFdef
4	Phe	Double Couple (1 RS)	agcbcdeafhFdef
5	Phe	Double Couple (1 RS)	agcbcdeafhFdef
6	Gln	Double Couple (1 RS)	agcbcdeafhFdef
7	Gln	Double Couple (1 RS)	agcbcdeafhFdef
8	Pro	Double Couple (1 RS)	agcbcdeafhFdef
9	Lys	Double Couple (1 RS)	agcbcdeafhFdef
10	Pro	Double Couple (1 RS)	agcbcdeafhFdef
11	Arg	Double Couple (1 RS)	agcbcdeafhFdef
12		Final Wash	hgc

6. Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibcdeafhFdef

Cy: 2 Rpt: 10 M: agcbcdeafhFdef

Cy: 12 Rpt: 1 M: hgc

Boc/HOBt/DCC 0.50 mmol Cycles with double couple of all Gln

To synthesize Substance P, starting with MBHA resin, double couple all Gln, and leave the final Boc group on the peptide-resin:

1. Open the “Boc/HOBt/DCC 0.50 mmol” Chemistry file. Click the Default Set button. Verify that the Default is Single Couple.
2. Select “End” in the AA column and choose the Insert command in the Edit menu. Click the new entry and choose “Gln” from the pop-up menu.
3. Choose Double Couple (1 RS) as the default Cycle for Gln. You may save this modified file with a new name.
4. Open a new Run file in SynthAssist Software.
5. Choose the **modified** Chemistry file and the “Substance P” Sequence file.
6. Choose MBHA resin. Enter the resin substitution and resin weight, and save the Run file.
7. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibcdef
2	Leu	Single Couple	agcbcdef
3	Gly	Single Couple	agcbcdef
4	Phe	Single Couple	agcbcdef
5	Phe	Single Couple	agcbcdef
6	Gln	Double Couple (1 RS)	agcbcdeafhFdef
7	Gln	Double Couple (1 RS)	agcbcdeafhFdef
8	Pro	Single Couple	agcbcdef
9	Lys	Single Couple	agcbcdef
10	Pro	Single Couple	agcbcdef
11	Arg	Single Couple	agcbcdef
12		Final Wash	hgc

8. Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

The 433A Run Editor menu contains the following information:

Cy: 1 Rpt: 1 M: aibcdef
 Cy: 2 Rpt: 4 M: agcbcddef
 Cy: 6 Rpt: 2 M: agcbcddefhFdef
 Cy: 8 Rpt: 4 M: agcbcddef
 Cy: 12 Rpt: 1 M: hgc

Boc/HOBt/DCC 0.50 mmol Cycles, with only one double couple

To synthesize Substance P, starting with MBHA resin, with only one double couple at Gln⁵, and without removal of the final Boc group:

1. Open the “Boc/HOBt/DCC 0.50 mmol” Chemistry file and click the Default Set button. Verify that the Default cycle is Single Couple.
2. Open a new Run file in SynthAssist Software.
3. Choose the “Boc/HOBt/DCC 0.50 mmol” Chemistry file and the “Substance P” Sequence file.
4. Choose MBHA resin. Enter the resin substitution and resin weight, and save the Run file.
5. Choose Cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibcdef
2	Leu	Single Couple	agcbcddef
3	Gly	Single Couple	agcbcddef
4	Phe	Single Couple	agcbcddef
5	Phe	Single Couple	agcbcddef
6	Gln	Single Couple	agcbcddef
7	Gln	Single Couple	agcbcddef
8	Pro	Single Couple	agcbcddef
9	Lys	Single Couple	agcbcddef
10	Pro	Single Couple	agcbcddef
11	Arg	Single Couple	agcbcddef
12		Final Wash	hgc

6. Select the Single Couple cycle on line 7. Click it again to make the pop-up entry field appear. Choose Double Couple from the pop-up menu and press the Return key.

The modified SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Met	Single Couple	aibcdef
2	Leu	Single Couple	agcbcddef
3	Gly	Single Couple	agcbcddef

AA		Cycle	Modules
4	Phe	Single Couple	agcbcddef
5	Phe	Single Couple	agcbcddef
6	Gln	Single Couple	agcbcddef
7	Gln	Double Couple (1RS)	agcbcddeafhFdef
8	Pro	Single Couple	agcbcddef
9	Lys	Single Couple	agcbcddef
10	Pro	Single Couple	agcbcddef
11	Arg	Single Couple	agcbcddef
12		Final Wash	hgc

- Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

The 433A Run Editor menu contains the following information:

Cy: 1	Rpt: 1	M: aibcddef
Cy: 2	Rpt: 5	M: agcbcddef
Cy: 7	Rpt: 1	M: agcbcddeafhFdef
Cy: 8	Rpt: 4	M: agcbcddef
Cy: 12	Rpt: 1	M: hgc

Acetylation of the N-Terminal Amine

To synthesize an N-terminal acetylated peptide, use the “Final N-terminal Acetylation” cycle for the end cycle.

Boc/HOBt/DCC (0.50 mmol) Cycles for N-terminal Acetylation

To synthesize Angiotensin with the N-terminal acetylated, starting with Boc-Leu PAM resin and single coupling, you must modify the Run file by changing the End cycle to the Final N-terminal Acetylation cycle.

To modify the Run file by changing the End cycle:

1. Open a new Run file in SynthAssist Software.
2. Choose the “Boc/HOBt/DCC 0.50 mmol” Chemistry file.
3. Choose the Angiotensin Sequence file.
4. Choose PAM resin. Enter the substitution and resin weight, and save the Run file.
5. Choose cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	DCM Wash	c
2	His	Single Coupling	aibcdef
3	Phe	Single Coupling	agcbcddef
4	Pro	Single Coupling	agcbcddef
5	His	Single Coupling	agcbcddef
6	Ile	Single Coupling	agcbcddef
7	Tyr	Single Coupling	agcbcddef
8	Val	Single Coupling	agcbcddef
9	Arg	Single Coupling	agcbcddef
10	Asp	Single Coupling	agcbcddef
11		Final Wash	hgc

With this Run file, the N-terminal amino acid will not be acetylated. You now must replace the last cycle in the Run with “Final N-terminal Acetylation.”

6. Double-click the cycle entry “Final Wash” to make it become a pop-up menu. Choose “Final N-terminal Acetylation” from the pop-up menu and press the Return key.

Now the Run file should look like this:

	AA	Cycle	Modules
1	Leu	DCM Wash	c
2	His	Single Coupling	aibcdef
3	Phe	Single Coupling	agcbcddef
4	Pro	Single Coupling	agcbcddef
5	His	Single Coupling	agcbcddef
6	Ile	Single Coupling	agcbcddef
7	Tyr	Single Coupling	agcbcddef
8	Val	Single Coupling	agcbcddef
9	Arg	Single Coupling	agcbcddef
10	Asp	Single Coupling	agcbcddef
11		Final N-terminal Acetylation	hgcbcdCc

7. Send this Run file to the ABI 433A instrument.

After you send the SynthAssist Run file to the ABI 433A instrument, the 433A Run Editor menu contains the following information:

Cy: 1	Rpt: 1M: c
Cy: 2	Rpt: 1M: aibcdef
Cy:3	Rpt: 8M: agcbcddef
Cy: 11	Rpt: 1M: hgcbcdCc

Removal of the Final Boc Group

When the End cycle of the Boc Chemistry Default Set is “Final Wash,” the synthesized peptide-resin has the final Boc group still on the N-terminal amino acid. If you want to synthesize a peptide with the final Boc group removed, change the End cycle in the Default Set to “Final Deprotection.”

When the End cycle is “Final Deprotection,” the final Boc group is removed and the N-terminal amino acid is neutralized before the DCM wash. If you want the N-terminal amine to remain a TFA salt, take module “d” out of the Final Deprotection cycle.

Boc/HOBt/DCC 0.50 mmol cycles for synthesis of a peptide-resin with the final Boc group removed

To synthesize Angiotensin starting with Boc-Leu Pam resin, remove the final Boc group, and neutralize the N-terminal amine, you must change the End cycle in the Boc/HOBt/DCC Chemistry Default Set to “Final Deprotection.”

1. Open the “Boc/HOBt/DCC 0.50 mmol” Chemistry file and click the Default Set button. Click the Default Set button.
2. Double-click the Cycle pop-up entry field labeled End to make the pop-up menu appear. Choose “Final Deprotection” from the pop-up menu. You may save this modified Chemistry file with a new file name.
3. Open a new Run file in SynthAssist Software.
4. Choose the **modified** “Boc/HOBt/DCC 0.50 mmol” Chemistry file and the “Angiotensin” Sequence file.
5. Choose Pam resin. Enter the resin substitution and resin weight, and save the Run file.
6. Choose cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	Single Coupling	aibcdef
2	His	Single Coupling	agcbcddef
3	Phe	Single Coupling	agcbcddef
4	Pro	Single Coupling	agcbcddef
5	His	Single Coupling	agcbcddef
6	Ile	Single Coupling	agcbcddef
7	Tyr	Single Coupling	agcbcddef
8	Val	Single Coupling	agcbcddef
9	Arg	Single Coupling	agcbcddef
10	Asp	Single Coupling	agcbcddef
11		Final Deprotection	hgcbcdc

7. Send the "Boc/HOBt/DCC 0.50 mmol" Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

The 433A Run Editor menu contains the following information:

Cy: 1	Rpt: 1M: aibcdef
Cy: 2	Rpt: 9M: agcbcddef
Cy: 11	Rpt: 1M: hgcbcdc

Boc/HOBt/DCC 0.50 mmol cycles for synthesis of a peptide-resin with the final Boc group removed and N-terminal amine as a TFA salt

To synthesize Angiotensin starting with Boc-Leu Pam resin, remove the final Boc group, and leave the N-terminal amine a TFA salt, you must modify the "Final Deprotection" cycle.

To modify the final deprotection cycle:

1. Open the "Boc/HOBt/DCC 0.50 mmol" Chemistry file and double-click the "Final Deprotection" cycle to open the window that displays the modules in the Final Deprotection cycle.
2. In the window that displays the Final Deprotection cycle, use the Delete command in the Edit menu to change the cycle to "h - b c c." See *Creating a Custom Chemistry File* in the SynthAssist user guide for directions. You may save this modified Chemistry file with a new file name.
3. Open a new Run file in SynthAssist Software.
4. Choose the **modified** Chemistry file and the "Angiotensin" Sequence file.
5. Choose Pam resin. Enter the resin substitution and resin weight.
6. Choose cycles from the pop-up menu. The new SynthAssist Run file should look like this:

	AA	Cycle	Modules
1	Leu	Single Coupling	aibcdef
2	His	Single Coupling	agcbcddef
3	Phe	Single Coupling	agcbcddef
4	Pro	Single Coupling	agcbcddef
5	His	Single Coupling	agcbcddef
6	Ile	Single Coupling	agcbcddef
7	Tyr	Single Coupling	agcbcddef
8	Val	Single Coupling	agcbcddef
9	Arg	Single Coupling	agcbcddef
10	Asp	Single Coupling	agcbcddef
11		Final Deprotection	hgcbcc

-
- Send the “Boc/HOBt/DCC 0.50 mmol” Chemistry to the ABI 433A instrument, if necessary. Then send this Run file to the ABI 433A instrument.

The 433A Run Editor menu contains the following information:

Cy: 1	Rpt: 1M: aibcdef
Cy: 2	Rpt: 9M: agcbcddef
Cy: 11	Rpt: 1M: hgcbcc

Modification of Individual Modules

You may change the Boc/HOBt/DCC 0.50 mmol Chemistry cycles to customize your synthesis by modifying individual modules.

Shorten the TFA Deprotection (module “b”)

The TFA deprotection step in the standard cycle runs for 18 minutes. When synthesizing short peptides (10 mer or less), you may decrease the deprotection time. To decrease deprotection time to 12 minutes, for example, perform the following modifications to module “b”:

Step 79, “Begin Loop,” change the value from 11 to 5.

Step 88, “Begin Loop,” change the value from 11 to 5.

Remove the Capping Step in the Single Couple Cycle

Replace module “g” in a Single Couple cycle with module “G.”

Single Couple /no capping: a - b c d e f /G c.

Reduce the number of NMP washes before coupling from 6 to 4

Change module “d” (DIEA Neutralization & NMP washes).

At Step 67, “Begin Loop,” change the value of T from 2 to 1.

Eliminate Steps 38 through 50 (13 deletions).

Remove the DMSO addition

Change module “f” (Coupling and DMSO addition).

Eliminate Steps 56 through 87 (32 deletions).

Remove the DIEA addition at the end of Coupling

Change module “g” (DIEA, Drain, RS, and Capping).

Eliminate Steps 1 through 17.

Shorten the Coupling time

Change module “f” (Coupling and DMSO addition) to shorten the coupling time by 10 minutes.

Step 28, “Begin Loop,” change the value of T from 26 to 16.

Step 51, “Begin Loop,” change the value of T from 26 to 16.

Take two resin samples instead of one

Change module “g” (DIEA, Drain, RS, and Capping)

Step 38, “Begin Loop,” change the value of T from 1 to 2.

The controller will now repeat Steps 38, “Begin Loop,” through 65, “End Loop,” twice. The fraction collector will need two test tubes for both resin samples, one for the wash and one for the sample.

Take a resin sample after coupling and after capping

Create a new module by modifying module “g.” For our example, we call it module “H” (Resin sampling).

Eliminate Steps 67 through 87 (21 deletions).

Eliminate Steps 1 through 32 (32 deletions).

Then add the module “H” to the Single Couple cycle.

Single Couple (2 RS): a - b c d e f/g H c.

Add Times and Chemical Usage

During synthesis, the resin volume increases. Add Time is a designated amount of time added to a step at each cycle as synthesis progresses to compensate for the increased resin volume. As an example, Add Time can be used to increase the time for each wash step in Module “c” to compensate for the additional swelling or mass of the peptide-resin as the synthesis progresses. Add Times are most useful in the following steps:

- Piperidine deprotection
- TFA treatment volumes
- Solvent wash volume - after TFA, after DIEA and after coupling
- RV draining times

If desired, an add time can be entered for each step. (Refer to *The Module Editor Menu* on page 9-6). Add time values typically range from 0 to 12. For your convenience, a table of Add Times is included on page 7-56.

Calculating the Amount of Time Added to a Step per Cycle

To calculate the Add Time per cycle, determine the amount of time you want to add per step and multiply that value by 10. For example, to add 0.3 seconds to the step at each cycle, enter an add time of 3.

To calculate the amount of time added to a step per cycle:

1. Divide the add time by 10 (remember you multiplied by 10 above).
2. Multiply this value by the synthesis cycle number. The nearest whole number will be added to the step. Round up fractions of a second to the nearest second (e.g., 1.5 seconds is rounded up to 2.0 seconds).

Example: The Add Time Value Entered is 3.

Add time of 3: $3 \div 10 = 0.3$ seconds

<i>Seconds</i>	$\times$	<i>Cycle #</i>	$=$	<i>Actual Time Added to step</i>
0.3		1	0.3	0
0.3		2	0.6	1
0.3		3	0.9	1
0.3		4	1.2	1
0.3		5	1.5	2
0.3		6	1.8	2
0.3		7	2.1	2
0.3		8	2.4	2
0.3		9	2.7	3

Explanation: An add time of 3 (0.3 seconds) was entered. Therefore, no time will be added to the step time for cycle 1; 1 second will be added to the step time for cycles 2, 3 and 4; 2 seconds will be added to the step time for cycle 5, 6, 7 and 8; etc.

Table 7-12. Added Time per Cycle

Cycle	Add Time (sec)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	0	0	1	1	1	1	1	1	1	1
2	0	0	1	1	1	1	1	2	2	2	2	2
3	0	1	1	1	2	2	2	2	3	3	3	4
4	0	1	1	2	2	2	3	3	4	4	4	5
5	1	1	2	2	3	3	4	4	5	5	6	6
6	1	1	2	2	3	4	4	5	5	6	7	7
7	1	1	2	3	4	4	5	6	6	7	8	8
8	1	2	2	3	4	5	6	6	7	8	9	10
9	1	2	3	4	5	5	6	7	8	9	10	11
10	1	2	3	4	5	6	7	8	9	10	11	12
11	1	2	3	4	6	7	8	9	10	11	12	13
12	1	2	4	5	6	7	8	10	11	12	13	14
13	1	3	4	5	7	8	9	10	12	13	14	16
14	1	3	4	6	7	8	10	11	13	14	15	17
15	2	3	5	6	8	9	11	12	14	15	17	18
16	2	3	5	6	8	10	11	13	14	16	18	19
17	2	3	5	7	9	10	12	14	15	17	19	20
18	2	4	5	7	9	11	13	14	16	18	20	22
19	2	4	6	8	10	11	13	15	17	19	21	23
20	2	4	6	8	10	12	14	16	18	20	22	24
21	2	4	6	8	11	13	15	17	19	21	23	25
22	2	4	7	9	11	13	15	18	20	22	24	26
23	2	5	7	9	12	14	16	18	21	23	25	28
24	2	5	7	10	12	14	17	19	22	24	26	29
25	3	5	8	10	13	15	18	20	23	25	28	30
26	3	5	8	10	13	16	18	21	23	26	29	31
27	3	5	8	11	14	16	19	22	24	27	30	32
28	3	6	8	11	14	17	20	22	25	28	31	34
29	3	6	9	12	15	17	21	23	26	29	32	35
30	3	6	9	12	15	18	21	24	27	30	33	36
31	3	6	9	12	16	19	22	25	28	31	34	37
32	3	6	10	13	16	19	22	26	29	32	35	38
33	3	7	10	13	17	20	23	26	30	33	36	40
34	3	7	10	14	17	20	24	27	31	34	37	41
35	4	7	11	14	18	21	25	28	32	35	39	42
36	4	7	11	14	18	22	25	29	32	36	40	43
37	4	7	11	15	19	22	26	30	33	37	41	44
38	4	8	11	15	19	23	27	30	34	38	42	46
39	4	8	12	16	20	23	27	31	35	39	43	47
40	4	8	12	16	20	24	28	32	36	40	44	48

Add Times ensure that more reagent or solvent is used as the cycle number increases. Depending on the chemistry being used, when a synthesis reaches a critical number of cycles, these reagents and solvents must be replaced.

Table 7-13 lists these four reagents, the chemistries they are associated with, and the cycle number at which new bottles of reagents should be added to ensure uninterrupted reagent delivery. For example, if you are using *FastMoc* 0.25 mmol chemistry with resin sampling to make an 80-mer, you would replace the NMP at cycles 26, 49, and 75. The values in Table 7-13 assume a two-bottle configuration for NMP in *FastMoc* and Fmoc chemistries, and a two-bottle configuration for DCM in Boc chemistry.

Table 7-13. Guidelines for Reagent Bottle Replacement by Cycle Number

	<i>FastMoc</i>				Fmoc		Boc	
	0.25 mmol	0.25 mmol + r.s.	0.1 mmol	1.0 mmol	0.25 mmol	0.1 mmol	0.5 mmol	0.1 mmol
NMP ^a	29	26	73	26	37	89	27	77
	54	49			69		53	
	77	75					77	
DCM ^a							39	91
							72	
Piperidine ^a	78	—		33	78	—		
TFA ^a							36	
							58	
							76	

a. These figures are based on chemical usage with average flow rates:

NMP, 2.5 mL/5 sec

DCM, 3.4 mL/5 sec

Piperidine, 1.0 mL/5 sec

TFA, 2.0 mL/18 sec

If the flow rates on a ABI 433A instrument are greater than these values, the number of cycles per bottle will be proportionately lower.



8 System Description

This chapter contains illustrations and explanations of several parts in the ABI 433A Peptide Synthesizer, a description of the instrument's chemical delivery system and a list of functions.

Contents

The Chemical Delivery System	8-3
Functions	8-25

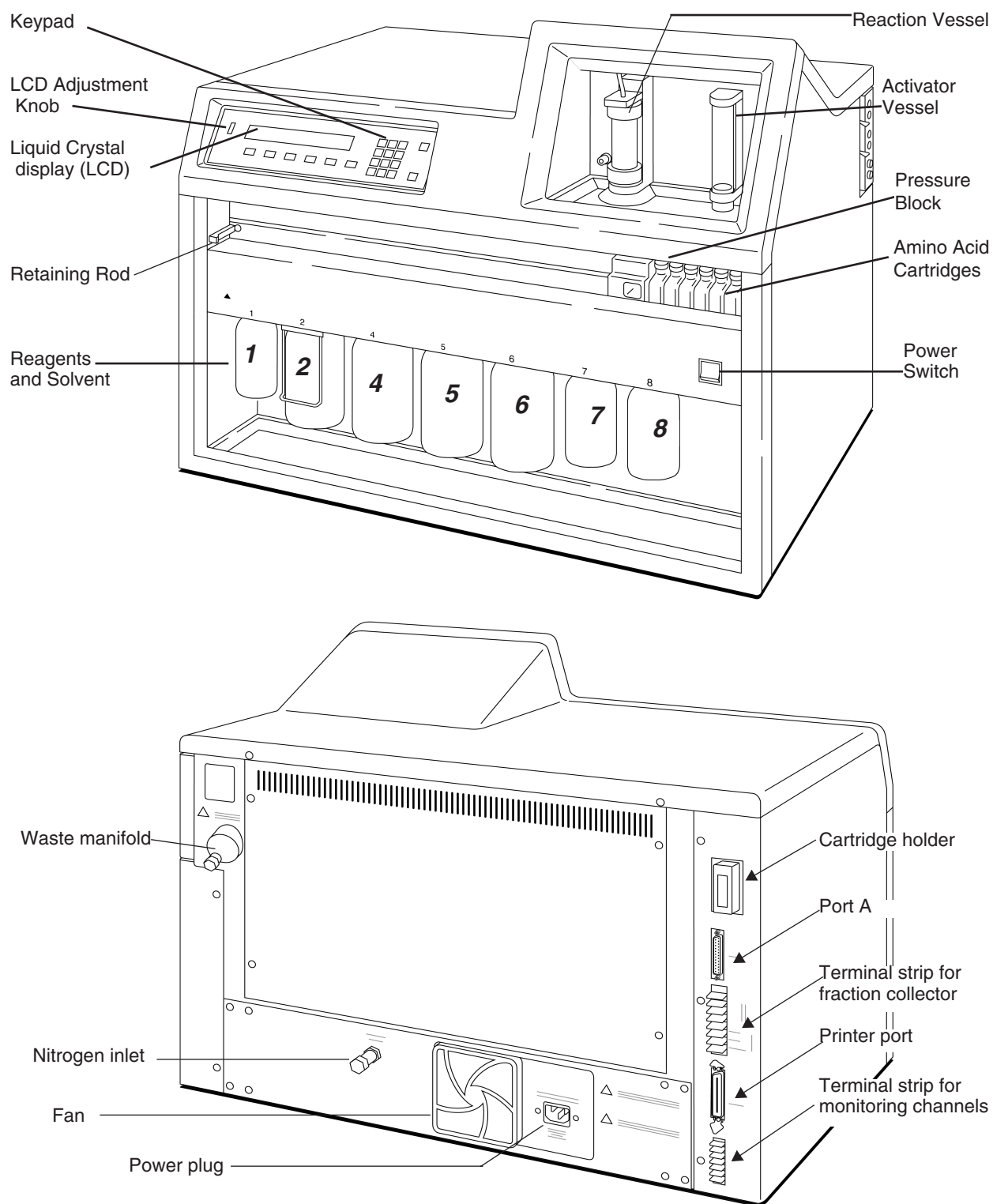


Figure 8-1. ABI 433A Peptide Synthesizer front and rear panels

The Chemical Delivery System

Chemicals

FastMoc[™], Boc/HOBt/DCC, and Fmoc/HOBt/DCC chemistries can be used with the ABI 433A Peptide Synthesizer. The chemicals used for these chemistries are listed in Table 8-1.

Seven glass chemical bottles, numbered 1, 2, 4, 5, 6, 7, 8, attach to the front of the synthesizer (see Figure 8-1). In addition, two external, four-liter bottle positions are located to the right of the ABI 433A instrument. In *FastMoc* and Fmoc/HOBt/DCC chemistries, two four-liter bottles of NMP in position 10 may be connected in parallel. In Boc/HOBt/DCC, two four-liter bottles of DCM in position 9 may be connected in parallel. Refer to “Reagent and Solvent Bottles” on page 8-7 for a description of bottle assembly and chemical delivery.

Table 8-1. Reagent and Solvent Contents by Bottle Number

Bottle	<i>FastMoc</i>	Fmoc/HOBt/DCC	Boc/HOBt/DCC
1	Piperidine	Piperidine	DIEA
2	(empty)	(empty)	TFA
4	DMAP	DMAP	Acetic Anhydride
5	HBTU	(empty)	DMSO
6	MeOH	MeOH	MeOH
7	2 M DIEA in NMP	1M HOBt in NMP	1M HOBt in NMP
8	1M DCC in NMP	1M DCC in NMP	1M DCC in NMP
9	DCM	DCM	DCM
10	NMP	NMP	NMP

WARNING **CHEMICAL HAZARDS.** *Chemicals used on the ABI 433A Peptide Synthesizer can be hazardous and cause injury, illness or death. Become completely familiar with the MSDS for each hazardous chemical before attempting to operate the instrument or use the reagents. When working with hazardous chemicals, wear all appropriate safety attire listed in the MSDSs. To minimize inhalation of the chemicals do not leave any chemical bottles uncapped.*

Chemical Flow

A positive-pressure chemical delivery system controls the flow of reagents, solvents and gas through the ABI 433A instrument. The system's components include:

- A regulated gas-pressure system
- Angar valves
- Valve blocks with their delivery valves
- Reagent and solvent bottles
- A vented waste system

All inner surfaces are made of inert materials such as Teflon, Kel-F, and Kalrez. Delivery lines (except for Bottle #2) are made of Teflon tubing with inert fittings. When the Boc/HOBt/DCC chemistry is used, Bottle #2 contains TFA. The delivery line for this bottle is polyethylene, which is less permeable to TFA vapors than Teflon.

Gas Pressure

Gas pressure should be provided by a tank of pre-purified (99.998%) nitrogen. A gas tank is connected to the inlet port at the rear of the ABI 433A instrument by oxygen-impermeable tubing and gas-tight connectors. The pressure regulator on the tank is set at approximately 65 psi.

WARNING ***PRESSURIZED GAS CYLINDERS ARE EXPLOSIVE. Attach pressurized gas cylinders firmly to the wall or a bench by means of approved brackets, chains or clamps. Always cap the gas cylinder when not in use.***

WARNING ***DAMAGE TO SYNTHESIZER AND LABORATORY. Do not operate the instrument without gas pressure. Damage can occur to the valves and regulators which could result in damage to the instrument and the laboratory.***

Gas travels from the tank through a 10- μ particle filter to two pressure regulators (upper and lower), the resin sampler, and the autodelivery system (Figure 8-2). Each of these components is discussed in the following pages.

WARNING **POTENTIAL EXPLOSION HAZARD.** Do not let the gas tank depressurize during instrument operation. If the pressure drops below 300 psi while the instrument is running, organic solvents could backflush into the pressure regulator and cause the regulator to fail. As a result, reagent bottles could be overpressurized and explode.

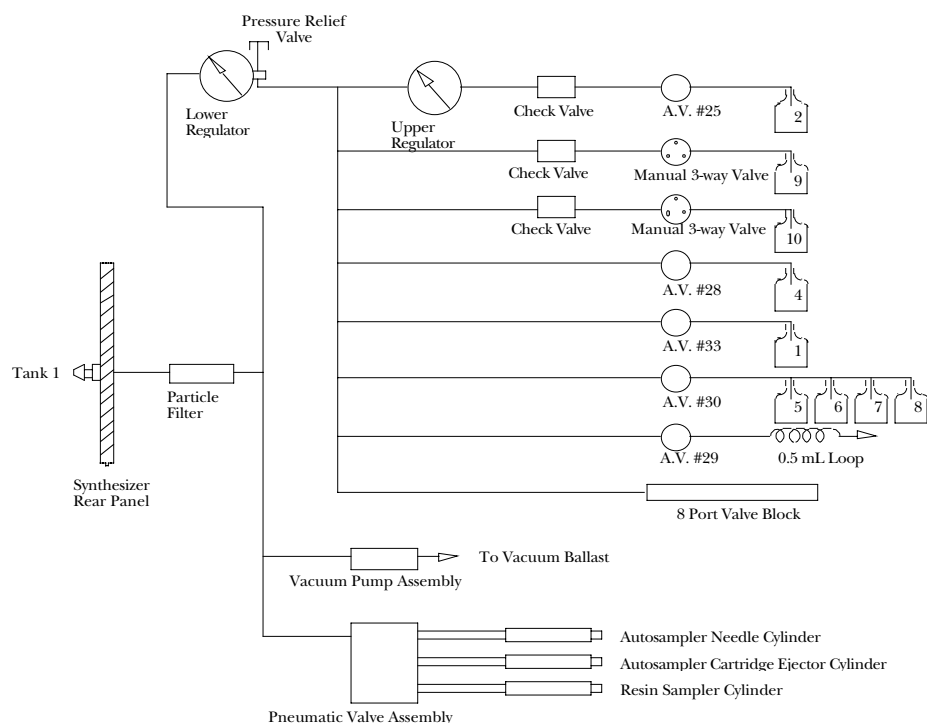


Figure 8-2. Gas Flow through the ABI 433A instrument

Regulators

There are two pressure regulators located behind the right panel covering. These regulators are calibrated at installation and should be checked routinely to ensure proper reagent and solvent deliveries. The *upper regulator* is set to monitor gas pressure for bottle 2 only. The *lower regulator* monitors gas pressure to the 8-port valve block at valve 17, to all chemical bottles (except bottle 2), and to the upper regulator. See “Calibrate Gas Regulators” on page 2-25.

Note *Both regulators have a safety lock that is set to prevent over-pressurization. The upper regulator lock is set at 3.5 psi; the lower regulator lock is set at 11 psi.*

Valves

Nine Angar valves (valves 25 through 33) and the zero-dead volume (delivery) valves located on three valve blocks (valves 1-3 and 6-23) manage the chemical delivery system. All valves are located behind the right side panel of the ABI 433A instrument. Refer to the schematic in Figure 8-3 for placement of each valve.

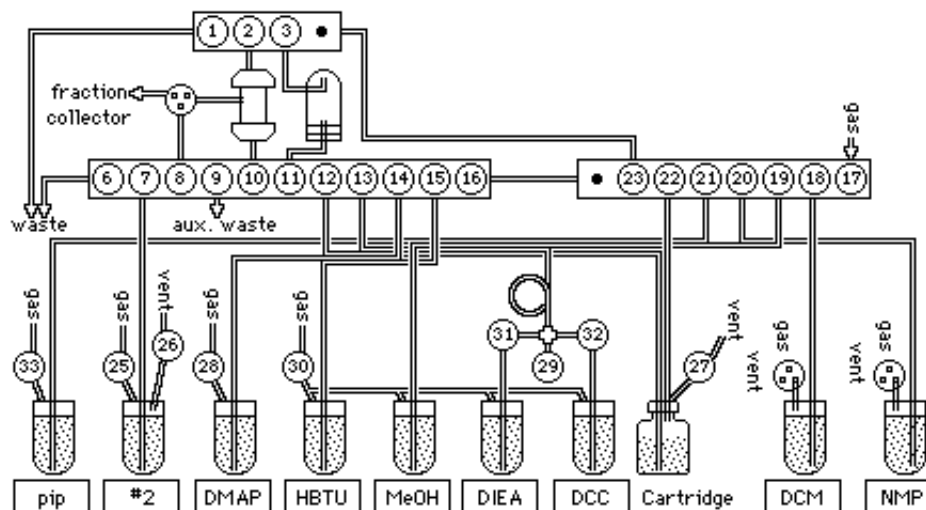


Figure 8-3. Delivery valve placement

Angar Valves

Four Angar valves (numbered 33, 25, 28 and 30) control gas flow to the bottles attached to the front of the synthesizer. When these valves are open, gas flows into the bottles, pressurizing them for subsequent delivery. Valves 26 and 27 control venting of bottle 2 and the cartridge. Three Angar valves (numbers 31, 32 and 29) control the calibrated deliveries of reagents from bottles 7 and 8.

When Boc/HOBt/DCC chemistry is used, bottle 2 contains TFA. Because this is a corrosive chemical, bottle 2 is slightly different than the other bottles. The pressure valve for bottle 2 is located between the upper regulator and the bottle. This valve opens to pressurize bottle 2 before TFA delivery and closes when delivery is complete. Valve 26 vents bottle 2 after delivery. In standard Boc/HOBt/DCC cycles, bottle 2 is only pressurized immediately before and during a TFA delivery.

Note

The Angar valves that control TFA pressure and venting are made of material resistant to TFA. The other valves on the instrument are not made of this material. Replace TFA valves only with valves specified for the TFA position.

Delivery Valves

Delivery valves are located on the valve blocks. See “Delivery Valves and Valve Blocks” in this section.

Reagent and Solvent Bottles

WARNING **CHEMICAL HAZARDS.** *Chemicals used on the ABI 433A Peptide Synthesizer can be hazardous and cause injury, illness or death. Become completely familiar with the MSDS for each hazardous chemical before attempting to operate the instrument or use the reagents. When working with hazardous chemicals, wear all appropriate safety attire listed in the MSDSs. To minimize inhalation of the chemicals do not leave any chemical bottles uncapped.*

See “About MSDSs” on page 1-17 for more details on obtaining MSDSs.

The seven glass chemical bottles, numbered 1, 2, 4, 5, 6, 7 and 8, screw snugly into threaded ratchet caps mounted on the synthesizer. A cap insert and bottle seal help maintain an air-tight seal between the cap and bottle (see Figure 8-5), except for Bottle 2 which uses a different bottle seal (not shown). There is no Bottle 3 position on the ABI 433A instrument. Place ethanolamine directly into the waste container to neutralize the waste.

Three external four-liter bottles, at positions 9 and 10, are placed at the right of the ABI 433A instrument. Bottle caps for Bottles 9 and 10 have a gasket that forms a seal between the bottle and the cap. In addition, a metal inlet filter at the end of each delivery line prevents particles from entering the delivery system.

IMPORTANT *The inlet filters must be in place at all times to prevent particles from entering and clogging the delivery system.*

Solvent capacity at positions 9 or 10 can be increased by adding connecting two four-liter bottles in parallel. This configuration increases the number of cycles that may be run unattended before reagent must be replaced.

IMPORTANT *Bottle caps for Bottles 9 and 10 must have a cap thread size of 38-430 to prevent leakage. Most solvent bottles use this size thread and all gallon bottles supplied by Applied Biosystems have this thread size. If bottles are purchased from other chemical manufacturers, check the thread size.*

Secondary Containment

To minimize danger of injury from broken glass and spilled chemicals, the four-liter bottles in positions 9 and 10 should be encased in a secondary container made of low-density polyethylene (ABI P/N 140041). Figure 8-4 shows a cross-section of a four-liter bottle encased in the secondary container. Note that when the metal handle on the secondary container is upright, it firmly locks the container cover in place.

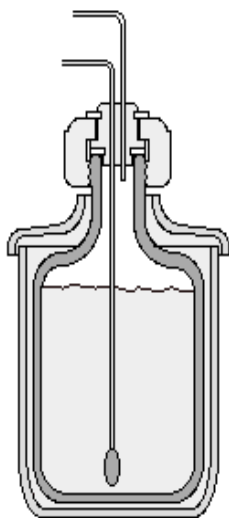


Figure 8-4. Cross-section of a four-liter bottle in secondary containment

The bottle design makes it easy to replenish chemicals. Simply remove the empty bottle and replace it with a new one. The chemical protocol used will determine the number of cycles the synthesizer can perform before the bottles empty.

A pressure line and a delivery line enter each bottle through the cap. The pressure line enters the bottle and remains above the liquid level, but the delivery line extends to the bottom of the bottle. Gas enters the bottle through the pressure line to pressurize the bottle headspace. When the bottle is pressurized and the delivery valve opens, liquid is pushed into the delivery line. The delivery schemes of each bottle differ, as discussed in the following paragraphs.

Refer to “Valves” on page 8-6 for valve descriptions and to Figure 8-3 on page 8-6 for valve placement.

Bottle 1

Angar valve 33 controls the flow of gas pressure to bottle 1. The Bottle 1 delivery line is connected to the 8-port valve block at valve 21.

Bottle 2

Angar valve 25 controls the flow of gas pressure to bottle 2. Gas flows from the upper regulator, through a one-way valve to valve 25 and into Bottle 2.

The delivery line from this bottle is connected to the 11-port valve block at valve 7.

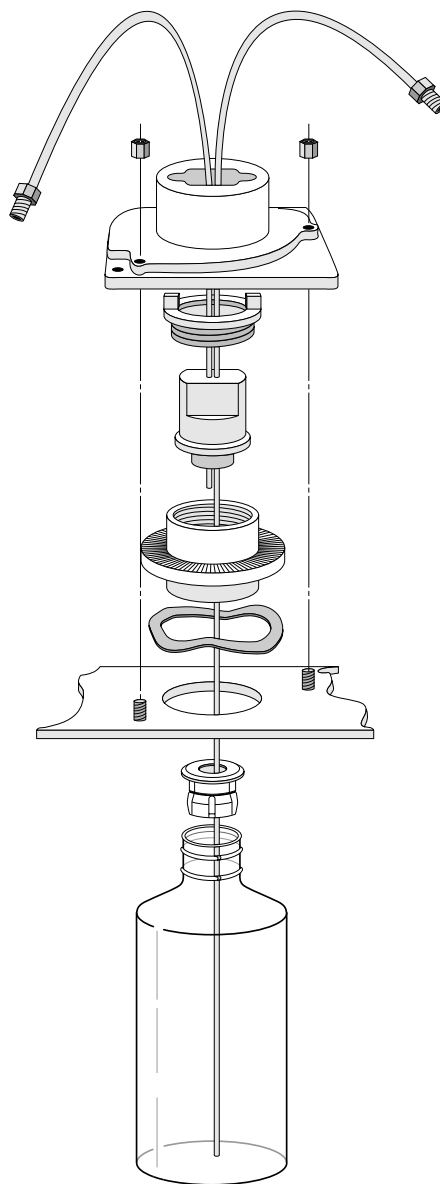


Figure 8-5. Bottle and bottle cap assembly for bottles 1 through 8 (except bottle 2)

Bottle 2 is used for TFA when the Boc/HOBt/DCC chemistry is used and has a third line, the 'vent' line. Venting is controlled by valve 26. When valve 26 is open, the TFA bottle vents directly to the waste system. The vent valve depressurizes the TFA bottle to back-flush the TFA delivery line. Any TFA left in the line after a delivery is blown back into the TFA bottle and then the

bottle is vented. TFA is never left in the delivery line, and the bottle is not under pressure except immediately before and during TFA delivery.

Bottle 4

Angar valve 28 controls the flow of gas to bottle 4. The delivery line is connected to the 11-port valve block at valve 14.

Bottles 5, 6, 7 and 8

Bottles 5, 6, 7 and 8 share a common Angar valve. All four bottles are pressurized when valve 30 opens. There are two steps to the metered delivery of Bottles 7 and 8 (DIEA or HOBt, and DCC, respectively). In the first step, reagent goes through the valve block to fill the 0.5 mL calibrated line (see Figure 8-6) and then on to waste. To measure the 0.5 mL DIEA or HOBt from Bottle 7, angar valve 31 and valves 13 and 6 (on the valve-block) open. For DCC delivery from Bottle 8, angar valve 32 and valves 13 and 6 on the valve-block open. In both cases, reagent fills the line and excess goes to waste.

The second step delivers the liquid in the 0.5 mL loop by using gas pressure to force it out of the loop (see Figure 8-6). Angar valve 29 and valve-block valve 13 open to provide gas pressure and deliver DIEA or HOBt, and DCC. Additional valve-block delivery valves must be opened to deliver these reagents.

Bottles 9 and 10

There are four-liter bottles for reservoirs 9 and 10. A 3-way valve controls the supply of gas pressure to each bottle. Liquid deliveries flow from bottle 9 to the 8-port valve block at valve 18. From bottle 10, liquid deliveries flow to the 8-port valve block at valve 20.

You can increase the capacity of bottle positions 9 and 10. In *FastMoc* and Fmoc/HOBt/DCC chemistries, you may connect two four-liter bottles of NMP at position 10 in parallel. In Boc/HOBt/DCC, you may connect two four-liter bottles of DCM at position 9 in parallel.

Auxiliary Bottle

An 'auxiliary' bottle can be connected and used as another waste or solvent container. The auxiliary line is connected to the 11-port valve block at valve 9.

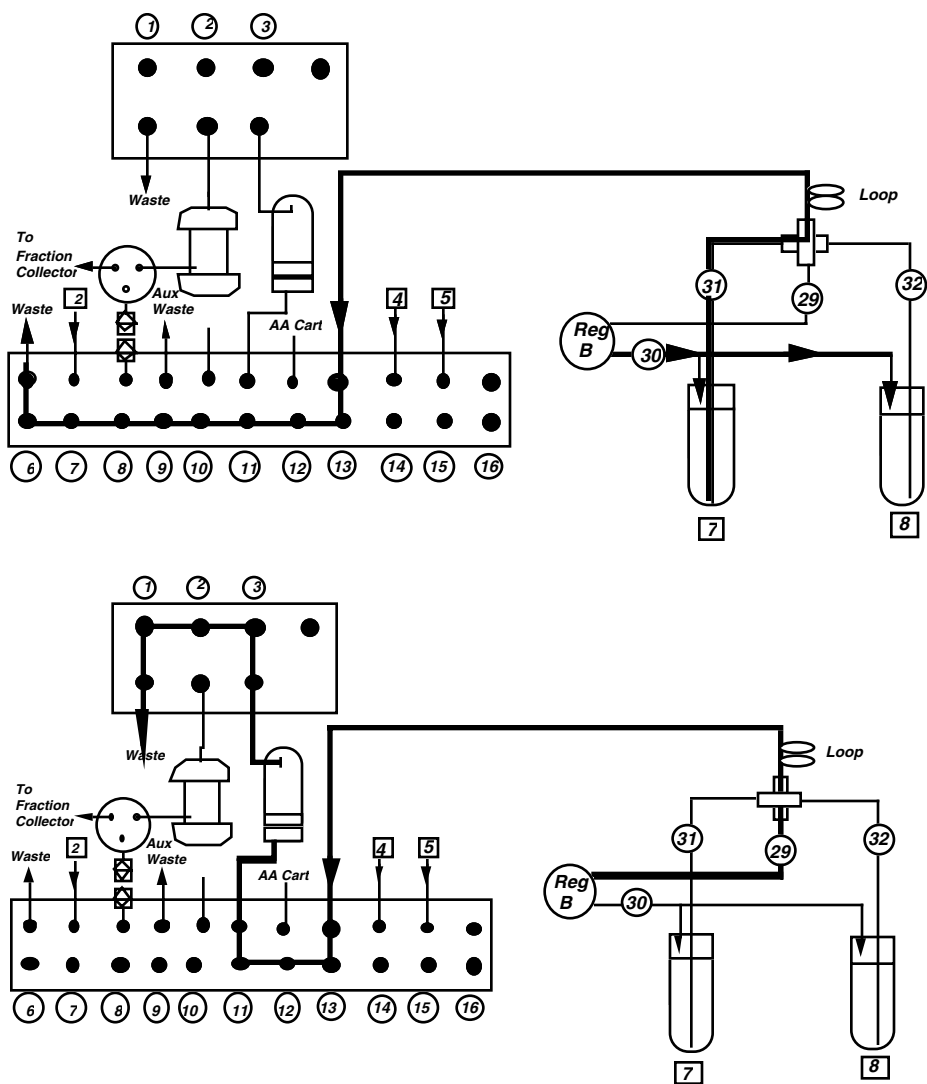


Figure 8-6. Calibrated Delivery of Bottle 7

Delivery Valves and Valve Blocks

The valve blocks control gas flows, and chemical flows to and from the activator vessel, the reaction vessel, the autodelivery system (including amino acid cartridges), the resin sampler, and to the waste system.

To eliminate cross-contamination from other chemicals, the valve block design provides zero dead volume. Delivery lines feed into each valve block and connect to the common pathway in the valve-block manifold via a manifold inlet line and a solenoid-controlled diaphragm valve (see Figure 8-7). Passage between the manifold inlet line and the common pathway is by way of an open solenoid valve.

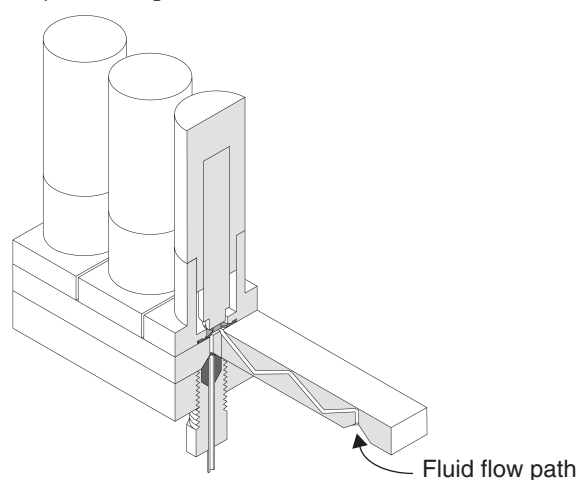


Figure 8-7. Valve block

When a valve opens, the solenoid piston pulls away from the valve block manifold, lifting the diaphragm into a domed shape. The domed chamber creates a passageway between the manifold inlet line and the common pathway. The common pathway zig-zags through the valve block manifold and passes other closed valves which are unaffected by the flow. The direction of flow is determined by the pressures on either end of the open valves.

Waste and Exhaust

WARNING **HAZARDOUS WASTE.** *Waste produced by the ABI 433A Peptide Synthesizer can be hazardous and cause injury, illness, or death. Handle all liquid, solid, and gaseous waste as potentially hazardous and wear protective clothing. Read all applicable MSDSs and waste profiles. Dispose of waste in accordance with all local, state, and federal health and safety regulations. Always handle hazardous materials beneath a fume hood that has been connected in accordance with all requirements.*

The waste and exhaust system is composed of a 2.5-gallon polyethylene bottle, a waste manifold, a waste line and an exhaust line (Figure 8-8). Fluid and gas waste from the instrument travels through the waste manifold and the waste line to the waste bottle. An exhaust line directs the gaseous waste to a fume hood or ventilation system for disposal.

WARNING **POTENTIAL EXPOSURE TO HARMFUL CHEMICALS.** *The polyethylene waste bottle becomes less impact-resistant and more susceptible to damage over time. Replace it whenever the bottle appears to be deteriorating or becoming soft. To reorder, use ABI part number 140040.*

IMPORTANT *The exhaust line must lead to the fume hood in a straight and upward direction. The line must not dip downward (creating low points) at any point. For proper ventilation, the fume hood must be turned 'on' when the instrument is operating (see the Safety Supplement).*

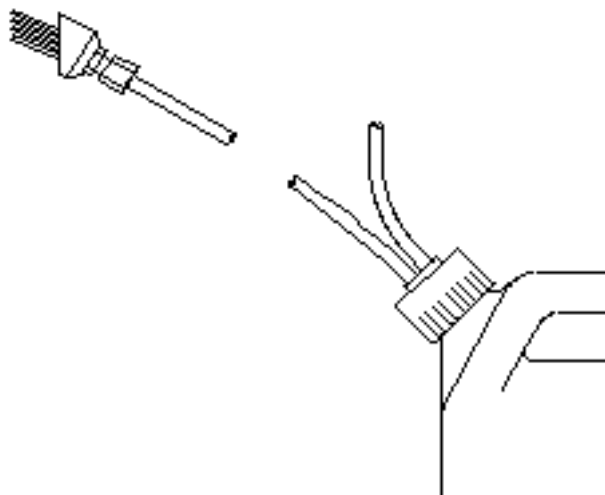


Figure 8-8. Waste system

The Autodelivery System

The autodelivery system determines how amino acid cartridges in a desired peptide sequence enter the synthesizer for dissolution and injection. It consists of a guideway and retaining rod, pressure block and latch, barcode reader, cartridge guide, needle assembly, ejector assembly, and cartridge disposal.

Amino Acid Cartridges

Cartridges containing dry amino acids are loaded into the guideway in the desired sequence from left to right, (that is, from the N- to the C- terminal). Up to 50 cartridges can be placed in the guideway in a single loading. Additional cartridges can be loaded later during the synthesis.

In addition to the pre-loaded cartridges containing dry amino acids, there are two types of empty pre-labeled cartridges available from Applied Biosystems: SP cartridges, and Empty cartridges.

SP Cartridges Cartridges labeled SP-1, SP-2, SP-3, and SP-4 are called “special” cartridges and are provided for use with non-standard amino acid derivatives.

Empty Cartridges These are generic cartridges. Each label specifies the amino acid, however, it does not specify the protecting groups. These cartridges can be used in the same manner as the pre-loaded cartridges supplied by Applied Biosystems.

When cartridges are correctly loaded into the guideway, the amino acid designation should face out, away from the instrument, with the barcode facing toward the instrument. An empty cartridge should be placed in the first position (closest to the needles) because the cartridge is ejected at the beginning of the cycle. Place the C-terminal amino acid cartridge next to the empty one, and the N-terminal amino acid cartridge at the extreme left.

Caution	<i>To prevent damage to delivery and pressure/vent needles, remove the metal tab covering each cartridge septum before placing the cartridge in the guideway.</i>

A spring-loaded pressure block slides back and forth in the cartridge guideway. When the pressure block is pushed to the extreme left position, it is held by a latch. Slide the pressure block using both hands, and verify that the block is latched securely by releasing one hand at a time.

WARNING	<i>POTENTIAL PHYSICAL INJURY. Sudden release of the pressure block will cause it to snap forcefully against fingers or hands in the guideway. Use two hands to slide the pressure block securely in place.</i>

With the cartridges in position, the retaining rod drops to hold cartridges upright and in place. The pressure block is placed against the last amino acid cartridge in the guideway and must be in position during synthesis to advance the cartridges for ejection and disposal.

Caution	<i>Be sure to release the pressure block before synthesis begins. If the pressure block is not released, and you have answered "NO" to the Interruption Option (see page 2-10), the barcode reader "reads air" and the instrument does not pause. As a result, chemicals are delivered on to the autosampler assembly.</i>

The Barcode Reader and the Cartridge Guide

The barcode reader (BCR) and the cartridge guide are located at the amino acid position that precedes the injection position (Figure 8-9). The cartridge guide holds the cartridge in the correct position so that the BCR can read the cartridge barcode (i.e., the black and white bands printed on the cartridge label). Figure 8-10 shows the barcodes for all amino acid cartridges.

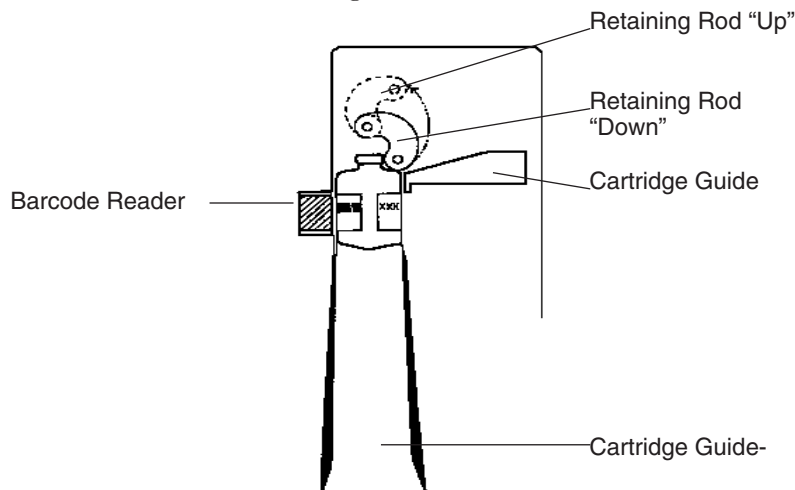


Figure 8-9. BCR and cartridge guide

Barcode Order	Assigned Name		
	Bottom	Top	
0			Air
1			SP1
2			SP2
3			SP3
4			SP4
5			Ala
6			Arg
7			Asn
8			Asp
9			Cys
10			Gln
11			Glu
12			Gly
13			His
14			Ile
15			Leu
16			Lys
17			Met
18			Phe
19			Pro
20			Ser
21			Thr
22			Trp
23			Tyr
24			Val
25			#25
26			#26
27			#27
28			#28
29			#29
30			C/N
31			Psh

Figure 8-10. Barcode label system

The Needle and Ejector Assemblies

The needle assembly consists of a pressure-driven needle arm that holds two needles in place. The needle arm moves down, forcing the needles to puncture the cartridge septum.

The ABI 433A instrument optically monitors the movement of the needle and ejector arm. The longer delivery needle delivers solvents for amino acid dissolution and gas for mixing the amino acid solution. Deliveries to and from the amino acid cartridge through this longer needle are controlled by delivery valve 12.

The shorter pressure/vent needle provides gas pressure and venting. Angar valve 27 is connected to the shorter needle and provides cartridge venting. It pressurizes the cartridge for delivery of solution to the activator vessel (ACT) through the long needle. Delivery valve 22 controls the gas and

solvent flow to the shorter cartridge needle for cartridge pressurization and cleaning.

WARNING **POTENTIAL INJURY HAZARD.** *Physical harm could result if contact is made with the needle arm, the moving needles or the ejector arm. Keep fingers away from the needle assembly while the instrument is in operation.*

Immediately before a new amino acid starts activation, the old cartridge is discarded into the disposal bin located to the right of bottle 8. This is accomplished by a pressure-driven ejector arm and spring-loaded positive ejector. The ejector arm moves out to slide the used cartridge into the disposal bin. The positive ejector slides up over the edge of the ejector arm and pushes down onto the cartridge to ensure that the cartridge drops out of the ejector arm and into the disposal bin.

The Activator Vessel (ACT)

The ACT is a 30-mL glass cylinder. Gas and chemical flows come from the top and bottom of the ACT. The 4-port valve block through valve 3 controls flow through the upper ACT line, while the 11-port valve block through valve 11 controls flow through the lower ACT line.

The upper ACT line ends in a glass nozzle that points upward to completely wash off the top and the sides of the vessel. A glass frit at the bottom of the vessel prevents any precipitated by-product from entering the valve block. A light behind the ACT makes it easier to visually examine reagent and solvent deliveries and look for precipitation.

During synthesis, the dissolved amino acid is transferred from the cartridge to the ACT where it is converted to its activated derivative. When activation is complete, the activated derivative solution is transferred to the reaction vessel (RV). Any precipitated by-product that has been filtered and left behind in the ACT is dissolved and washed out to waste with a solution of MeOH and DCM.

The Reaction Vessel (RV)

The RV is a Kel-F cylinder with screw-on caps at both ends. The caps' wetted material is Teflon and Kal-Rez. A hole through each cap allows gas and chemicals to enter at the top or bottom of the vessel. Zitex membranes, held in place by the Teflon caps, form a gasket between the cap and the vessel body and keep the resin in the RV. A Kal-Rez O-ring in each cap forms a secondary seal directly behind the Zitex membrane. In-line filters are placed in the upper and lower lines of the RV to protect the valve block in case of resin leaks from the vessel.

There are three sizes of RVs: 0.1 mmol, 0.25 mmol, and 1.0 mmol (See Figure 2-7 on page 2-35).

There are two types of RVs: non-resin sampling and resin-sampling. The resin-sampling RV, used in conjunction with the resin sampler, has a line attached to the vessel body which connects to the bulkhead fitting. This fitting then connects to the resin sampler.

IMPORTANT ***DO NOT** use the resin-sampling RV with the non-resin sampling cycles. Doing so may adversely affect the success of a synthesis. In resin-sampling cycles, the line is rinsed frequently to prevent residual resin, TFA or coupling solution from contaminating any subsequent portion of the synthetic chemistry. These steps do not occur in non-resin sampling cycles.*

Flows through the upper vessel line are controlled by the 4-port valve block through valve 2. Flows through the lower RV line are controlled by the 11-port valve block through valve 10.

The RV is mounted onto the synthesizer by a spring-loaded metal holder. Lift the protruding tab at the top of the holder to place an RV into the holder. The recesses in the RV caps fit into the ferrules that protrude from the top and bottom of the holder. Kal-Rez gaskets located in the cap recesses ensure a leak-proof connection.

The resin support is placed in the RV which is then mounted on the synthesizer. Deliveries of the activated amino acids, reagents and solvents are made to the RV to accomplish deprotection, neutralization, washing, and coupling.

Thorough mixing of the resin beads with reagents and solvents in the RV is essential for efficient coupling and washing. Vortex mixing prevents resin agglomeration and enables the use of small amounts of solvent by distributing the fluid evenly for total fluid-resin interaction.

Assembling the Reaction Vessels

To assemble the reaction vessels:

1. Clean the “knife edge” at both ends of the vessel and the Kal-Rez gaskets in both caps to remove old resin or other contamination.
2. Hold the RV vertically and place a filter into the opening of the vessel, seating it on the interior “knife edge” found just inside the RV opening.

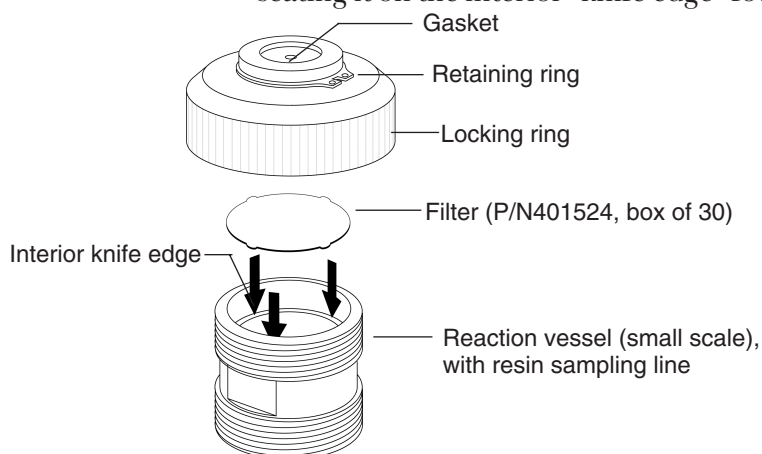


Figure 8-11. Placing a filter on the interior “knife edge” of the reaction vessel

3. Screw the bottom cap over the RV opening with the filter, making sure to hold the RV in a vertical position at all times.

Note *Always hold the RV in a vertical position while tightening the cap.*

Tighten the cap until you feel a firm resistance, which indicates that the primary seal is forming between the filter and the recessed knife edge. The surface of the filter should be flat and smooth, with no protrusions beyond the knife edge.

Caution *Do not use the black, open-ended wrench to tighten the RV caps. Avoid cracking the RV caps by hand-tightening the cap.*

Invert the RV. Look through the RV to check that the filter is flat and smooth, with no edges protruding away from the vessel walls.

4. Invert the reaction vessel to add resin.

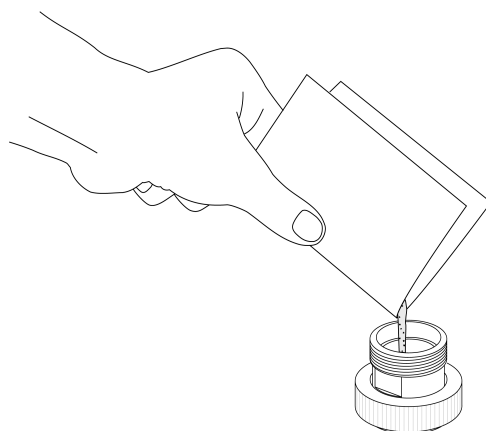


Figure 8-12. Filling the reaction vessel with resin

5. Place a filter into the top opening and seat it flat against the “knife edge.” Hold the RV vertically and finger-tighten the top cap.

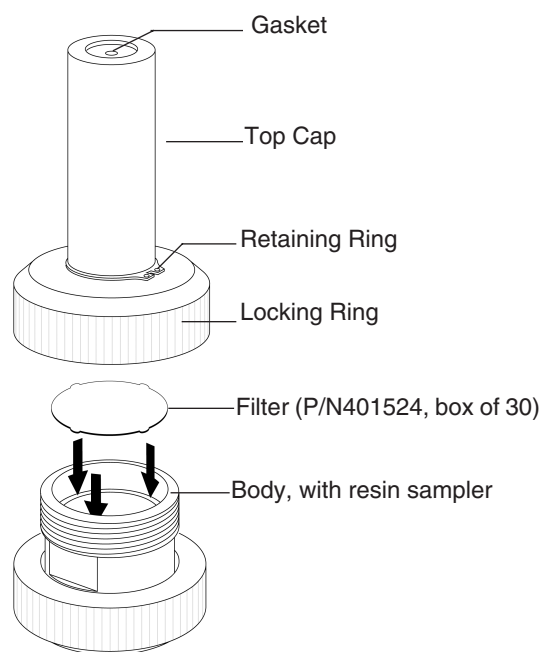


Figure 8-13. Placing a filter in the top of the small scale reaction vessel

Testing for Leaks in the Reaction Vessel

Test the reaction vessel for leaks before synthesis begins. The following section describes two procedures: one that uses the Manual Control menu and a second method that requires careful observation during Flow Test 9 or Flow Test 10.

<i>Note</i>	<i>Wear protective lab coats, gloves and safety glasses when testing reaction vessels for leaks.</i>

To test for leaks from the Manual Control menu:

1. Install the small scale reaction vessel and attach resin-sampling line, if applicable.
2. From the Manual Control menu, activate Fxn 55 until DCM flows into the waste.
3. Activate valves 2, 17, and 23 to pressurize the reaction vessel. Check for leaks around the reaction vessel locking rings (see Figure 8-13) and, if the reaction vessel has a resin-sampling line, check at the bulkhead fitting.
4. Activate Fxn 42 to drain the reaction vessel.

To Test for leaks with Flow Test 9 or Flow Test 10:

1. Begin either flow test. Check for leaks around the reaction vessel locking rings during solvent delivery.
2. If the reaction vessel has a resin sampler, perform a partial Flow Test 19 to check for leaks in the resin sampler system. Start Flow Test 19 and pause when it reaches step 8. Jump to step 17 and continue the flow test to the end. This bypasses the resin sampling steps. Check the resin-sampler line at the bulkhead fitting.
3. If no leaks are found, you may proceed with the synthesis.

The Resin Sampler

The Resin Sampler automatically removes resin samples and delivers them to a fraction collector. The resin sample can then be analyzed for stepwise coupling yields.

The resin sampler consists of a rotary valve with three lines connected to it (see Figure 8-14). The line connected to the 11-port valve block at valve 8 has two in-line filters to prevent resin from entering the valve block. A second line connects to a fraction collector, and the third line connects to the bulkhead fitting which connects to the RV. The rotary valve has two positions. Gas pressure moves the valve back and forth.

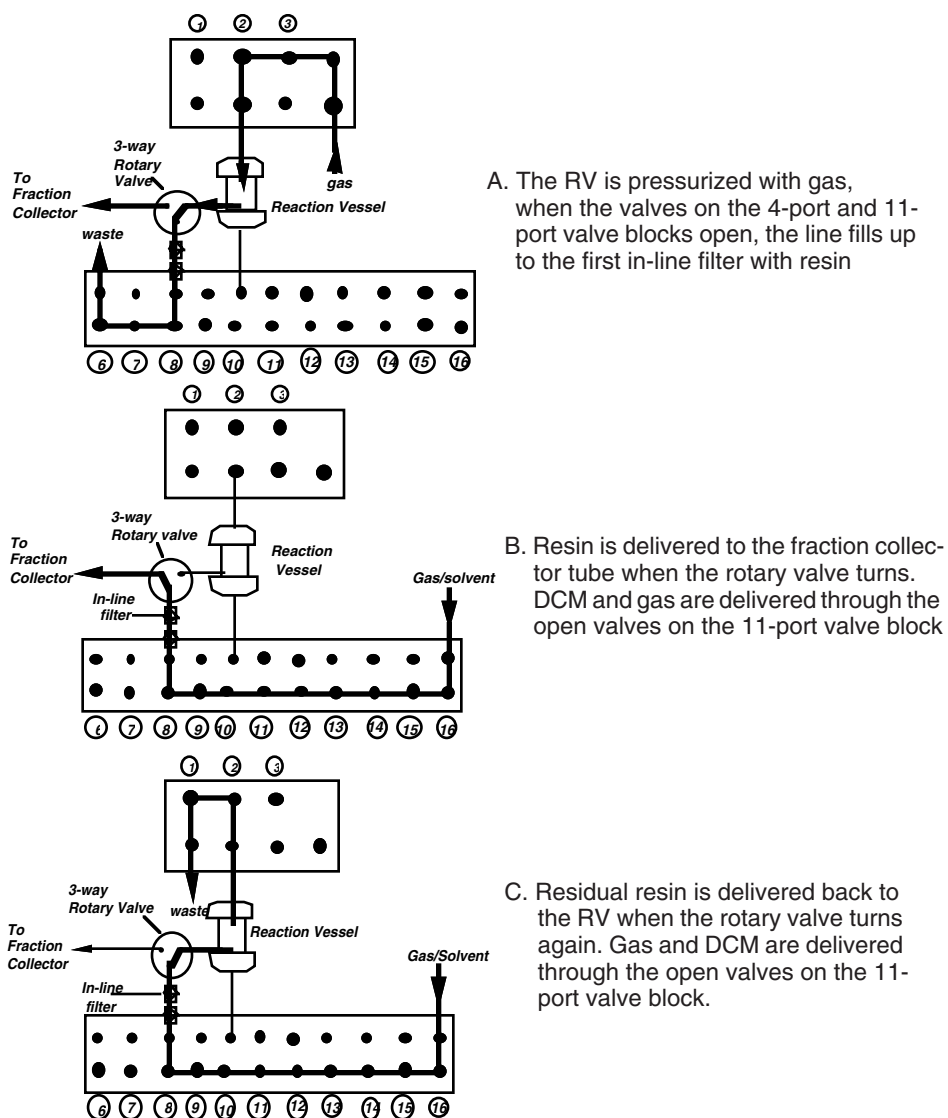


Figure 8-14. The steps of resin sampling

To collect a resin sample, the resin-sampler valve must open to let the resin sample flow from the RV. When the RV is pressurized with gas and valve 8 is open, the resin and solvent slurry flows from the RV, through the rotary valve and up to the first in-line filter (see Figure 8-14 A). Resin remains in the line while solvent flows through to waste. The rotary valve then changes position creating a path to the fraction collector (FC). When gas or solvent delivery goes through the 11-port valve block to valve 8, the resin in both the rotary valve and the line, up to the in-line filter, is delivered to the FC (see Figure 8-14 B). Approximately 2 to 8 mg of resin flows to the FC.

Residual resin in the line between the rotary valve and the RV can be delivered back to the RV when the rotary valve changes position again. When gas or solvent delivery is made through the 11-port valve block and valve 8 is open, resin will flow back to the RV (see Figure 8-14 C). Refer to “The Resin Sampler” on page 8-23.

Caution	<i>POTENTIAL FOR INSTRUMENT DAMAGE. When using a non-resin sampling RV, cover the bulkhead fitting with the sliding cover to protect against accidental delivery of reagents. When using the RV with a resin sampling line, check that the fitting on the line screws <u>easily</u> into the bulkhead to prevent stripping the threads. If the fitting does not screw in easily, back it off and try again.</i>

Conductivity Cell

The cylindrical, flow-through conductivity cell is connected to the chemical delivery system between valve port 10 and the in-line filter at the bottom of the reaction vessel. Contents of reagent bottles may flow through the conductivity cell on the way to the reaction vessel and, in the reverse direction, contents of the reaction vessel may flow from the reaction vessel through the conductivity cell on the way to liquid waste. Constructed of Kel-F with an aluminum thread plate, the flow cell measures approximately 1 x 0.5 in. It contains two gold-plated axial electrodes, that are separated by a Teflon spacer. Conductivity is measured as current flows between the two electrodes and through the fluid in the cell.

Functions

There are 152 functions, each designated by a number and a name (Table 8-2), which control all processes necessary for synthesis. Some functions activate a switch or set of switches to deliver solutions or to perform a specific task. Functions that control deliveries to similar areas of the synthesizer have been grouped together in Figure 8-15. Functions 128 - 148 control conductivity or spectrophotometric monitoring.

Table 8-2. ABI 433A instrument functions

Function #	Function Name	Valves	Description
1	WAIT	————	————
2	VORTEX ON	————	RV mixes
3	VORTEX OFF	————	RV stops mixing
4	SAVE CART	————	Compare cartridge label to list
5	NEEDLE DWN	————	Needle down
6	NEEDLE UP	————	Needle up
7	EJECT CART	————	Eject cartridge
8	ADVAN CART	————	Advance cartridge
9	GAS T VB	1, 17, 23	Gas thru top valve block
10	GAS B VB	6, 16, 17	Gas thru bottom valve block
11	#9 T VB	1, 18, 23	#9 thru top valve block
12	#9 B VB	6, 16, 18	#9 thru bottom valve block
13	#10 T VB	1, 20, 23	#10 thru top valve block
14	#10 B VB	6, 16, 20	#10 thru bottom valve block
15	#1 T VB	1, 21, 23, 33	#1 thru top valve block
16	#1 B VB	6, 16, 21, 33	#1 thru bottom valve block
17	#4 B VB	6, 14, 28	#4 thru bottom valve block
18	#5 B VB	6, 15, 30	#5 thru bottom valve block
19	#6 B VB	6, 16, 19, 30	#6 thru bottom valve block
20	MIX ACT	1, 3, 11, 16, 17	Mix Activator Vessel contents
21	VENT ACT	1, 3	Vent Activator Vessel at top
22	DRAIN ACT	3, 6, 11, 17, 23	Drain Activator Vessel contents
23	ML TO ACT	1, 3, 11, 13, 29	Measuring loop contents to ACT
24	CART TO AC	1, 3, 11, 12, 17, 22	Cartridge contents to ACT
25	#9 T ACT	3, 18, 23	#9 to ACT thru top valve block
26	#10 T ACT	3, 20, 23	#10 to ACT thru top valve block
27	#6 T ACT	3, 19, 23, 30	#6 to ACT thru top valve block
28	GAS T ACT	3, 17, 23	gas to ACT thru top valve block
29	#9 ACT-DRN	3, 6, 11, 18, 23	#9 to ACT thru top while draining
30	#10 A-DRN	3, 6, 11, 20, 23	#10 to ACT thru top while draining
31	#1 B ACT	1, 3, 11, 16, 21, 33	#1 to ACT thru bottom valve block
32	#4 B ACT	1, 3, 11, 14, 28	#4 to ACT thru bottom valve block

Function #	Function Name	Valves	Description
33	#5 B ACT	1, 3, 11, 15, 30	#5 to ACT thru bottom valve block
34	#6 B ACT	1, 3, 11, 16, 19, 30	#6 to ACT thru bottom valve block
35	#9 B ACT	1, 3, 11, 16, 18	#9 to ACT thru bottom valve block
36	#10 B ACT	1, 3, 11, 16, 20	#10 to ACT thru bottom valve block
37	ACT TO RVc	3, 10, 11, 17, 23	Transfer ACT to RV (RV Top closed)
38	ACT TO RVo	1, 2, 10, 11	Transfer ACT to RV (RV Top open)
39	RELAY 0		Momentarily closes Relay 0
40	MIX RV	1, 2, 10, 16, 17	Mix Reaction Vessel
41	VENT RV	1, 2	Vent Reaction Vessel at top
42	DRAIN RV	2, 6, 10, 17, 23	Drain Reaction Vessel
43	ML TO RV	1, 2, 10, 13, 29	Measuring loop contents to RV
44	CART TO RV	1, 2, 10, 12, 17, 22	Cartridge contents to RV
45	#9 T RV	2, 18, 23	#9 to RV thru top valve block
46	#10 T RV	2, 20, 23	#10 to RV thru top valve block
47	#1 T RV	2, 21, 23, 33	#1 to RV thru top valve block
48	GAS T RV	2, 17, 23	Gas to RV thru top valve block
49	#9 RV-DRN	2, 6, 10, 18, 23	#9 to RV thru top while draining
50	#10 RV-DRN	2, 6, 10, 20, 23	#10 to RV thru top while draining
51	#1 B RV	1, 2, 10, 16, 21, 33	#1 to RV thru bottom valve block
52	#4 B RV	1, 2, 10, 14, 28	#4 to RV thru bottom valve block
53	#5 B RV	1, 2, 10, 15, 30	#5 to RV thru bottom valve block
54	#6 B RV	1, 2, 10, 16, 19, 30	#6 to RV thru bottom valve block
55	#9 B RV	1, 2, 10, 16, 18	#9 to RV thru bottom valve block
56	#10 B RV	1, 2, 10, 16, 20	#10 to RV thru bottom valve block
57	RV TO ACTc	2, 10, 11, 17, 23	Transfer RV to ACT (Top ACT closed)
58	INTERRUPT		Temporarily interrupts synthesis
59	RELAY 1		Momentarily closes Relay 1
60	MIX CART	12, 16, 17, 27	Mix amino acid cartridge contents
61	VENT CART	27	Vents the amino acid cartridge
62	DRAIN CART	6, 12, 17, 22	Drain amino acid cartridge contents
63	ML TO CART	12, 13, 27, 29	Measuring loop contents to cartridge
64	#9 CART	12, 16, 18, 27	#9 to amino acid cartridge
65	#10 CART	12, 16, 20, 27	#10 to amino acid cartridge
66	#9 SML N	6, 12, 18, 22	#9 to small cartridge needle
67	#10 SML N	6, 12, 20, 22	#10 to small cartridge needle
68	MEAS #7	6, 13, 30, 31	#7 through measuring loop
69	MEAS #8	6, 13, 30, 32	#8 through measuring loop
70	PURGE ML	6, 13, 29	Loop contents thru bottom valve block
71	#2 B VB	6, 7, 25	#2 thru bottom valve block to waste
72	#2 B RV	1, 2, 7, 10, 25	#2 thru bottom valve block to RV

Function #	Function Name	Valves	Description
73	VENT #2	26	Vent #2 bottle
74	FLUSH #2	7, 16, 17, 26	Flush #2 delivery line contents back to #2
75	GAS-VENT #2	25, 26	Gas thru #2 pressure line to clear vapors
76	PRS #2	25	Pressurize bottle #2
77	PRS #4	28	Pressurize bottle #4
78	PRS #M	30	Pressurize manifold (bottles 5,6,7 and 8)
79	PRS #1	33	Pressurize bottle #1
80	GAS TO AUX	9, 16, 17	Gas to auxiliary waste
81	ACT TO AUX	3, 9, 11, 17, 23	ACT contents to auxiliary waste
82	RV TO AUX	2, 9, 10, 17, 23	RV contents to auxiliary waste
83	CART TO AX	9, 12, 17, 22	Cartridge contents to auxiliary waste
84	#9 TO AUX	9, 16, 18	#9 to auxiliary waste
85	#9 RV-AUX	2, 9, 10, 18, 23	#9 thru top of RV, to auxiliary waste
86	#10 RV-AUX	2, 9, 10, 20, 23	#10 thru top of RV, to auxiliary waste
87	TAKE SAMPL	2, 6, 8, 17, 23	Take a resin sample
88	RS TO RV		Resin sample switch to RV
89	RS TO FC		Resin sample switch to fraction collector
90	#9 TO RS	1, 2, 8, 16, 18	#9 to resin sampler
91	#10 TO RS	1, 2, 8, 16, 20	#10 to resin sampler
92	#1 TO RS	1, 2, 8, 16, 21, 33	#1 to resin sampler
93	GAS TO RS	1, 2, 8, 16, 17	Gas to resin sampler
94	#5 TO CART	12, 15, 27, 30	#5 to amino acid cartridge
95	VENT ML	6, 13	Vent measuring loop
96	CRT TO RVc	10, 12, 17, 22	Cartridge to RV (Top closed)
97	#7 TO MLc	30, 31	#7 to loop (closed at end)
98	BEGIN LOOP		
99	END LOOP		
100	User Fxn A	User Defined	User Defined
101	User Fxn B	User Defined	User Defined
102	User Fxn C	User Defined	User Defined
103	User Fxn D	User Defined	User Defined
104	User Fxn E	User Defined	User Defined
105	User Fxn F	User Defined	User Defined
106	User Fxn G	User Defined	User Defined
107	User Fxn H	User Defined	User Defined
108	User Fxn I	User Defined	User Defined
109	User Fxn J	User Defined	User Defined
110	begin loop		
111	end loop		

Function #	Function Name	Valves	Description
112	—		Not functional
113	TOGGLE OFF		Gas pressure Toggle function off
114	RELAY 0 ON		Relay 0 on
115	RLY 0 OFF		Relay 0 off
116	RELAY 1 ON		Relay 1 on
117	RLY 1 OFF		Relay 1 off
118	#10 TO AUX	9, 16, 20	#10 to aux. waste
119	#9 ACT-AUX	3, 9, 11, 18, 23	#9 thru top of Activator to aux. waste
120	AUX TO ACT	1, 3, 9, 11, 34	Aux. solvent to Activator Vessel
121	AUX TO CRT	9, 12, 27, 34	Aux solvent to cartridge
122	AUX TO RV	1, 2, 9, 10, 34	Aux solvent to RV
123	AUX TO WST	6, 9, 34	Aux solvent to waste
124	#1 TO CART	12, 16, 21, 27, 33	#1 to amino acid cartridge
125	#2 TO CART	7, 12, 25, 27	#2 to amino acid cartridge
126	#4 TO CART	12, 14, 27, 28	#4 to amino acid cartridge
127	#6 TO CART	12, 16, 19, 27, 30	#6 to amino acid cartridge
128	Mon1stPk-X		Set conductivity baseline + algorithm
129	Mon1stPk		Apply spectrophotometric algorithm
130	MonPrevPk		Apply conductivity algorithm
131	Mon Stop		Record last highest data value
132	Save MonPk		Print last highest data value
133	MonBegLoop		Set max. number of loops
134	MonEndLoop		Set T/10% and count loops
135	Mon Reset		Clear and reset to zero
136	SkipModMon		Skip module if conditions not met
137	Do Mod Mon		Perform module if conditions not met
138	Int MaxMon		Interrupt module if conditions not met
139	IntConduct		Channel 1 data collection interrupt
140	Int Chnl 2		Channel 2 data collection interrupt
141	Int Chnl 3		Channel 3 data collection interrupt
142	MonDrain X		Enter limiting conductivity value
143	MonRVWaste		Set maximum RV drain to waste
144	MonRV toAux		Set max. RV drain to aux. waste
145	Test X' > Pk		Set value for comparison to peak
146	Test X' < Pk		Set value for comparison to peak
147	SkipOnTest		Skip module if Test True
148	Do On Test		Perform module if Test True
149	Int OnTest		Interrupt module if Test True
150	MATCH CART		Compare cartridge label to last read
151	TOGL USER		Toggle on any User Function
152	ATTENTION		Sound alarm

Miscellaneous	Solvent and Gas through Valve Block	Activator Vessel	Reaction Vessel
1 WAIT	9 GAS T VB	20 MIX ACT	40 MIX RV
2 VORTEX ON	10 GAS B VB	21 VENT ACT	41 VENT RV
3 VORTEX OFF	11 #9 T VB	22 DRAIN ACT	42 DRAIN RV
4 SAVE CART	12 #9 B VB	23 ML TO ACT	43 ML TO RV
5 NEEDLE DWN	13 #10 T VB	24 CART TO AC	44 CART TO RV
6 NEEDLE UP	14 #10 B VB	25 #9 T ACT	45 #9 T RV
7 EJECT CART	15 #1 T VB	26 #10 T ACT	46 #10 T RV
8 ADVAN CART	16 #1 B VB	27 #6 T ACT	47 #1 T RV
39 RELAY 0	17 #4 B VB	28 GAS T ACT	48 GAS T RV
58 INTERRUPT	18 #5 B VB	29 #9 ACT-DRN	49 #9 RV-DRN
59 RELAY 1	19 #6 B VB	30 #10 A-DRN	50 #10 RV-DRN
98 BEGIN LOOP		31 #1 B ACT	51 #1 B RV
99 END LOOP		32 #4 B ACT	52 #4 B RV
110 begin loop		33 #5 B ACT	53 #5 B RV
111 end loop		34 #6 B ACT	54 #6 B RV
114 RELAY 0 ON		35 #9 B RV	55 #9 B RV
115 RLY 0 OFF		36 #10 B ACT	56 #10 B RV
116 RELAY 1 ON		37 ACT TO RVc	57 RV TO ACTc
117 RLY 1 OFF		38 ACT TO RVo	96 CRT TO RVc
150 MATCH CART			
151 TOGL USER			
152 ATTENTION			

Cartridge	Measuring Loop	Pressure	Monitoring
60 MIX CART	68 MEAS #7	77 PRS #4	128 Mon1stPk-X
61 VENT CART	69 MEAS #8	78 PRS #M	129 Mon1stPk
62 DRAIN CART	70 PURGE ML	79 PRS #1	130 MonPrevPk
63 ML TO CART	95 VENT ML	112 Not functional	131 Mon Stop
64 #9 CART	97 #7 TO MLc	113 TOGGLE OFF	132 Save MonPk
65 #10 CART			133 MonBegLoop
66 #9 SML N	#2	Resin Sampler	134 MonEndLoop
67 #10 SML N	71 #2 B VB	87 TAKE SAMPL	135 Mon Reset
94 #5 TO CART	72 #2 B RV	88 RS TO RV	136 SkipModMon
124 #1 TO CART	73 VENT #2	89 RS TO FC	137 Do Mod Mon
126 #4 TO CART	74 FLUSH #2	90 #9 TO RS	138 Int MaxMon
127 #6 TO CART	75 GAS-VENT #2	91 #10 TO RS	139 IntConduct
	76 PRS #2	92 #1 TO RS	140 Int Chnl 2
	125 #2 TO CART	93 GAS TO RS	141 Int Chnl 3
			142 MonDrain X
			143 MonRVWaste
			144 MonRVtoAux
			145 Test X > Pk
			146 Test X < Pk
			147 SkipOnTest
			148 Do On Test
			149 Int OnTest

Auxiliary Waste	User	Auxiliary Solvent
80 GAS TO AUX	100 USER FXN A	120 AUX TO ACT
81 ACT TO AUX	101 USER FXN B	121 AUX TO CRT
82 RV TO AUX	102 USER FXN C	122 AUX TO RV
83 CART TO AUX	103 USER FXN D	123 AUX TO WST
84 #9 TO AUX	104 USER FXN E	
85 #9 RV-AUX	105 USER FXN F	
86 #10 RV-AUX	106 USER FXN G	
118 #10 TO AUX	107 USER FXN H	
119 #9 ACT-AUX	108 USER FXN I	
	109 USER FXN J	

Figure 8-15. Functions, grouped according to related task

Instrument Function Categories

ABI 433A instrument functions can be divided into the following categories:

- Valve-activated
- Toggle
- Loop
- Relay
- Momentary Interrupt
- Resin Sample
- Barcode-reading
- User
- Monitor

Valve-activated Functions

These functions activate a valve or set of valves to accomplish a delivery. The valves turn on at the beginning of a step and turn off at the end of a step. The majority of the functions are valve-activated functions.

The flow path created by a valve-activated function can be traced using the flow schematic (Figure 8-3). As an example, Figure 8-16 illustrates the flow path of function 31, “#1 B Act.”

With valve 33 open, gas enters and pressurizes bottle 1. When valves 21, 16, 11, 3 and 1 are open, reagent can flow to the ACT. Refer to “Reagent and Solvent Bottles” on page 8-7 and “Valves” on page 8-6 for further delivery explanations.

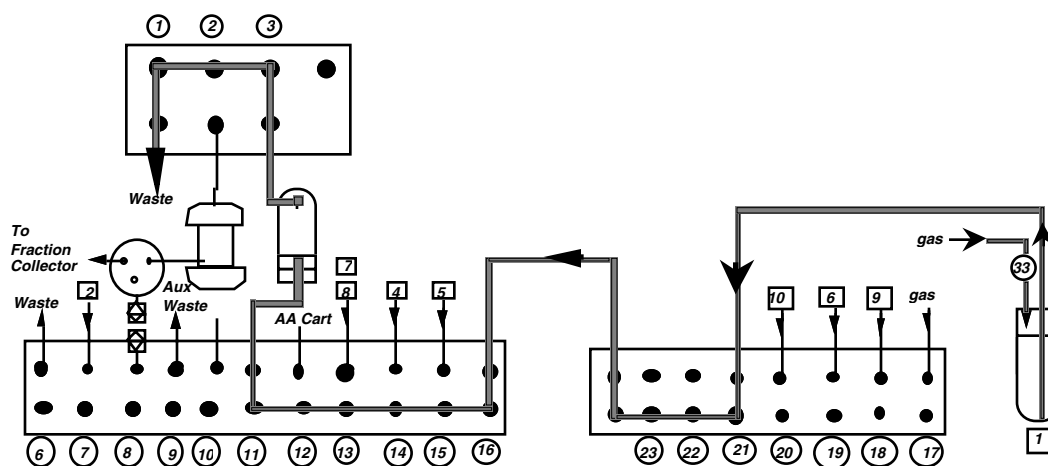


Figure 8-16. Function 31, “#1 B Act”

Toggle Functions

When these functions are activated, they remain “on” until the corresponding “off” function is activated, regardless of other activated functions. For example, when function 2 “Vortex On” is activated, the RV will continue to vortex until function 3, “Vortex Off” occurs.

<i>Note</i>	<i>If you press jmp stp after a ‘toggle on’ function and jump over the corresponding ‘toggle off’ function, the ‘toggle on’ function remains active.</i>

Toggle functions include:

Function 2: Vortex On

Function 3: Vortex Off

Function 5: Needle Down

Function 6: Needle Up

Function 7: Eject Cartridge

Function 8: Advance Cartridge

Function 88: Resin Sample to RV

Function 89: Resin Sample to Fraction Collector

Function 113: TOGGLE OFF

Function 114: RELAY 0 ON

Function 115: RLY 0 OFF

Function 116: RELAY 1 ON

Function 117: RLY 1 OFF

Function 151: TOGL USER

Function 113: TOGGLE OFF

Needle up, needle down, eject cartridge and advance cartridge also have sensors that detect if the operation is complete. When the operation is complete the controller automatically advances to the next step. If the operation is not completed in 10 seconds, the controller puts the synthesis in pause mode.

Function 114 (Relay 0 ON) and Function 116 (Relay 1 ON) turn on (close) the relays 0 and 1. The relay stays closed until Function 115 (RELAY 0 OFF) or Function 117 (RELAY 1 OFF) is reached. If the relay only needs to be turned on for a short pulse, such as for a fraction collector, then use Function 39 (RELAY 0) or Function 59 (RELAY 1).

Insert Function 151, TOGL USER, to turn on any user-defined function that turns on a set of valves or relays. Use Function 113 to turn off all functions.

Loop Functions

Begin Loop (Function 98) and End Loop (Function 99) allow the synthesizer to repeat a series of steps. When the Begin Loop function occurs in a step, the value entered in the time (“T”) field represents the number of times the synthesizer repeats the steps after Begin Loop and before End Loop. When loop functions are operating, the number of loops remaining appears on the LCD as the two digits after the letter L.

The Cycle Monitor Menu shown here reports the controller is on loop 5:

```

S: 12 L5      Fxn 1: WAIT      T: 9/100
hold  |  jmp stp |  pause  |  nxt stp |  more

```

Another set of loop functions—begin loop (Function 110) and end loop (Function 111)—operate like Function 98 and Function 99, but do not have a loop counter. You may place these Functions 110 and 111 inside or outside Functions 98 and 99, so that you have loops within loops (nested loops).

When used to monitor deprotection, Functions 133 and 134—MonBegLoop and End Mon Loop—work together to supervise and regulate the number of times the deprotection steps are repeated.

You can use Add Times with the loop functions to add loops. To determine the added loops per cycle, refer to Table 7-12 on page 7-56.

Momentary Relay Functions

Function 39 (RELAY 0) and Function 59 (RELAY 1) control the corresponding switches on the back of the instrument. When either of these functions is activated, its relay contact momentarily closes. If T=1, the controller sends a 0.1-second pulse to a device connected to the ABI 433A instrument through the relay. When T=5 for one of these functions, the pulse is half a second.

Function 39 is typically connected to a fraction collector. When the function is activated, the fraction collector moves the tubes to the next position.

Do not assign T a value greater than 99. If you want the relay to remain activated for more than two seconds, use the toggle relay functions: 114 and 115, or 116 and 117.

Interrupt Functions

Two functions—Fxn 149, ATTENTION, and Fxn 58, INTERRUPT—direct the controller to temporarily stop the ABI 433A instrument.

Function 149, ATTENTION

When the controller encounters Function 149, ATTENTION, in a module, a short, warbling alarm sounds and the synthesizer waits for the value of “T” in seconds, a very short interruption. Insert this function before a series of steps or action occurs that you want to observe.

Function 58, INTERRUPT

Function 58, INTERRUPT, can be used in two ways: to interrupt synthesis after a powerfailure or to pause a synthesis.

Powerfailure interrupt: When the time is set to 0, Function 58 is a powerfailure interrupt. If a power outage occurs and lasts longer than the time entered in the Powerfail Menu (see page 9-16), when the controller encounters Function 58, the instrument pauses.

For example, assume a Function 58 with T=0 is in the beginning of module a. Also, the Powerfail Menu is set for 30 minutes and an event shuts off the power for 45 minutes. When power returns and the controller encounters the next module a, the synthesizer pauses and sounds a brief alarm. The ABI 433A instrument does not continue synthesis until you press the ***pause** soft key.

If the controller encounters a Function 58 with a value of 0 after a power outage, and the power outage lasts less than the minutes entered in the Power-fail Menu, the instrument will continue to synthesize without interruption.

Synthesis Pause: If Function 58, T= 1, when the controller encounters Function 58, the instrument pauses until you press the ***pause** soft key

displayed on the screen. This operation is not dependent on the Powerfail Menu entry.

Resin Sample Functions

Resin Sample Functions direct the process of delivering resin samples from the RV to a fraction collector. Some of these functions are valve-activated functions and others are toggle functions.

Function 87: Take Sample

Function 88: Resin Sample to RV

Function 89: Resin Sample to Fraction Collector

Function 90: #9 to Resin Sample

Function 91: #10 to Resin Sample

Function 92: #1 to Resin Sample

Function 93: Gas to Resin Sample

Use the resin-sampling RV for resin deliveries. Most modules contain resin sampler functions.

Turn off the resin-sampling functions in the module when you use an RV without resin-sample tubing. To set up the synthesis from the Cycle Monitor Menu, respond 'no' to the prompt "Take Resin Sample?" Refer to "Resin Sampling" on page 2-40.

Barcode-reading Functions

Function 4, SAVE CART, and Function 150, MATCH CART, take barcode readings of cartridges. Function 4 compares the barcode readings to the list of cartridges in the sequence that was created in SynthAssist® Software on the computer. If a cartridge is missing or out of sequence, the controller momentarily interrupts synthesis (the ***pause** soft key becomes active).

Function 150, MATCH CART, compares the barcode reading of the current cartridge to the one that immediately preceded it. If the two cartridge labels do not match, the controller momentarily interrupts synthesis (the ***pause** soft key becomes active). Insert this function after Function 137, Do Mod Mon, to precede functions for a double couple.

User Functions

You can define 10 User functions (100 through 109) by indicating the valves to be activated by that function. Use these functions to accommodate protocols you have designed. Use Function 151, TOGL USER, to toggle on any user-defined function that turns on one or more valves or relays.

Monitoring Functions

Functions 128 through 149 regulate monitoring by setting conditions and checking the monitoring data to see if it meets those conditions.

For most monitoring functions, “T” does not represent time. Instead, T may represent repetitions, monitoring data, percentage, or some another value described in the following paragraphs. With the exception of Function 133, the controller ignores the function when the value of T equals zero.

IMPORTANT *With the exception of Function 133, always assign some value other than zero for T in the monitoring functions. The controller ignores the function when T=0.*

Monitoring functions can be used to monitor conductive species after deprotection and coupling, or to monitor the spectrophotometric absorbance of the fulvene-piperidine adduct during Fmoc deprotection. To facilitate explanation, this section discusses how monitoring functions can be used to monitor Fmoc deprotection. Read Section 5, “Monitoring,” for a thorough explanation of how the monitoring functions are used in the pre-defined Chemistry files.

Function 128, Mon1stPk-X, Function 129, Mon1stPk, and Function 130, MonPrevPk each apply an algorithm to the monitoring data. The algorithm in Function 128 and Function 129 compares the data peak from the **most recent** deprotection to the **first data peak** and calculates the percentage difference between the two peaks. The algorithm for Function 130 compares **the last two data peaks in a series** and calculates the percentage difference between them.

The value of “T” assigned in **Function 134** determines when deprotection stops. The controller ends the deprotection when the value of “T” in Function 134, divided by ten and expressed as a percentage, is equal to or greater than the percentage difference between the peaks designated by the chosen algorithm.

Use **Function 128, Mon1stPk-X**, as an alternative to Function 130 with conductivity monitoring. “T” in this function is the conductivity baseline divided by ten. For example, to assign a conductivity baseline of 1200, the value of T is 120. The conductivity data is baseline-corrected before the algorithm is applied. In each deprotection, the first data peak usually reflects 90 percent of the deprotection activity, with the successive peaks much smaller than the first. When all these peaks are baseline-corrected, sensitivity of the percentage difference calculation increases.

Use **Function 129, Mon1stPk**, to start Channel 2 monitoring and apply the spectrophotometric-monitoring algorithm to the data. With Function 129, the first data point collected acts as the baseline value and is subtracted from

all subsequent data points for that peak. The controller stops collecting data points for a peak when it encounters Function 131. Assign T=2 for this function to collect data from Channel 2.

Use **Function 130, MonPrevPk**, to start conductivity monitoring and apply the conductivity-monitoring algorithm to the data. This function does not apply a data baseline. T=1 to signal the controller to perform this function.

Function 131, Mon Stop, tells the controller to stop examining data points for the current deprotection. It stores as the data peak the largest data value collected after monitoring began. By default, T=1 and does not have any other significance.

Function 132, Save MonPk, reports the data peak for each monitoring loop to the Macintosh and, if you choose Print Events on the Cycle Monitor menu, to the printer attached to the ABI 433A instrument. T=1 to signal the controller to perform this function.

Function 133, MonBegLoop, determines the maximum number of times (“T”) the deprotection loop is repeated. Typically, T=2 is sufficient for most deprotections; however, you may use T=4, 5, or 6 as a precaution against difficult deprotections. If the conditions dictated by the monitoring algorithm are not met at the end of “T” repetitions, deprotection stops anyway in the Basic Monitoring cycles. In the Conditional Monitoring Chemistry cycles, if the conditions dictated by the monitoring algorithm are not met at the end of “T” repetitions, extended deprotection begins. See Section 5, “Monitoring,” for a description of Basic and Conditional Monitoring cycles.

The value of “T” in **Function 134, MonEndLoop**, divided by ten, sets the percentage value used by Function 128, 129, and 130 to determine when to stop the deprotection. For example, if you use Function 129 to monitor deprotection, and give “T” in Function 134 a value of 45, then deprotection stops when the last data peak is equal to or less than 4.5% of the peak that immediately preceded it.

Three data-input channels are available for monitoring. Channel 1 is the internal channel that receives conductivity data. Channel 2 and channel 3 may receive conductivity data, spectrophotometric data, or an alternate source of input. **Function 135, Mon Reset**, clears the data collected and resets its value to zero. “T” represents the number of the input-channel collecting the data—either 1, 2, or 3.

When **Function 136, SkipModMon**, is active, the controller skips all the steps that follow it in the current module. When **Function 137, Do Mod Mon**, is active, the controller performs all the steps that follow it in the current module. When **Function 138, Int MaxMon**, is active, the controller interrupts the current module. Functions 136, 137, and 138 all depend on the relationship between Function 134 and Function 133. The controller looks at the number of times deprotection was actually repeated (Function

134) and compares it to the value of “T” in Function 133. If these values are equal, then it allows Functions 136, 137, and 138 to be active. For all three functions, T represents the number of the input-channel collecting the data—either 1, 2, or 3.

For example, you might put Function 138 at the beginning of a coupling module. Then, if deprotection was repeated 5 times and, in Function 133, T=5, Function 138 becomes active and interrupts coupling.

Functions 139, 140, and 141 all interrupt synthesis when a sampled monitoring data value exceeds or equals ten times “T” ($10 \times T$). **Function 139, IntConduct**, monitors the data received on Channel 1; **Function 140, Int UV**, monitors the data received on Channel 2, **Function 141, Int Chnl 3**, monitors the data received on Channel 3. With all three functions, the monitored data value is not saved.

Function 142, MonDrain X, sets a conductivity value ($10 \times T$) that Function 143 or 144 uses for comparison. Use Monitor Check (page 6-20) to determine conductivity of various solutions and of air on your synthesizer.

Function 143, MonRVWaste, sets the maximum time for the RV drain to waste. The conductivity of the draining solution is typically a four-digit number. Draining continues for T seconds, or until the conductivity is equal to or less than the conductivity value ($10 \times T$) assigned in Function 142.

Function 144, MonRVtoAux, operates like Function 143 to monitor RV drain time to an auxiliary waste.

Function 145, Test $X' > Pk$ and **Function 146, Test $X' < Pk$** , both compare the data peak value saved by Function 132, SaveMon, to X' , where $X' = 10 \times T$. The controller defines Function 145 as True if X' exceeds or equals the peak value. The controller defines Function 146 as True if X' is less than or equals the peak value. When a Function 135, Mon Reset, follows Function 145 or Function 146, the controller defines those two functions as False, regardless of the peak data value.

Functions 147, 148, and 149 become active if the controller has defined either Function 145 or 146 as True. **Functions 147, SkipOnTest**, skips the module steps that follow it, **Function 148, Do On Test**, performs the module steps that follow, and **Function 149, Int OnTest**, interrupts the module.



9 Software Menus

This chapter describes how to use the software menus.

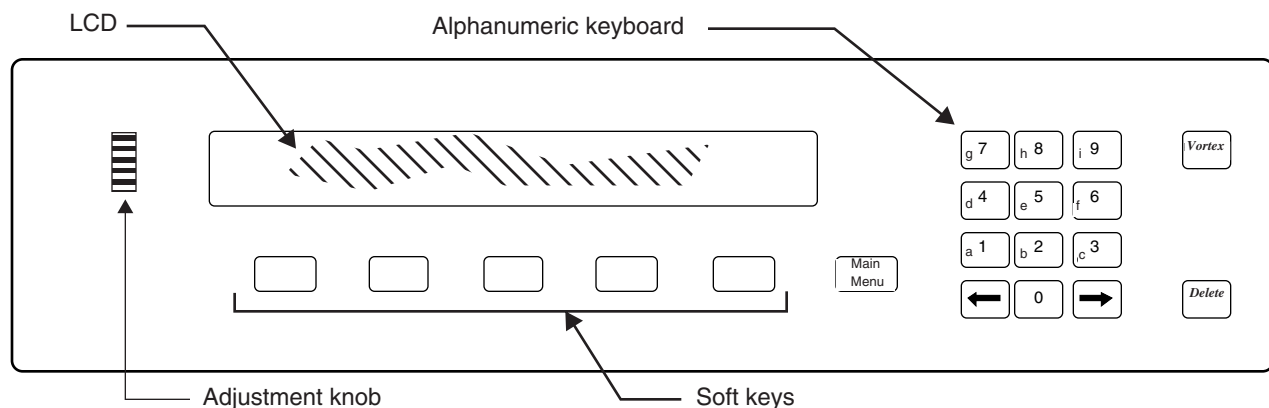
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Hardware Components

The LCD and Keyboard

The LCD (liquid crystal display) shows available operations. Five raised keys under the LCD correspond to selections. To operate the instrument, press the key directly under your choice. The dark knurled knob to the left of the screen adjusts the contrast for optimal viewing.



Use the alpha-numeric keyboard to make numeric and letter entries. The keyboard contains the **vortex** and **delete** keys and the arrow keys (<— and —>) that control the cursor. Repeatedly press any of the keys on the keyboard to select that key continuously.

The Keys

Use the number keys (**0–9**) and the letter keys (**a–i**) to respond to prompts on the screen. The position of the cursor, which appears as a bar (—), indicates where the selected numbers and letters appear on the LCD.

- Use the number keys to indicate quantity.
- Use the letter keys to indicate modules.

—> <— These keys move the cursor across the screen in the direction of the arrows.

Delete Press the **delete** key to erase an entry at the cursor position. When the cursor is not under an entry, the deletion is made to the left of the cursor.

Main Menu This key can be used at any time to return to the Main menu choices.

Vortex This key is like a toggle switch that controls RV mixing. When the RV **is not** mixing, press this key to mix the contents of the RV. Mixing stops when this key is released. When the RV is mixing, press this key to suspend RV mixing. Mixing continues when this key is released.

Menus

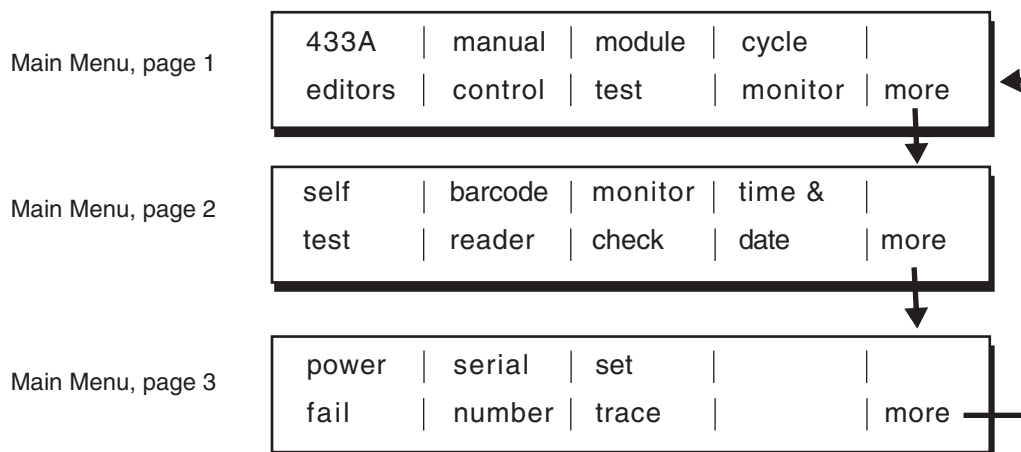
ABI 433A PEPTIDE SYNTHESIZER				
Chemistry: NOT DECLARED!!!			Main Menu ->	

When the ABI 433A Peptide Synthesizer is first turned on, this screen appears.

Press the Main menu key to see the first page of the Main menu.

Main Menu

Main Menu displays the available options. There are three Main Menu 'pages' (see page 2-6). Press **more** from any Main Menu page to view the next page. Any selection you choose from the Main Menu brings up a new 'menu' on the screen. For example, move to the 433A Editors Menu by pressing **433A editors**. From the 433A Editors Menu, press the **main menu** key to return to the Main Menu.



433A Editors Choose this menu to edit the Run Editor, the Module Editor, or user-defined functions.

Manual Control Use this menu to manually control individual valves or functions and facilitate testing or manipulation of fluid flows through the instrument. In addition, the vortexer and the autosampler can be manually controlled to test proper operation. If a synthesis is underway, you must first press the pause soft key before using the Manual Control Menu.

Module Test Use this menu to select and run any module, especially when running Flow Tests or modules that check instrument performance.

Cycle Monitor Start synthesis from this menu. After synthesis is started, you can view the current step of the synthesis including the function number, countdown, and the add time. From this menu, you can terminate a

synthesis, make the synthesizer pause at the current step, use the **set interrupt** key to make the synthesizer pause at some future step, jump to a different step in the cycle, or hold (prolong) a step.

Self Test Use Self Test to verify proper operation of the instrument's electrical and mechanical components.

Barcode Reader Use this menu to calibrate the barcode reader and check the barcode labels on the amino acid cartridges against the peptide sequence in the SynthAssist® Software Run.

Monitor Check Use this menu to check ground, voltage reference, conductivity voltages, channel 2 and channel 3.

Time & Date Set the hour, minute, month, day, and year in this menu.

Powerfail Use this menu to designate the amount of time (1 to 99 minutes) that an interruption in power must last to pause a synthesis. If the duration of a power failure is greater than the time entered in the powerfail menu, synthesis is interrupted at the start of an activation.

Serial number Open this menu to see the instrument's serial number. This number was determined during manufacturing.

Set Trace Use this menu to select the level of data detail to be recorded in the SynthAssist® Software log.

433A Editors Menu

Use the 433A Editors menu to edit runs, modules, and functions that have been transferred from SynthAssist Software to the ABI 433A instrument software cartridge.

433A	manual	module	cycle	
editors	control	test	monitor	more
↓				
	run	module	fxn	
	editor	editor	editor	

Press **run editor** to add or delete modules in the Run. Press **module editor** to add or delete steps in a module. Press **fxn editor** to define User Functions—Functions 100-109.

The Run Editor Menu

The Run Editor menu displays each cycle (C) in the run, how many times the cycle is to be repeated (Rpt), and the modules (M) in each cycle. The Run Editor holds up to 30 cycles.

Number of cycle repetitions		Module sequence in cycle		
Cycle number				
Run Editor menu				
C: 1	Rpt: <u>1</u>	M: a i b c d e f		
next	prev	delete	a...i	insert

Cycle 1 automatically appears first in the Run Editor menu, with the cursor in the “Rpt” (repetitions) field. You may modify the number of times you want the cycle to be repeated or change the order of modules in the cycle.

next: Press **next** to see the next cycle in the run. The cycle number increases to represent the total number of cycles in the run. For example, if cycle 1 repeats 3 times, the next cycle is number 4.

prev: Press **prev** to review or modify the preceding cycle in the run.

delete: Press the **delete** key to erase the character at the cursor position.

a...i: Press this key to toggle between lower-case letters (a through i) and upper-case letters (A through I).

insert /replace: When you press the **insert** soft key, it toggles to display **replace**. Press **insert** to add a module at the cursor position. Press **replace** and the character at the cursor position will be replaced by the new entry.

The Module Editor Menu

The Module Editor menu displays the modules currently available on the ABI 433A instrument software. Use SynthAssist Software to transfer additional modules or change the modules currently available.

Module Editor menu

Select action for:		MOD a (15 steps)		
edit		copy		print prev next

edit: You can edit a module by inserting a step, by deleting a step, or by changing the function, time, or add time of a step.

copy: Press this key to copy all the steps in any module into a module with no steps.

print: Press this key to generate a printed report of the steps in the selected module.

prev: Press this key to display the previous module.

next: Press this key to display the next module letter in alphabetical order.

Copying A Module

You can create a new module by copying and editing an existing module.

Select action for:		MOD a (93 steps)		
edit		copy		print prev next
↓				
Copy MOD a to: MOD <u>A</u>				
done				prev next enter
↓				
MOD A	Stp: 1	Fxn: 11 #9 T VB	T:1 +0	
prev		next		insert delete done

To copy a module:

1. Press the **next** and **prev** keys to display the module letter that corresponds to the module you wish to copy.
2. Press **copy**. The **LCD** display the message “Copy MOD X to MOD ____.”
3. Press the **next** or **prev** keys. Press the new module letter and press **enter**. The LCD displays the first step of the copied module. You can now edit the copied module.

Editing a Module

You can edit a module by inserting a step, by deleting a step, or by changing the function, time or add time of a step.

Select action for: MOD A (93 steps)				
edit	copy	print	prev	next
↓				
MOD A	Stp: 1	Fxn: 11 #9 T VB	T: 1 +0	
prev	next	insert	delete	done
↓				
MOD A	Stp: 2	Fxn: _	T: +	
			cancel	

To edit a module:

1. Press the **next** or **prev** key to display the letter of the module to be edited after the words **Select action for:**.
2. Press **edit**. The LCD now displays step 1 of the module to be edited along with the function (Fxn) number, function name, the step time (T), and the add time (+).
3. Press the **next** or **prev** keys to display the number of the next step you want to edit.

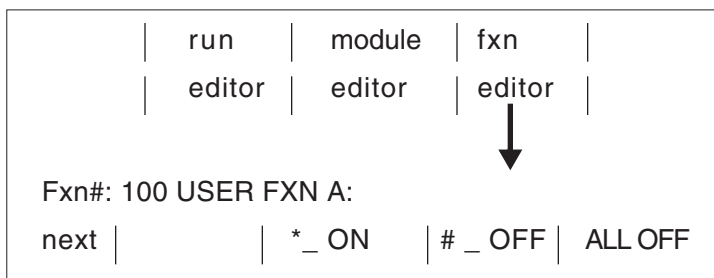
Use the arrow keys to move the cursor to the function number field. Use the alphanumeric keys to select the new function number. To change the time or add time of a step, move the cursor to the time or add time field and enter the new number.

To insert a new step, go to the step number that precedes the new step and press **insert**. You can also use the **insert** key to add a step to the end of the module. The LCD displays the module letter and the step number. With the cursor in the function number field, enter the function number and the function name automatically appears. Move the cursor to the time (T) field and enter the amount of time for this step to occur. Then move the cursor to the add time (+) field and enter the amount of add time for this step. (Refer to *Add Times and Chemical Usage* on page 7-54.)

To delete a step, press the **next** or **prev** keys to go to the step number to be deleted, and then press **delete**.

The Function Editor Menu

The Function Editor menu displays only the User Functions, Functions 100-109. (See page 8-34 for a description of User Functions.) You define a User Function by assigning valves to the Function and designating if the valves should be opened or closed (on or off).



next Use to select another User Function to be defined.

#_ ON/#_ OFF Use these keys to assign the open valves to the user function. One at a time, enter a valve number, then press either **ON** or **OFF**. The valves you designate **ON** appear on the top line of the LCD, after the User Function number. Up to six valves may be turned on simultaneously in the Function Editor menu.

ALL OFF Press this key to turn off all the valves listed in the User Function.

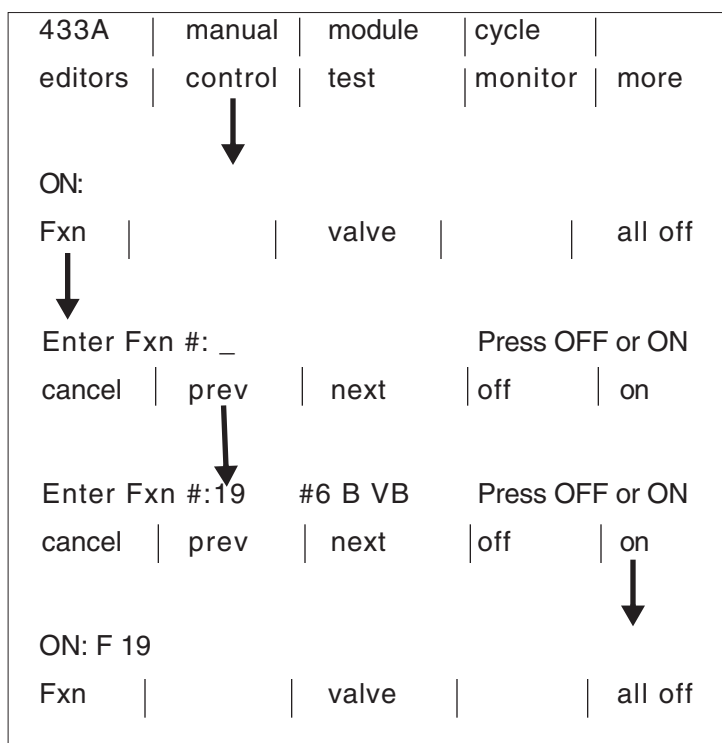
The Manual Control Menu

From this menu, you can manually activate and deactivate functions and valves to deliver chemicals, vortex the reaction vessel, etc. This menu is typically used for troubleshooting purposes. During a run, you must interrupt the synthesizer before you can use the Manual Control menu.

Activate a function

Only one valve-activating function can be activated at a time. A toggle function can simultaneously be activated with a valve-activated function. Refer to *Functions* on page 8-25 for descriptions of each function.

Manual Control menu



To activate a function:

1. Press **Fxn** in the Manual Control menu.
2. Enter the number of the function to be activated.
3. Press **on** to activate the function.

The LCD returns to the Manual Control menu. The activated function appears after the word **ON**.

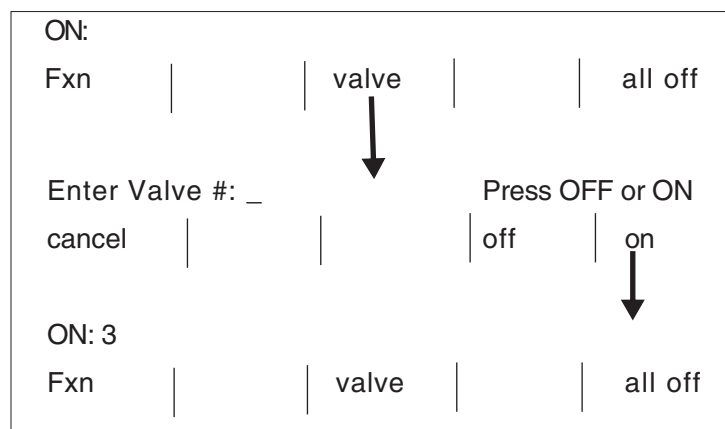
Deactivate a function

There are two ways to deactivate a function:

- Press the **all off** key to close all valves and deactivate the function.
- Press **Fxn**, enter the function number, and then press **off**.

Activate valves

Use this procedure to activate one valve, or as many as nine valves, or one function and numerous valves.



To activate a valve:

1. Press **valve** in the Manual Control menu.
2. Enter the number of the valve to be activated and press **on**.

The LCD returns to the Manual Control menu. The number of the valve to be activated appears after the word **ON**.

Deactivate valves

There are two ways to deactivate valves:

- Press the **all off** key to close all valves.
- Press the **valve** key, enter the valve number, and then press **off** to deactivate a single valve.

Module Test Menu

Use the Module Test menu to activate individual modules, such as those used for flow tests. See *Load Flow Tests from SynthAssist Software* on page 2-20 for a complete description of how to run flow tests. See Chapter 6 for a detailed description of Flow Test steps. Use Flow Tests to:

- Adjust the gas regulators
- Check for proper chemical flows
- Flush chemicals through the lines
- Troubleshoot the instrument

Note You cannot interrupt a synthesis to perform a flow test.

Cycle Monitor Menu

Use the Cycle Monitor menu to start and monitor synthesis. During synthesis you can control synthesis operations by interrupting or ending synthesis, holding a step, and jumping to the next step or to a different step.

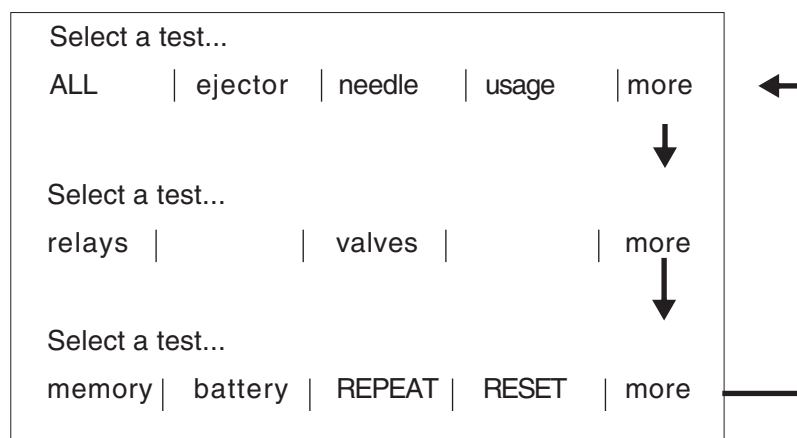
See *Start Synthesis in Cycle Monitor Menu* on page 2-40 for an explanation of how to use the Cycle Monitor menu to begin a synthesis.

See *Monitoring and Controlling Synthesis Operations* on page 2-42 for a description of how to use the Cycle Monitor menu to interrupt or end a synthesis, hold a step or jump to another step in the run.

Self Test Menu

The Self Test menu key appears on page two of the Main menu. Self Test options include: ALL, ejector, needle, relays, valves, memory, and battery. When you select **ALL**, the instrument automatically performs the tests for ejector, needle, relays, values, memory, and battery. You may run any one of the Self Tests separately. Usage is not a self test, but a record of the number of cartridges used during instrument operation.

Note **ALL** does not check the monitoring voltages.



ALL When you press this key, testing begins and the screen displays the message “**All tests in progress.**” When testing is complete and all tests pass, the screen displays the self test menu.

If one or more tests failed, a message displays a number that corresponds to the failed component with the highest number and other necessary information. For example, if both valve 32 and valve 14 failed, the message “**VALVES test failed: Valves 32**” is displayed. The failed message remains on display until you press **continue** and the Self Test menu appears. For troubleshooting purposes, keep a record of component failures.

To select a single Self Test, press the **more** key until the desired option appears on the screen, then select it. Testing begins and the screen displays a message to indicate testing is in progress.

ejector: When this test is in progress, the ejector arm moves ‘out.’ After a 2-second delay, the ejector moves ‘in.’ Sensors check each movement. The ejector test fails if the sensors do not detect the ejector in the proper position within 10 seconds.

needle: When this test is in progress, the needle moves to the ‘down’ position and then pulls ‘up.’ Sensors check each position.

usage: Press this key and the LCD displays the number of cartridges that have been used in the lifetime of the instrument. This number does not include barcode calibration readings. It is derived by counting the times the needle down function is activated during either a synthesis or a module test.

relays: This test checks the closure of the two relays. After both relays close they are checked to ensure that they were driven completely closed. Then the relays open. This test cannot check whether the relay mechanism is working properly.

valves: Press this key to test all valves for proper operation.

memory: Checks all of RAM (random access memory), and each of the four ROM (read only memory) chips.

battery: Checks for sufficient power in the back-up battery.

REPEAT: When you select the **REPEAT** key, all Self Tests are performed continuously, until you select the **cancel** key.

RESET: Press this key to restart the ABI 433A instrument.

<i>Note</i>	<i>When you use the reset button to restart the instrument, all of the modules in the Run file and all user-defined functions are erased.</i>

With the SynthAssist Software, you can store all user-programmable functions and modules permanently on the computer.

When you select the **reset** button, the question “**Are you sure you wish to reset?**” appears on the screen and a beep sounds. If you press **yes**, the screen becomes blank while the software is reloaded. Then, the power-up menu is displayed along with a prompt to select **main menu**.

Press the Main menu key to return to the Main menu.

The Barcode Reader Menu

self	barcode	monitor	time &	
test	reader	check	date	more
↓				
Interrupt when barcode incorrect:				YES
calib				YES/NO

Barcode Interruption Option

The barcode reader detects 5 bands on the cartridge (the barcode) and determines whether they are black or white. In the Barcode menu, you can direct the controller to check the barcodes against the peptide sequence you have entered in SynthAssist Software for any synthesis. Press the **YES/NO** key to choose your response to the Barcode menu statement “Interrupt when barcode incorrect:.” If you choose Yes, the ABI 433A instrument controller interrupts synthesis if the barcode reader detects a cartridge label that doesn’t match the run’s peptide sequence.

Barcode Calibration

Calibrate the barcode reader before your first synthesis. Barcode calibration standardizes the channel readings so that all channels accurately detect the black and white bands on the amino acid cartridge labels. See page 6-18 for the barcode calibration procedure. See page 8-17 for an illustration of the barcode label system for amino acids.

Monitor Check Menu

Use this menu to check the voltages associated with conductivity and spectrophotometric monitoring, and ground.

A/D Reading (Conduct.): 970

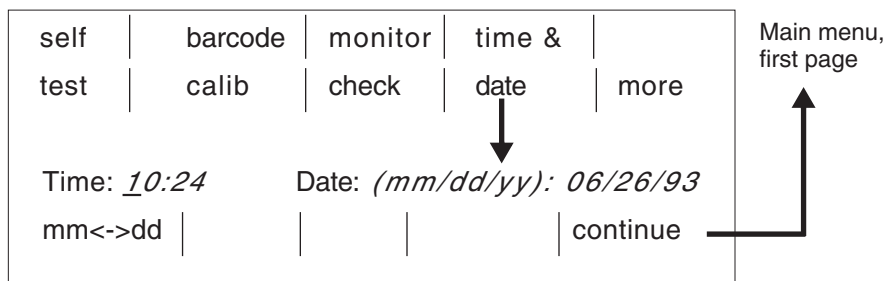
Conduct | Chnl 2 | Chnl 3 | V Ref | Ground

Press the monitoring options that are appropriate for your particular system. The value after the words **A/D Reading** on the top line of the LCD changes as you select options. Table 9-1 lists typical voltages for each option. Press Main menu to return to the Main menu.

Table 9-1. Table of Typical Monitoring Values

Menu Selection	Value
Cond	950- 1100
Chnl 2	dependent on input
Chnl 3	dependent on input
VRef	16328
Ground	0

Time & Date Menu



Time is displayed as four numbers, using the 24-hour clock. The date is displayed as 6 digits which may represent month/day/year (mm/dd/yy) or day/month/year (dd/mm/yy). Press the **mm<->dd** key to toggle between the two date formats.

To set time and date:

1. Press the **time & date** soft key.
The Time and Date Menu appears. The cursor appears under the first digit of the time. If no time has been previously entered, the cursor appears on the first empty entry field.
2. To set the time, move the cursor to the first digit of the Time entry field. Enter a number from the keyboard.
The cursor moves when a number is selected.
3. Enter the remaining digits for the time.
4. To set the date, use the arrow key to move the cursor to the date entry field.
5. Press the **mm<->dd** soft key to switch the order of the numbers in the date. The date may be displayed as *mm/dd/yy* or *dd/mm/yy*.
6. Enter the date from the keyboard.
7. Select **continue** to return to the Main menu.

Powerfail Menu

The Powerfail Menu is used with Function 58, the interrupt function, when Function 58 has a time of zero (0). Fxn 58, time = 0, appears at Step 2 of the first module in a cycle, usually module “B” or module “a.”

You may enter any number from 1 to 99 in the Powerfail Menu. This value represents minutes. When a powerfail occurs, if it lasts longer than the time entered in the Powerfail menu, the ABI 433A instrument pauses when the controller reads Function 58, Time = 0. If the value of the Powerfail Menu is zero when a powerfail occurs, the instrument does not pause when the controller reads Function 58, Time = 0.

Maximum powerfail minutes: _ _	done

After a Powerfail that lasts longer than the time entered in the Powerfail Menu, you may decide to discontinue synthesis when the extended exposure to reagents may compromise the quality of the final product.

Serial number

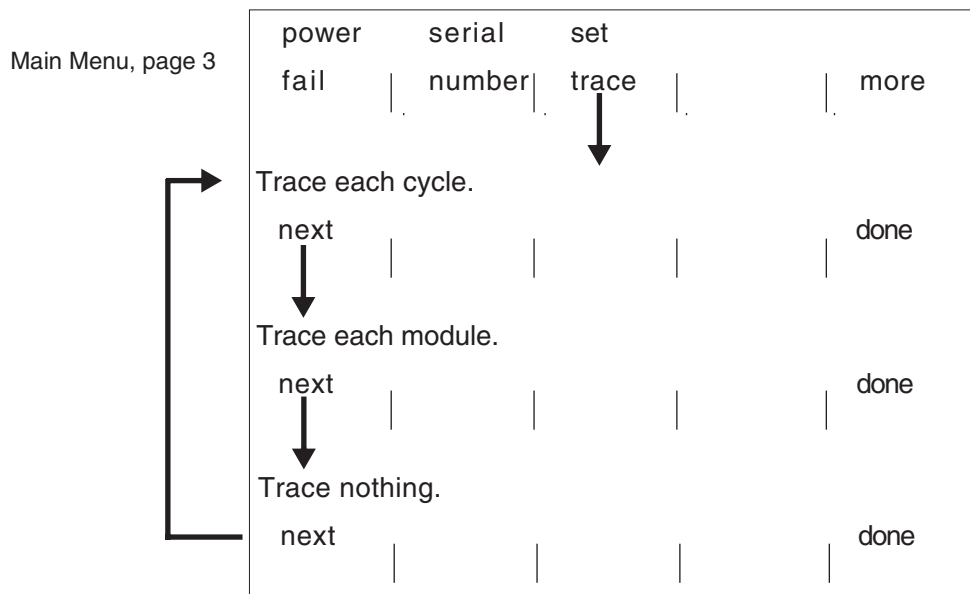
The ABI 433A instrument serial number appears on this display. The first four digits represent the year and the month the instrument was manufactured. This serial number was entered during manufacturing and cannot be edited.

Instrument serial number:	done

Set Activity Trace Menu

Choose from three levels of detail for the synthesis log:

- **Trace each module** includes peak and amino acid data, along with a complete listing of all modules in each cycle (maximum detail).
- **Trace each cycle** includes peak, amino acid, and first module data for each cycle (moderate detail).
- **Trace nothing** includes only peak and amino acid data (minimum detail).



To select an activity trace option:

1. Press the **Main Menu** key to return to the Main Menu.
2. Select **set trace** from the 3rd page of the Main Menu.
3. Press the **next** key to cycle among the three available trace activity options.
4. Press **done** when the trace activity option you require displays on the LCD.



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