ABI™ 433A Peptide Synthesizer and Series 200 UV Detector

UV Monitoring Guide

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

ABI 433a Peptide Synthisizer products are covered by U.S. Patents 5,273,715, 5,186,898 and 4,816,513.

TRADEMARKS:

Part Number 4352299 Rev. A
07/2004

Contents

Preface

Safety and EMC Compliance Information

Chapter 1 Introduction

Chapter 2 Setting Up the System for UV Monitoring

Appendix B UV Chemistry Folders and Files

Appendix C Standard Scale S200 UV Detector Modules

Appendix D Millimole-Scale Chemistries

Appendix E S200 UV Detector Flow Connections

Preface

How to Use This Guide

Purpose of This Guide

The *ABI™ 433A Peptide Synthesizer and Series 200 UV Detector UV Monitoring Guide* provides information on setting up and using the 433A Peptide Synthesizer with the S200 UV Detector for the UV monitoring of Fmoc deprotection.

Audience

This guide is intended for novice and experienced ABI 433A Peptide Synthesizer users who want to use UV monitoring with the Series 200 UV Detector.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page x.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- ***ABI 433A Peptide Synthesizer User Guide*** – Describes the ABI 433A Peptide Synthesizer hardware and software and provides information on preparing, maintaining, and troubleshooting the system.

- ***ABI 433A Peptide Synthesizer Site Preparation and Safety Guide*** – Provides instrument specifications and site requirements for the installation of the ABI 433A Peptide Synthesizer.

Using Documents on Demand

To download a PDF version of this document:

1. Go to **www.appliedbiosystems.com**.
2. Click **Support**.
3. Scroll to the bottom of the page and click **Product and Service Literature**.

Note: For additional documentation, see "How to Obtain Support."

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

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.

Safety and EMC Compliance Information

This section includes the following topics:

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! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– 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.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– 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.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems instruments* (see "Safety Symbols" on page xi).

Examples

The following examples show the use of safety alert words:

IMPORTANT! You must create a separate a Sample Entry Spreadsheet for each 96-well plate.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

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.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

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 *s*ymbols 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 xii). 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.
	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.

Symbol	Description
	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.

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangeureux. 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 régulation 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.
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.
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.

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 xiv.

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.

Cleaning or Decontaminating the Instrument

CAUTION Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

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 "About MSDSs" on page xiv.)
- 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 **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

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

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.

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

Fuses

ELECTRICAL SHOCK HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

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

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

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

Overvoltage Rating

The Applied Biosystems Series 200 UV Detector system has an installation (overvoltage) category of II, and is classified as portable equipment

Physical Hazard Safety

Ultraviolet Light

ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

ULTRAVIOLET LIGHT HAZARD. The deuterium lamp emits ultraviolet radiation. Exposure to ultraviolet radiation can cause permanent damage to the eyes, including blindness. The door that covers the flow cell prevents the emission of ultraviolet radiation. Do not open this door while the detector and UV lamp are powered on.

Moving Parts

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.

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.

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:

- 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 **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. 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.

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

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 standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

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."

Introduction 1

This chapter covers:

About Monitoring Fmoc Deprotections

The Series 200 UV Detector has been used for many years on the Applied Biosystems 49X Procise® Sequencers and has established its sensitivity and reliability. Use of the S200 UV Detector with the ABI™ 433A Peptide Synthesizer is a major advancement in the monitoring of Fmoc deprotections during SPPS.

Connecting the System

The S200 detector has two connections to the 433A synthesizer:

- Cable connecting the REC 10 mV (+, -) output of the S200 detector to the Channel 2 input (+, -) of the 433A synthesizer
- Teflon tubes connecting valve 9 of the 433A synthersizer to the flowcell of the S200 detector, then back to the Aux A port of the 433A synthesizer

The S200 detector has its own 110 V power cord.

How the System Monitors

During deprotection cycles, the S200 UV Detector monitors the pip-fulvene byproduct at a wavelength of 301 nm.

Sensitivity of the System

UV monitoring at 301 nm has a higher signal-to-background ratio than conductivity monitoring.

	UV	Conductivity
Signal-to-Background Ratio	60 to 1 or higher	8 to 1

Advantages of UV Monitoring

At 301 nm, UV monitoring is not sensitive to impurities that generate excessively high conductive backgrounds. Conductive impurities undercut the decision-making of the 433A synthesizer software and lead to an excessive consumption of reagents.

The sensitivity of UV monitoring provides a clearer distinction between residues that are easy versus difficult to deprotect. There is a clearer start/stop signal to the conditional deprotections and extended couplings on the 433A synthesizer. The ability of UV monitoring to make this distinction provides:

- More structural information about the amino acid sequence
- A more efficient use of reagents
- An improved peptide product

In particular, the synthesis of long-mer sequences is most effectively carried out on the 433A instrument with UV monitoring.

Absorbance of the Piperidine-Fulvene Adduct

Piperidine is involved in two steps that are important to UV monitoring:

- The initial removal of the Fmoc group
- The subsequent formation of the Pip-fulvene adduct

The Series 200 UV Detector monitors at 301 nm where the adduct has a unique absorbance. See Figure 1-1 for an outline of the reactions that take place during the removal of the Fmoc-group from an N-terminal amino acid by piperidine.

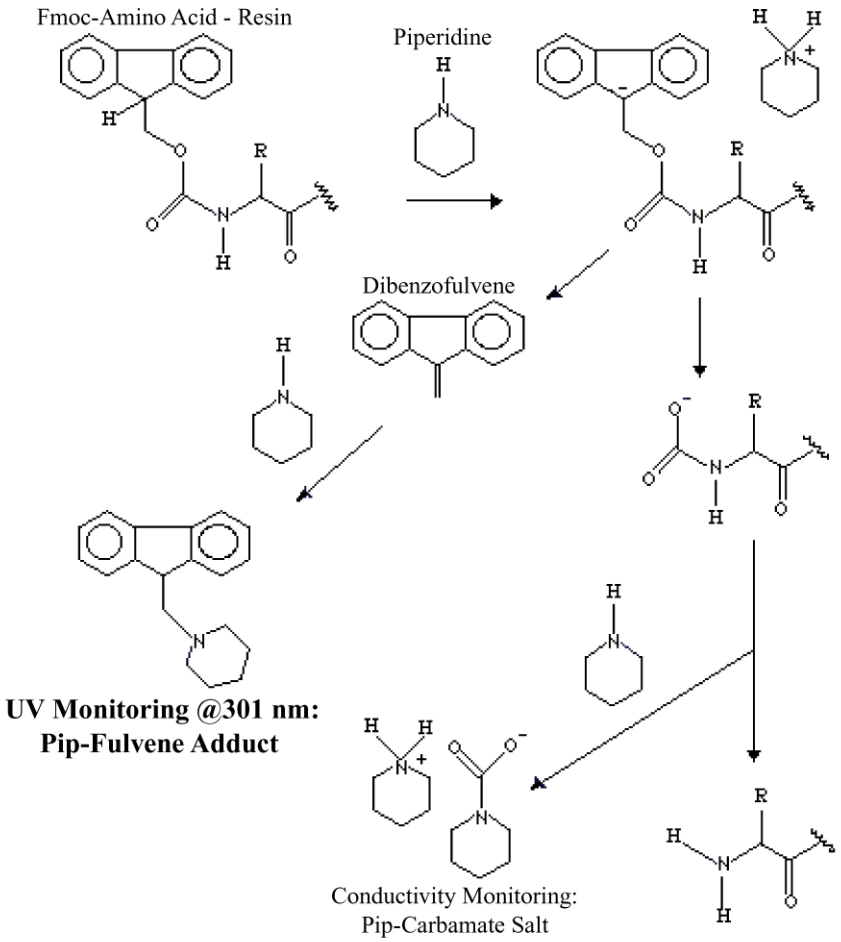


Figure 1-1 The reaction of piperidine and dibenzofulvene generates the Pip-Fulvene Adduct.

About the UV Sample

Piperidine and NMP are delivered to the 433A synthesizer Reaction Vessel to provide a solution of approximately 20% piperidine.

After a 2-minute cycle of deprotection in Module B, a sample of the Reaction Vessel liquid is drained through the lower valve block (VB) to waste, filling the VB between port 9 (UV) and port 10 (RV) with approximately 13 µL of the Fmoc solution. The sample of the Fmoc solution (the UV plug) is then directed to the flow cell of the S200 UV detector. When conditional (overtime) deprotections develop in Module b, the UV plug is withdrawn from the Reaction Vessel after each 10-minute cycle. See Figure 1-2 for the location of the Reaction Vessel sample (color).

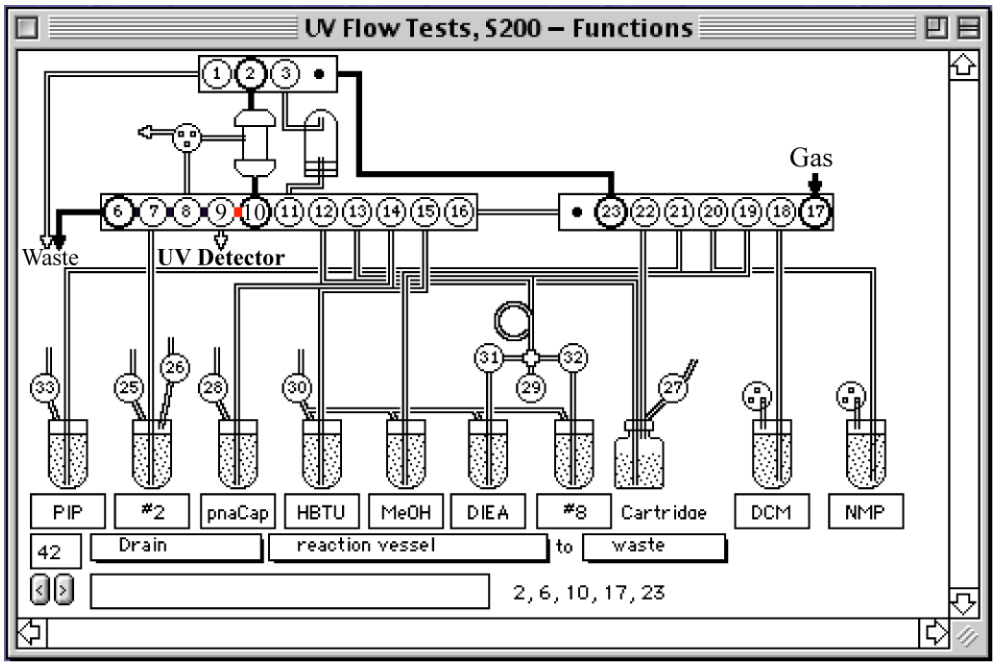


Figure 1-2 The small volume of Fmoc-solution that is directed to the UV detector is shown in red.

Detection of the UV Plug

When the NMP flow to the detector starts, there is a 5-second delay (Wait 1) . During Wait 1 you should see a stable, low-absorbing UV baseline. UV monitoring begins with the activation of Function 129, which zeros the data register. During the next 25 seconds (Wait 2) the data register accumulates a single value for the maximum of the 301-nm absorbance. When Function 131 is activated, data entry is terminated. See Figure 1-3 for an outline of the 433A synthesizer monitoring process for the UV plug as it passes through the detector.

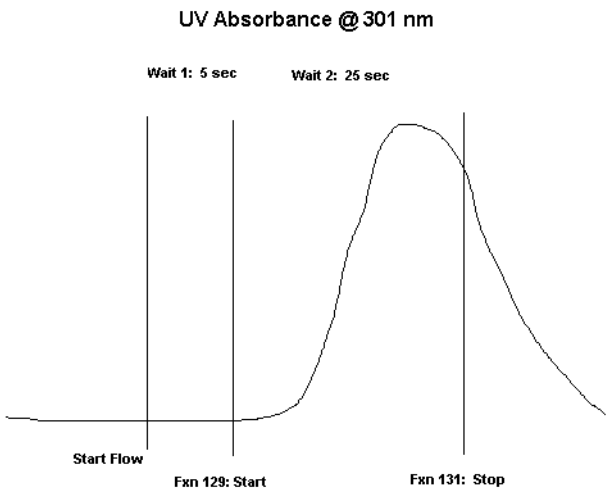


Figure 1-3 The steps of the monitoring process at 301 nm for the UV plug as it passes through the S200 flow cell.

The numerical value for a UV peak is the difference between the maximum recorded during the 25 seconds and the baseline zero at Function 129. The numerical value of the UV peaks that are displayed on a monitor trace or printed in the Log represent this difference measurement.

For more information on Wait 1 and Wait 2, see Chapter 3, "Using the ABI 433A Peptide Synthesizer with the S200 UV Detector." For Troubleshooting information on Wait 1 and Wait 2, see Chapter 4, "Troubleshooting."

Setting Up the System for UV Monitoring

2

This chapter covers:

Setting Up the ABI 433A Synthesizer for UV Monitoring

NMP Delivery

UV monitoring depends on the proper flow of NMP to the 433A instrument. The NMP flow, in turn, pushes the UV plug to the S200 detector.

To set the NMP delivery to the 433A instrument , adjust nitrogen pressure (using the lower regulator on the 433A instrument) to give the expected deliveries for Modules A and B (Flow Tests 1-18 in the SynthAssist® Software — see the *ABI 433A Peptide Synthesizer User Guide*, PN 904855).

IMPORTANT! Once you have established the proper NMP flow, do not change the nitrogen pressure.

Note: Always start the flow tests with full bottles of NMP.

Capping Solution at Bottle 4

The UV chemistry files run in the conditional mode, meaning that a bottle of capping solution must be installed at Bottle 4 during synthesis runs.

There are two capping solutions recommended for position 4:

- PNA Capping Solution (PN GEN 063102)

  or
- Capping cocktail (see page 7-14 of the *ABI 433A Peptide Synthesizer User Guide*, Vol. 1).

Choose the solution that is most convenient for you to use.

Priming Bottle 4

Before starting a synthesis run, prime Bottle 4.

To prime Bottle 4:

1.	Using Manual Control on the 433A instrument, run Function 77 for 10 seconds.
2.	Run the following for 2 to 3 seconds each: • Function 17 • Function 14 • Function 10

Methanol at Bottle 6

The UV chemistry files use methanol to clear bubbles in the flow path and to auto-zero the S200 detector.

Priming Bottle 6

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.

Always keep fresh methanol in Bottle 6. Discard residual methanol and install fresh methanol on a regular basis, or at least once a month.

To prime Bottle 6:

1.	Using Manual Control on the 433A instrument, run Function 78 for 10 seconds.
2.	Run the following for 2 to 3 seconds each: • Function 19 • Function 14 • Function 10

Setting up the Series 200 UV Detector

UV Flow Tests

Send the UV Flow Tests to the 433A instrument.

Zeroing the S200 Detector

Zero the S200 detector using Module **e** two or three times to be certain that all bubbles are cleared from the cell and that the zero is stable.

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.

Note: Zeroing with methanol gives the NMP a slightly positive reading of 0.05-0.10 A (see "Manual Autozeroing of the S200 UV Detector" on page A-2).

To zero the S200 detector:

1.	Using Module Test at the 433A screen, run Module **e** (methanol, NMP to Aux).
2.	On the 433A instrument screen, watch for Step 7, the 60-second delivery of methanol.
3.	When there are 5 to 10 seconds remaining in Step 7, press the **Auto-Zero** key on the S200 detector.

Note: On occasion the NMP absorbance may fall below 0.0000 A after the S200 detector has been set to Auto-Zero due to UV-absorbing contaminants in the methanol. Use a fresh bottle of dry methanol to resolve this problem.

IMPORTANT! If UV-free methanol is not available, autozero the S200 detector twice, first during methanol flow and then during NMP flow.

S200 Detector Settings

The UV detector has been configured at installation with the settings given in Table 2-1. Note that the absorbance range increases for syntheses at the 1.0-mM scale, where the Fmoc-concentration is higher than at the other scales.

Table 2-1 UV detector settings

	0.10 and 0.25 mM Scales	1.0 mM Scale
Absorbance Range	0.025 A	0.030 A
Wavelength	301 nm	
Rise Time	2 seconds	

Measuring the NMP Flow

Applied Biosystems recommends checking the NMP flow through the UV lines when new bottles of NMP or a new tank of nitrogen are installed, or if any flow problems develop. The flow through the UV detector should be 0.80 to 0.90 mL per minute.

To measure the NMP flow:

1.	Send the UV Flow Tests to the 433A instrument.
2.	Run Function 101 (methanol to Aux) for 60 seconds.
3.	Run Function 118 for 60 seconds to fill the UV lines with NMP.
4.	Disconnect the UV line at the AUX Waste port (see Figure E-1) and place the fitting into an open (decapped), tared cartridge.
5.	Run Module B (NMP 5 min) and weigh the cartridge. An expected value is 4.3 g. **Note:** Alternately, place the fitting into a 10-mL graduated cylinder, then run module B to deliver NMP for 5 minutes.

Zeroing the S200 Detector

Zero the S200 detector using Module **e** two or three times to be certain that all bubbles are cleared from the cell and that the zero is stable.

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.

Note: Zeroing with methanol gives the NMP a slightly positive reading of 0.05-0.10 A.

To zero the S200 detector:

1.	Using Module Test on the 433A instrument, run Module **e** (methanol, NMP to Aux).
2.	On the 433A instrument screen, watch for Step 7, the 60-second delivery of methanol.
3.	When there are 5 to 10 seconds remaining in Step 7, press the **Auto-Zero** key on the S200 detector.

Note: On occasion the NMP absorbance may fall below 0.0000 A after the S200 detector has been set to Auto-Zero due to UV-absorbing contaminants in the methanol. Use a fresh bottle of dry methanol to resolve this problem.

IMPORTANT! If UV-free methanol is not available, autozero the S200 detector twice, first during methanol flow and then during NMP flow.

Using the ABI 433A Peptide Synthesizer with the S200 UV Detector

3

This chapter covers:

The UV Flow Tests

The two UV Flow tests discussed in this section are:

- UV Fmoc-Phe
- UV Fmoc-Resin

Before running the tests, the ABI™ 433A Peptide Synthesizer must be set to UV monitoring and the Series 200 UV Detector must be zeroed. Send the UV Flow Tests to the 433A instrument.

Setting the Instrument to UV Monitoring

To set the 433A instrument to UV monitoring:

1.	Using the Module Test on the 433A instrument screen, run Module **g**. **Note:** Module g switches the 433A instrument to UV monitoring (Channel 2).
2.	Using Manual Control on the 433A instrument screen, run the following for 60 seconds each: a. **Function 101** (methanol to Aux) b. **Function 118** (NMP to Aux)

About the UV Fmoc-Phe Test

The Fmoc-Phe Test is a cycle (UV Fmoc-Phe Test) in the UV Flow Test Chemistry file. The Fmoc-Phe Test is a timing test to verify the proper flow of NMP through the UV lines. Expect to see 10 (tall) peaks in the 5000 to 7000 range. The peaks need not be identical but they must be tall and roughly equal in value.
See Figure 3-1.

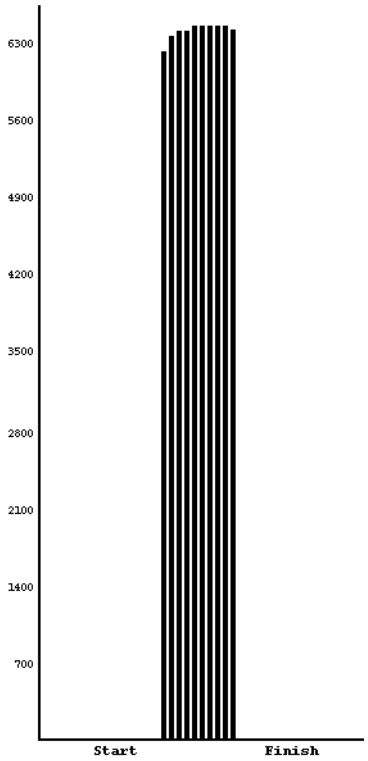


Figure 3-1 The UV Fmoc-Phe Test determines whether the NMP flow to the S200 is consistent and whether the timing (Wait 1 and Wait 2) is appropriate to capture the UV peak maximum.

Watch the Absorbance display on the S200 detector while simultaneously watching the time countdown on the 433A instrument screen for Wait steps 18 and 20.

- **Step 18**. During the 5-second Wait the baseline absorbance should be a low, stable value.
- **Step 20**. During the 25-second Wait there should be no absorbance change for 4 to 5 seconds. The absorbance should then rise rapidly to a maximum and start to decrease by the end of the 25 seconds. The UV absorbance need not fall to baseline during the 25 seconds.

Note: You must incorporate any changes to Wait 1 or Wait 2 in the UV Flow Test chemistry (Module H) into the deprotection modules B and b in the UV Chemistry files. See Table 3-1 on page 3-3.

Table 3-1 Wait steps in module H

Step	Function		Time
16	42	Drain RV	3
17	151	Toggle user Function on	108
18	**1**	**Wait**	**5**
19	129	Monitor first peak	1

Table 3-1 Wait steps in module H

Step	Function		Time
20	**1**	**Wait**	**25**
21	131	Monitor stop	1

Running the UV Fmoc-Phe Test

The Fmoc-Phe Test determines whether the flow of the UV plug to the S200 cell is consistently within specifications. If the NMP flow to the UV cell is not within the expected range or is inconsistent, the peak heights vary significantly.

Piperidine at Bottle 1 and NMP at Bottle 10 are required for this test.

To run the UV Fmoc-Phe test:

1.	Weigh out 100 mg of Fmoc-Phe-OH (PN 400645) into the 40 mL Reaction Vessel.
2.	Set up and start a new run using the UV Flow Tests as the chemistry. • Sequence — **none** • Cycle — **UV Fmoc-Phe Test**
3.	Start the new synthesis. The expected monitor display is ten peaks of approximately the same height in the 5000 to 7000 range. See Figure 3-1.

About the UV Fmoc-Resin Test

The Fmoc-Resin Test is a cycle (UV Fmoc-Resin Test) in the UV Flow Test Chemistry file. The Fmoc-Resin Test measures the UV signal-to-background values for a clean deprotection where the resin has not encountered any other reagents and no sequence dependent issues are present.

The expected signal-to-background ratio should the 60:1 to 100:1 range. It is important that the background-to-peak ratio falls below the Function 134 setting of 3.5% (standard). See Figure 3-2.

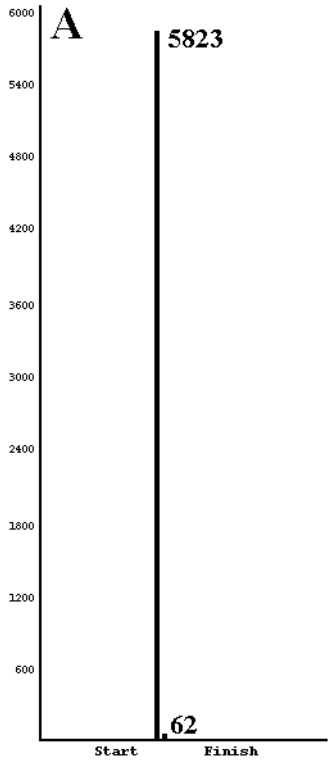


Figure 3-2 The UV monitor trace for the deprotection of an Fmoc-resin. The peak:background ratio is >90:1.

Running the UV Fmoc-Resin Test

The Fmoc-Resin Test monitors peak-to-background values at a wavelength 301 nm. The peak-to-background ratio should be at least 60:1 and is typically much higher.

Piperidine at Bottle 1 and NMP at Bottle 10 are required for this test.

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.

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.

To run the Fmoc-Resin test:

1.	Weigh 0.10 mmole of Fmoc-Gly Resin (PN 401421) into the 8-mL Reaction Vessel.

To run the Fmoc-Resin test: ***(continued)***

2.	Set up and start a new run with the UV Flow Tests as the chemistry: • Sequence — **none** • Cycle — **UV Fmoc-Resin Test**
3.	Check the results and verify that the second deprotection peak is in the 50 to 150 range, and that the ratio of the second peak to the first peak is <3.5%. See the example, Figure 3-3 on page 3-7.
4.	If the ratio exceeds 3.5%: a. Install fresh bottles of NMP and Piperidine. b. Prime both lines. c. Increase the value of Function 134 in the H module to 5% (50) or to a value larger than observed in the previous Fmoc-Resin test. d. Repeat the Fmoc-Resin test.

UV Monitoring Examples

About the Chart Recording

Figures 3-3 and 3-4 show two UV traces for a 12-mer peptide illustrating the monitor display and the corresponding strip chart recording, respectively. Note that there is a faithful correspondence between the peak heights of the monitor display and the strip chart recording.

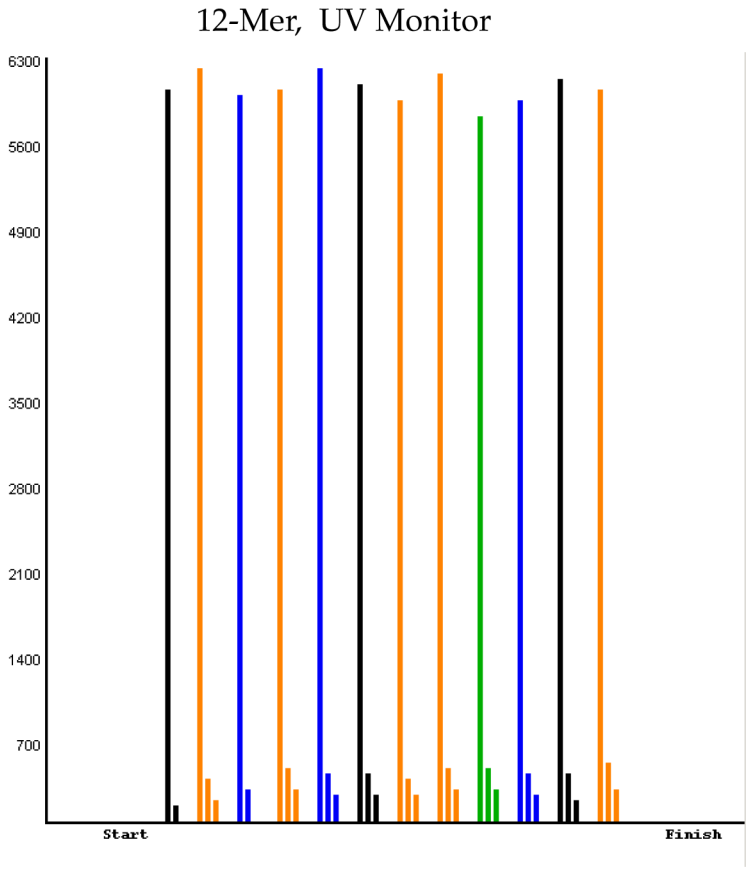


Figure 3-3 UV Monitor trace for a 12-mer peptide.

In the strip-chart tracing (Figure 3-4 on page 3-8) make note of the:

- Methanol and NMP baselines
- Flat baseline preceding each of the large/small UV peaks
- Bubble

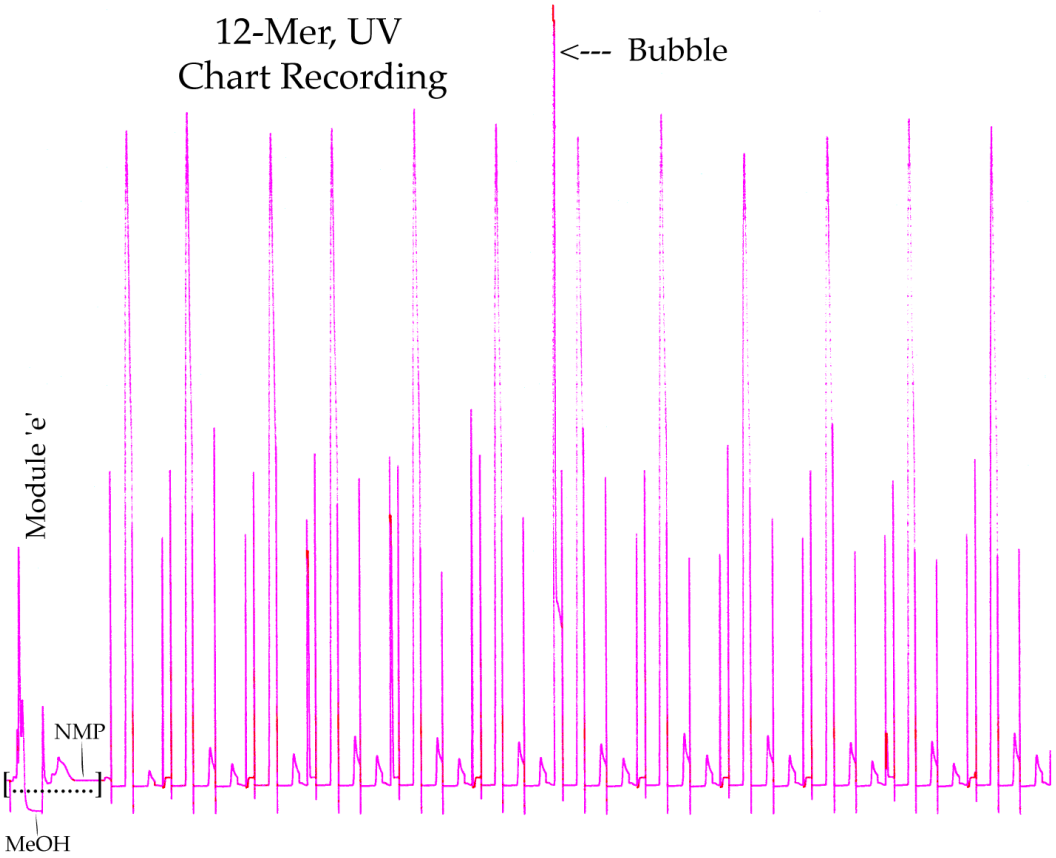


Figure 3-4 The strip-chart recording for the 12-mer peptide shows the methanol and NMP baselines (Module e), the deprotection peaks, the washouts, and the bubble.

Note: You need not re-zero the detector a second time with NMP flowing through the cell. Auto-zeroing on methanol clears bubbles from the flow cell and provides a slightly positive baseline value for the NMP absorbance. The absorbance of NMP is sometimes drops below zero after auto-zeroing with methanol. Installing a fresh bottle of (dry) methanol generally corrects the problem.

Removing Bubbles

Methanol is used to flush the lines before every UV plug is sent to the S200 detector, assuring that bubbles are swept away and pre-peak baselines remain flat.

Preventing the formation of bubbles in the UV lines is impractical. Removing the bubbles with methanol is the preferred resolution of the bubble problem.

Necessary and Unnecessary UV Deprotections

Necessary Deprotections

Extended deprotections are necessary for the last several residues, where the first deprotection peaks are smaller than those of the initial residues in the synthesis (see Figure 3-5 on page 3-9). The conditional deprotections begin on the fifth peak for the final residues.

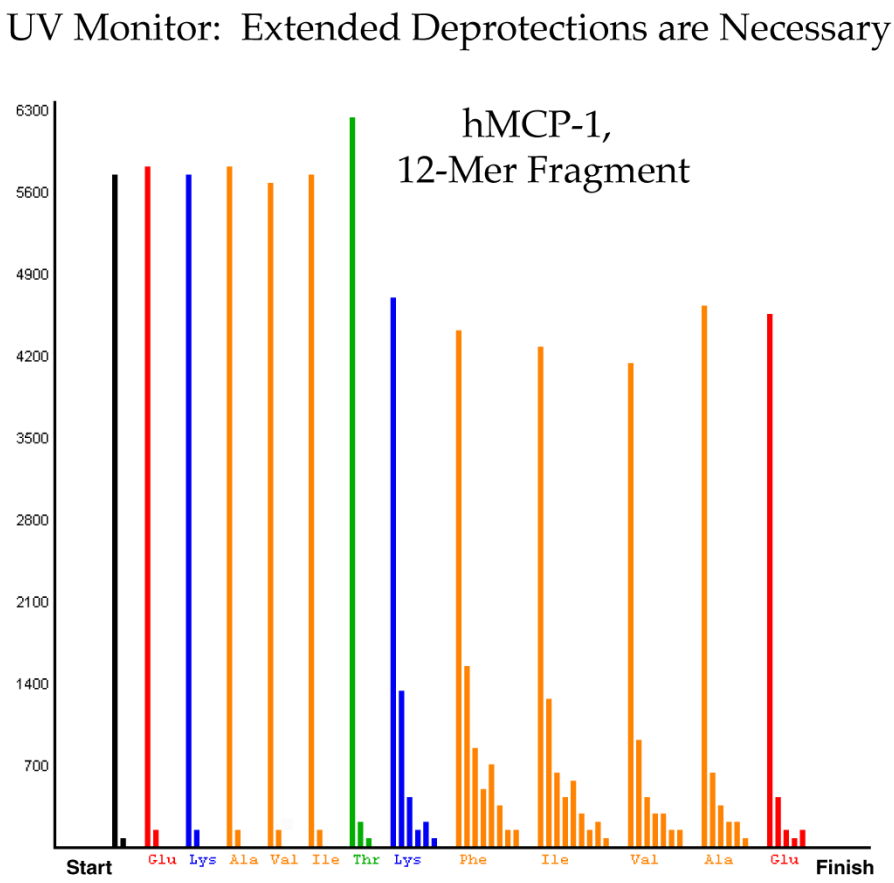


Figure 3-5 The UV monitoring of a 12-mer fragment from hMCP-1 clearly shows the necessity of extended deprotections starting several residues from the C-terminus.

Unnecessary Deprotections

Figure 3-6 on page 3-10 shows a synthesis where none of the residues are difficult to deprotect. In this example, the value of Function 134 (Module B) was increased from 35 to 40 to avoid extra deprotections, which are wasteful of time and reagents.

Extra deprotections can arise because of UV absorbing materials left over from the coupling reagents. Note that the cartridge-derived amino acids are affected, not the starting resin. UV is much less sensitive to contaminants than conductivity, but even UV cannot completely escape the effects of problem reagents.

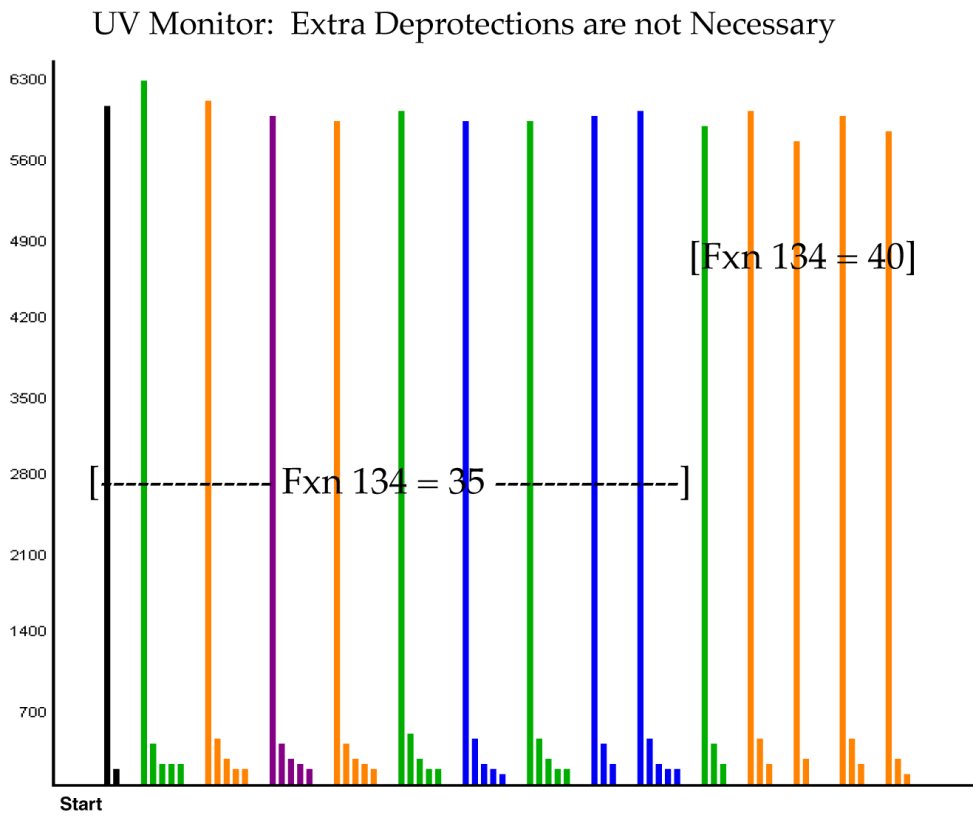


Figure 3-6 The extra deprotections for the C-terminal residues are unnecessary. Increasing the value of Function 134 to 4.0% resolves the problem.

Example of the Value of UV Monitoring

UV monitoring provides more accurate information about easy versus difficult residues in a sequence, better control of a synthesis, and a better peptide product than conductivity monitoring.

The 26-Mer Conductivity Trace

The conductivity profile (Figure 3-7) shows a very large increase in the initial deprotection peak at Asn(14) compared to the first peaks of the several C-terminal residues. The first deprotections following Asn are also very tall and then the peaks decline in height until they approach the profile displayed at the beginning of the synthesis.

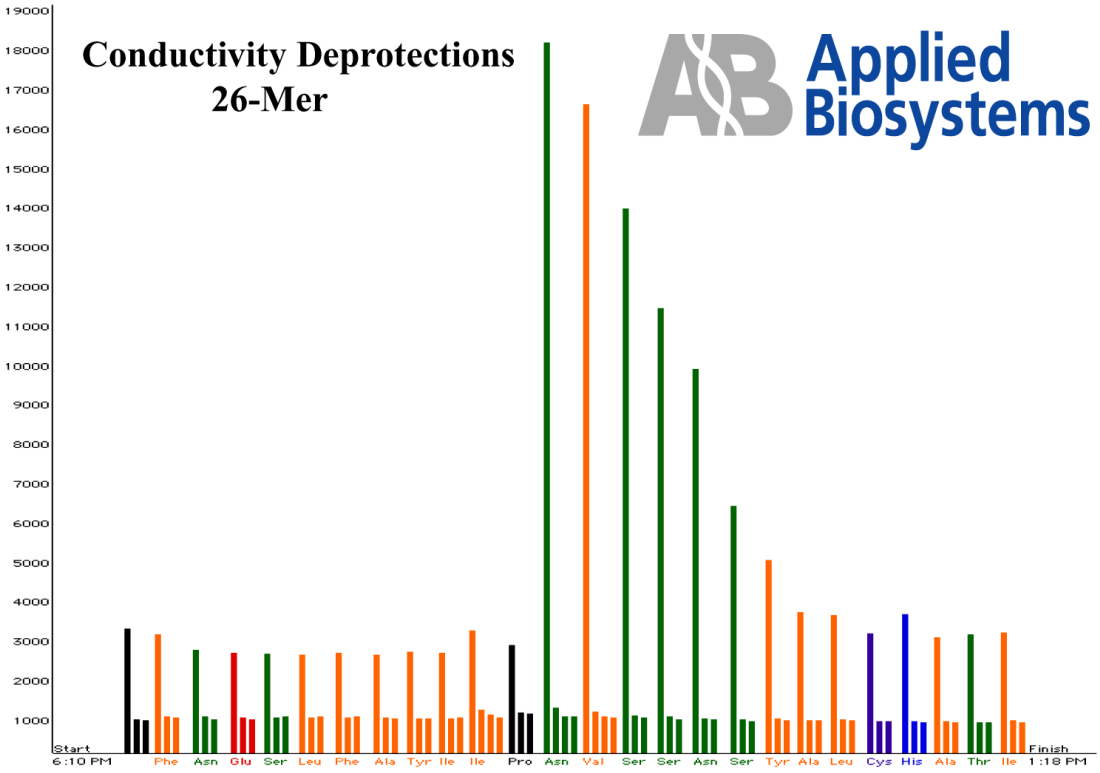


Figure 3-7 A major change in the conductivity deprotections of the ICAT® reagent 26-mer takes place at Asn (14).

The conductivity deprotection profile shows that starting at Asn(14) the normal washout of conductive species from the resin (during the coupling cycle) is significantly impaired. A base washout of the resin, that is, piperidine deprotection, is needed to flush out the conductive species.

The conductivity trace indicates that the resin is condensed or collapsed at Asn(14) and does not recover until much later in the synthesis. The trace shows a significant synthesis problem this point that is not solved until much later in the sequence, if at all.

The 26-Mer UV Trace

The UV profile (Figure 3-8 on page 3-12) gives a different picture of the 26-mer synthesis. The UV trace shows easy deprotections for the early residues, as expected. Ile(16) and Pro(15) require overtime deprotections (with extended coupling and then capping). The subsequent residues become easy to the end of the synthesis.

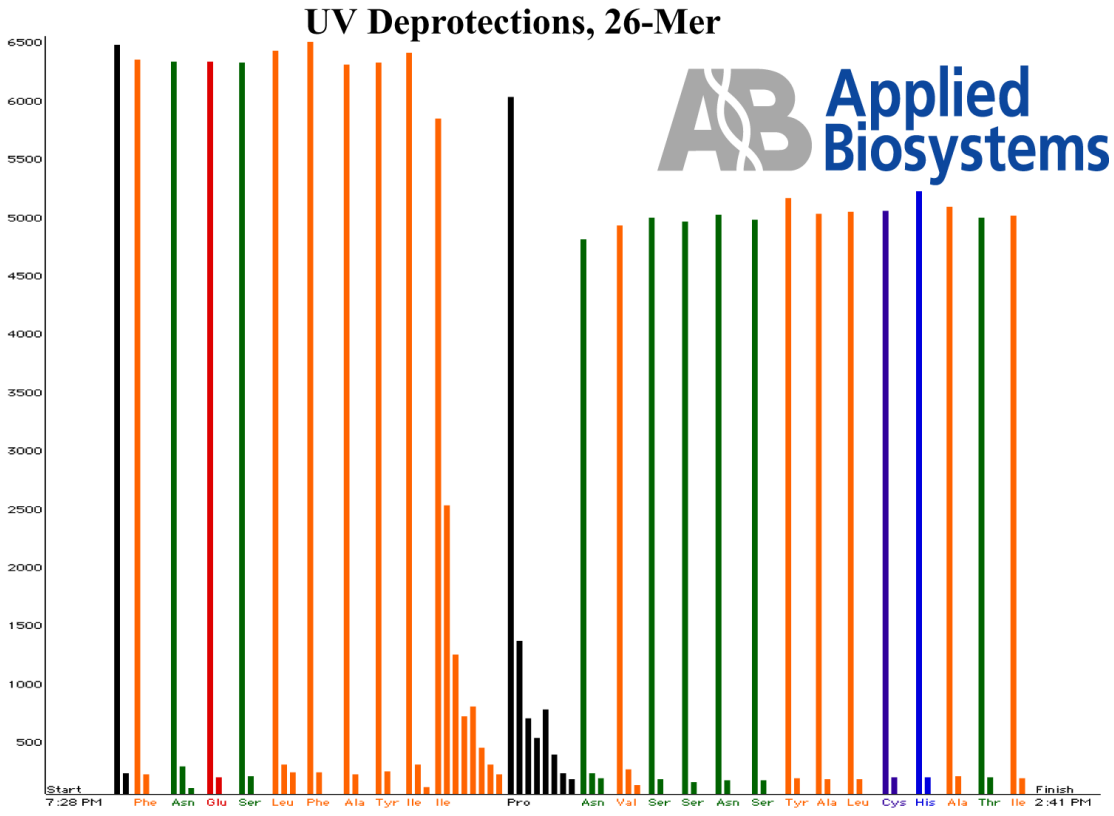


Figure 3-8 The UV trace of the 26-mer synthesis. The trouble begins at Ile(16), not at Asn(14).

The UV monitoring detected the difficult residues in the sequence and has taken the appropriate steps to solve the problem. In comparison, conductivity monitoring failed to recognize the difficult residues and then was forced to work with a collapsed resin.

UV Chemistries for the 3 mL Reaction Vessel

UV chemistries have been developed for the 3 mL Reaction Vessel on a 433A instrument that is running with the S200 UV Detector.

Small Scale Advantages

The 3 mL Reaction Vessel has been available for several years with both tBoc (PNA) cycles and Fmoc-conductivity chemistry cycles. These cycles carry out syntheses on the 433A synthesizer at 5-, 10- or 20-µmole scales. The small scale of the 3 mL syntheses allow the incorporation of peptide residues that are very expensive and/or very difficult to prepare. The 3 mL Reaction Vessel chemistries permit the synthesis of peptides that would be prohibitively expensive to carry out at the standard scales of 0.10 to 1.0 mmoles.

3 mL Reaction Vessel Kit

The chemistries for the 3 mL Reaction Vessel can be used only after the 3 mL Reaction Vessel Kit (PN 402067) has been installed. Replacement of the 0.50-mL metering loop with a new 0.125-mL loop for Bottles 7 and 8 is a required modification to the 433A instrument. See the *ABI 433A Peptide Synthesis 3 mL Reaction Vessel User's Manual* (PN 904923) for more information.

The 3 mL UV Chemistry Files

The 3 mL UV cycles are FastMoc chemistries (HBTU/HOBt) at the 5-, 10- or 20-µmole scales (see Figure 3-9). The monitoring functions in Modules B and b are set to values appropriate for the 3 mL scales. The values are different from those in Modules B and b of the standard scale chemistries (0.10 to 1.0 mmoles). The rationale for setting the values applies to both sets of the UV chemistries. You may wish to change the default values of Functions 134 or 145 based on the numbers recorded for the UV baselines. At the outset, however, the 3-mL chemistries should be used as provided.

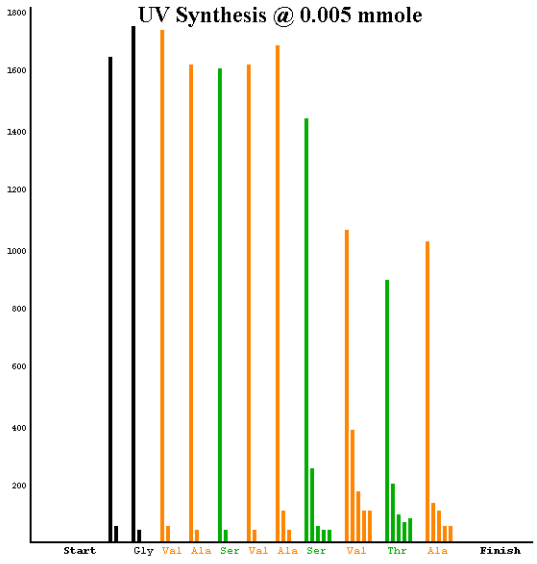


Figure 3-9 The UV deprotection profile for a 10-mer peptide at the 5 µmole scale, starting with 7 mg of Fmoc-Amide resin.

Troubleshooting 4

This chapter covers:

Trouble-Shooting NMP Flow Problems

Note: Before attempting to carry out any of the solutions in Table 4-1, always verify that the NMP:

- Delivery to the ABI™ 433A Peptide Synthesizer is in spec (Flow Tests 10 and 11), and
- Flow to the S200 detector is in spec (0.80 to 0.90 mL/min).

Table 4-1 NMP flow problems

Problem	Solution
The Abs starts increasing late during the 25-second Wait and barely starts to fall before the time expires.	The NMP flow is too slow. Either: • Increase the 25-second Wait to 30 seconds or • Use a shorter piece of restriction tubing
The Abs starts increasing later during the 25-second Wait and does not begin to fall before the time expires.	The NMP flow is too slow. Use a shorter length of restriction tubing.
The Abs starts increasing before the 5-second Wait begins.	The NMP flow is too fast. Use a longer length of restriction tubing.
The Abs starts increasing during the 5-second Wait.	
The Abs starts increasing at the start of the 25-second Wait.	The NMP flow is too fast. Either: • Decrease the 5-second Wait to 1 or 2 seconds or • Use a longer length of restriction tubing

For more information, see Figures 4-1and 4-2.

NMP Flow Rates

The Effects of a Slow NMP Flow Rate

If the NMP flow to the detector is too slow, the zeroing of the register at Function 129 is normal, but the peak reaches a maximum after the monitoring stops at Function 131. The data register fails to capture the correct maximum value of the peak (see Figure 4-1).

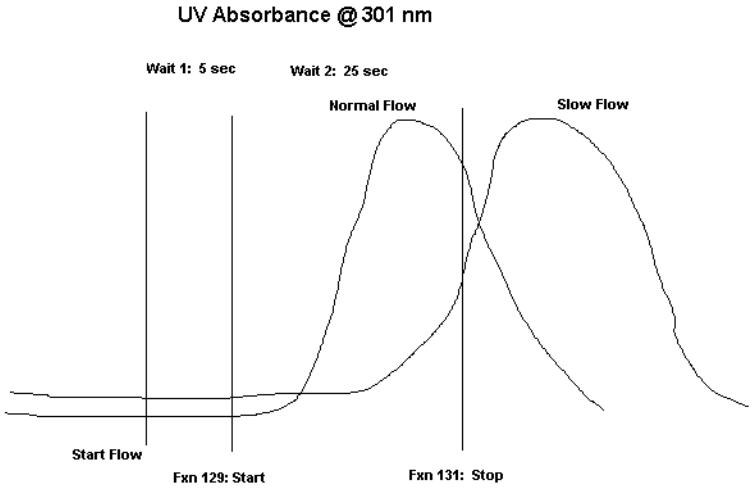


Figure 4-1 If the NMP flow to the cell is too slow, the maximum value of the UV peak is not captured.

The Effects of a Fast NMP Flow Rate

If the NMP flow to the detectgor is too fast, the peak maximum may fall within Wait 2 but the register zeroing at Function 129 is incorrect. The maximum-to-baseline value of the peak is truncated (see Figure 4-2).

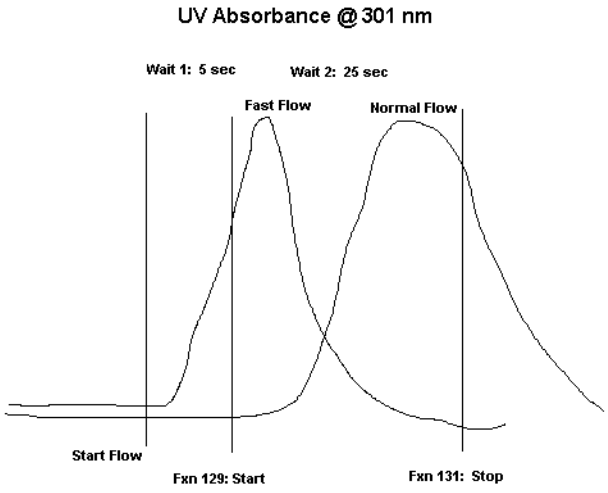


Figure 4-2 If the NMP flow to the cell is too fast, the maximum value of the UV peak may be captured but the Function 129 zeroing is incorrect.

Other Troubleshooting Issues

Absorbance Out of Range

The absorbance display on the S200 detector starts to flash whenever the UV signal falls outside the range of approximately +1.5 A to –0.1 A. The flashing display for a deprotection peak does not signify a loss of data. If a strip-chart recorder is connected to the system, the pen tracing shows that the rising UV peak is not truncated during such times. See Figure 4-3.

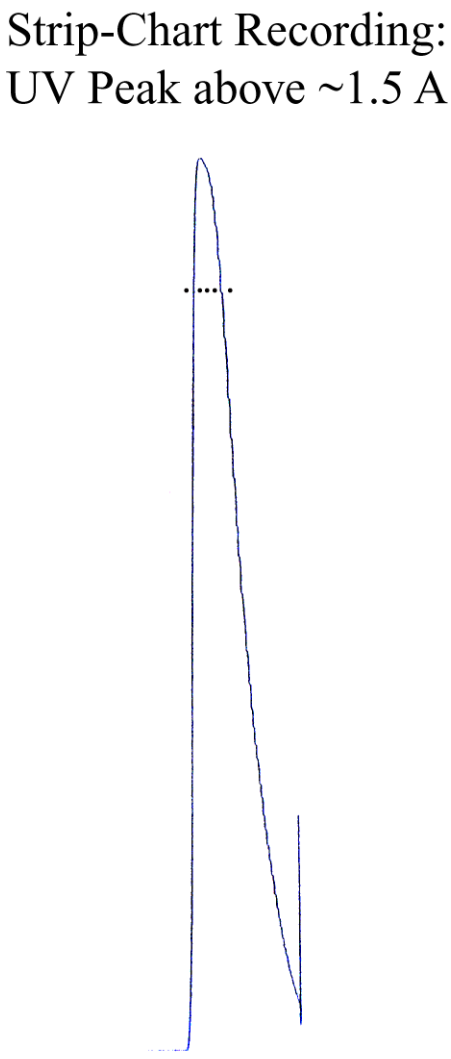


Figure 4-3 The strip-chart recording of a UV peak that exceeds the approximately1.5 A range of the S200 detector panel display. The dotted line shows the approximate start/stop position where the absorbance display flashes. The peak is not truncated.

Excessively Large Peaks for the First Deprotection

If the UV Fmoc-Resin Test is giving first-peak heights of >10,000 units, or if the UV peaks during syntheses are >10,000 units, increase the absorbance range of the S200 detector to 0.030 A and repeat the Fmoc-Resin Test.

Excessively large values for the first deprotection peaks may allow all subsequent peaks to fall below the Function 134 limit, potentially preventing overtime deprotections from taking place. Therefore, it is important to maintain the signal-to-background ratio at a level that permits overtime deprotections to switch on when they are required.

If the UV peaks are consistently in the 10,000 to 20,000 range, resin may have leaked from the Reaction Vessel. Resin leaks can occur when the caps of the Reaction Vessel are not properly tightened.

Zeroing the S200 Detector

The UV signal that is recorded is a relative measure of peak height, beginning with Function 129 and ending with Function 131 (see Figure 1-3). The data register is zeroed by Function 129. The largest UV signal recorded within the Wait period of 25 seconds is the value displayed in the monitor trace. The recorded value of the UV peak is the same whether measured between 0.00 A and 0.50 A or between 0.10 A and 0.60 A.

It is not necessary for the S200 detector to display 0.0000 A for the methanol flow. Methanol zero values in the 0.0000 to 0.1000 A range provide adequate starting points for UV monitoring. What is most important is that the zero value be low and stable during the methanol flow.

About UV Monitoring A

This appendix covers:

Manual Autozeroing of the S200 UV Detector

The procedure for manually autozeroing the S200 detector (see "Zeroing the S200 Detector" on page 2-4) gives a stable, low-absorbance value for the NMP that passes through the detector.

Note: The 301-nm NMP background absorbance value need not be 0.0000. The numerical value for a UV peak is the same for maximum-to-baseline measurements of 0.2500 and 0.0000 as it is for 0.3000 and 0.0500. It is more important that the baseline absorbance be stable, that is, non-varying, rather than at an absolute zero during the 5-second interval (Wait 1).

The auto-zeroing procedure for the S200 detector (Module e) runs methanol followed by NMP to the flow cell. Methanol is used to sweep bubbles out of the cell and to autozero the detector. NMP flow through the cell typically gives a 301-nm UV absorbance of about 0.0100 higher than methanol.

The most important step to insure proper UV monitoring is setting the NMP flow to its optimum range (see "Measuring the NMP Flow" on page 2-5). See Chapter 4, "Troubleshooting," for procedures for fine-tuning the time values in Wait 1 and Wait 2.

UV Monitoring and the Conditional Cycle

Peaks Being Compared

The UV monitoring process in Module B compares the second (and each subsequent) deprotection peak to the first peak. If the second peak is less than 3.5% of the first peak, the deprotection process is considered complete, the deprotection cycle is terminated, and the synthesis moves forward to the activation of the next residue and the NMP washing of the resin.

For example, in Figure A-1 the second peak is 62/5823 = 1.06% of the first deprotection peak.

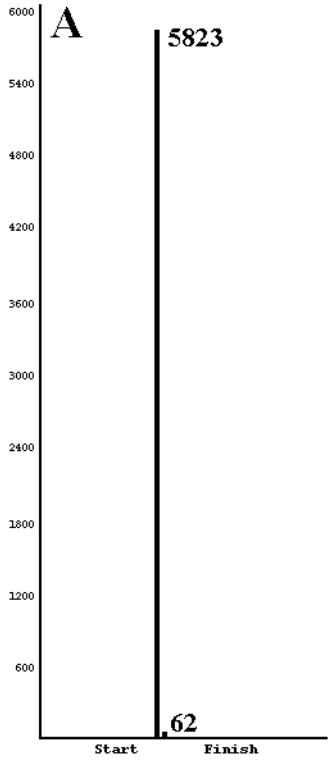


Figure A-1 The UV monitor trace for the deprotection of an Fmoc-resin. The peak:background ratio is >90:1.

If the 3.5% limit is not reached, the deprotections in Module B continues to generate peaks and to test (up to a maximum of three times) whether they are below the 3.5% goal. If Module B reaches the maximum, deprotections go conditional and continue into Module b until a UV background limit is reached (see Figure A-2).

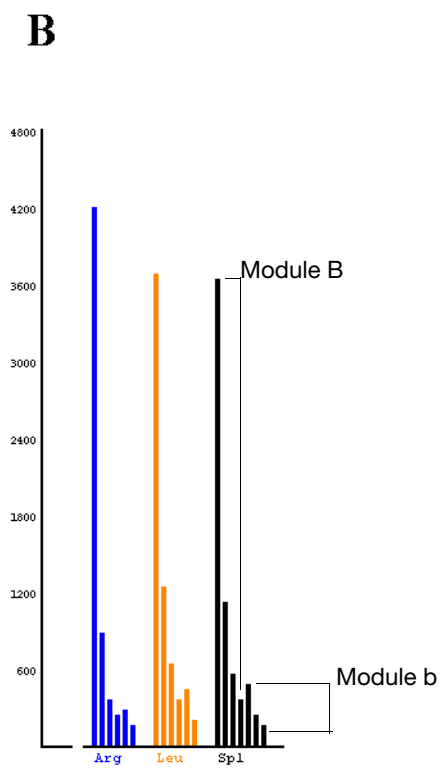


Figure A-2 UV deprotections that fail the Module B conditions and that require the extended deprotections in Module b: two for Arg and Leu; three for Sp1.

Establishing Values for Functions 133 and 134

Functions 133 and 134 in Module B set the conditions for testing the deprotection peaks. Function 133 sets the number of deprotection loops and Function 134 sets the limiting goal. Residues that are easy to deprotect generally reach the 3.5% goal in one or two out of three deprotection cycles under standard operating conditions. (Note that "3.5%" is entered in Function 134 as "35.")

The deprotections in Module b run for a maximum of six loops, 10 minutes each, to reach an absorbance minimum. The goal of Module b is an absorbance falling below a preset value (Function 145). Module b no longer compares absorbances to the first deprotection peak of Module B.

For example, Figure A-3 shows the UV monitoring trace for an 11-mer sequence from a histone protein. The first eight residues of the synthesis are all easy to deprotect, but the final three residues are difficult. The observation of easy deprotections for the early C-terminal residues is common to every sequence. The later residues may become difficult to deprotect because of structural problems that may develop in a longer chain, such as in alpha-helix formations, beta-sheet formations, and peptide-peptide or peptide-resin interactions.

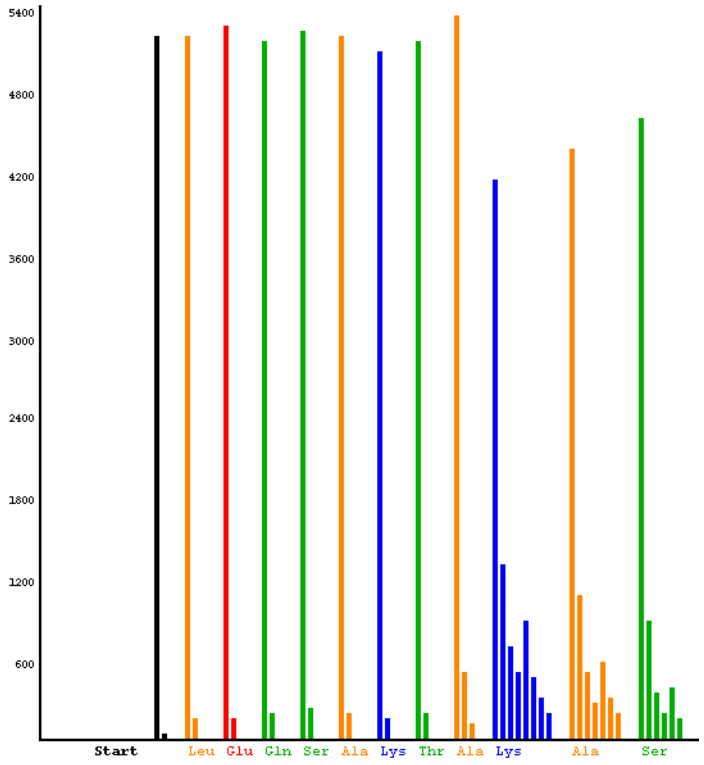


Figure A-3 The UV monitor trace for an 11-mer sequence showing easy deprotections for the eight early residues and difficult deprotections for the final three residues.

You must set the values for Functions 133 and 134 in Module B so that the UV deprotections look like Figure A-3, that is, easy for the early residues and difficult for the later residues. If Functions 133 and 134 are set so that all deprotections are difficult, then the synthesis wastes reagents and time. In addition, important information about where the sequence converts from easy to difficult is lost.

Note: If you change the values for Functions 133 and 134, synthesize the 11-mer sequence in Figure A-3 as a test to verify the usability of the new settings.

Note: The values for Functions 133 and 134 are the same at the 0.10 and 0.25 mmole scales of synthesis. The value of Function 134 at the 1.0 mmole scale is increased (7.5%) due to the higher concentration of Fmoc-species at this scale. The values for Functions 133 and 134 in the 3 mL Reaction Vessel chemistries are different from those in the standard scale chemistries due to the smaller amounts of UV species.

Note: Remember that the 3.5% value for Function 134 represents the comparison of peak heights: the next deprotection peak relative to the first (large) deprotection peak. The 3.5% value does *not* represent a quantity of Fmoc-group remaining on the resin.

Establishing Values for Function 145 (Module b)

Function 145 in the conditional deprotection Module b is set by default to an absorbance background level of 200, the value commonly observed at the end of extended deprotections. If this value is too high, the extended deprotections terminate too soon and the Fmoc-groups may not be completely removed. If the value is too low, the extended deprotections are unnecessarily prolonged.

The UV baseline peaks for the easy residues at the start of a synthesis, such as the C-terminal residues 1 through 8 in Figure A-3, provide the best estimate of an appropriate value for Function 145. If an average value for the early baseline peaks is, for example, 150 units, then add 50 units and set Function 145 in Module b to a value of 200 units by entering 20 in the Time column of Function 145.

Nonlinearity of UV Monitoring

The UV signal-to-background ratio demonstrated in Figure A-1 is greater than 90:1. The ratio typically falls between 50:1 and 100:1, depending on the synthesis conditions. The first (large) UV deprotection peak at each residue is drawn from a concentrated Fmoc solution in the Reaction Vessel. A plot of UV peak height versus SFmoc concentration is not linear (see Figure A-4). Under most circumstances the Fmoc-groups are easily removed from the N-terminus of a peptide chain and their high concentration exceeds the limits of linearity in the UV detector.

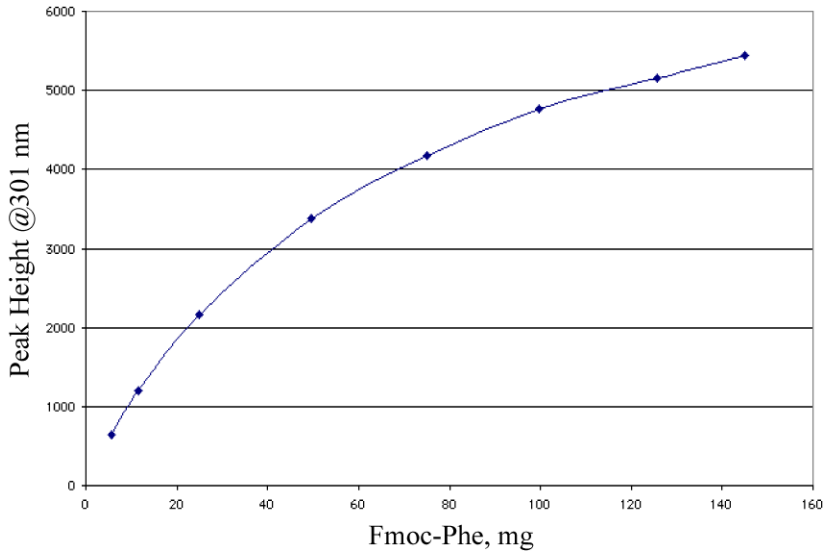
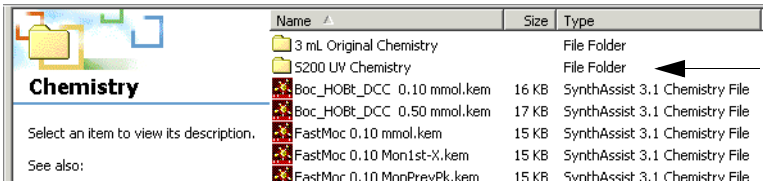


Figure A-4 A plot of UV peak height against Fmoc concentration is nonlinear.

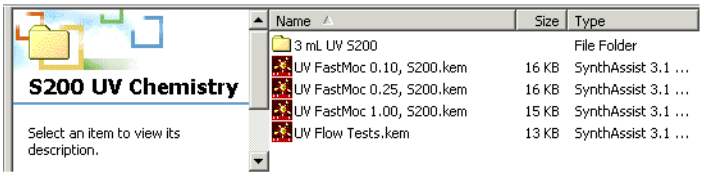
UV Chemistry Folders and Files B

Organization of the UV Chemistry Folders and Files

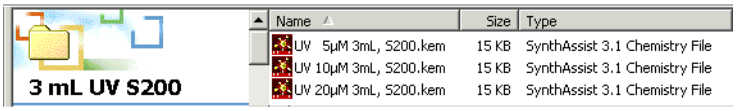
The "S200 UV Chemistry" folder is in the Chemistry folder of the SynthAssist® v3.1 software. The 3 mL Original Chemistry folder contains the tBoc (PNA) and Fmoc-conductivity chemistry cycles.



The "S200 UV Chemistry" folder contains both the standard scale UV chemistries and the 3 mL UV chemistries. The standard chemistries run at the 0.10-, 0.25- and 1.0-mmole scales.



The "3 mL UV S200" folder contains the 5-, 10- and 20-µmole scale chemistries.



Standard Scale S200 UV Detector Modules

C

This appendix covers:

UV Flow Test Chemistry Modules

The UV Flow Test chemistry contains two cycles and eighteen modules (Figure C-1). The modules are designed for:

- Setting the proper NMP flow to the S200 detector
- Running the Fmoc-Phe Test cycle
- Running the Fmoc-Resin Test cycle

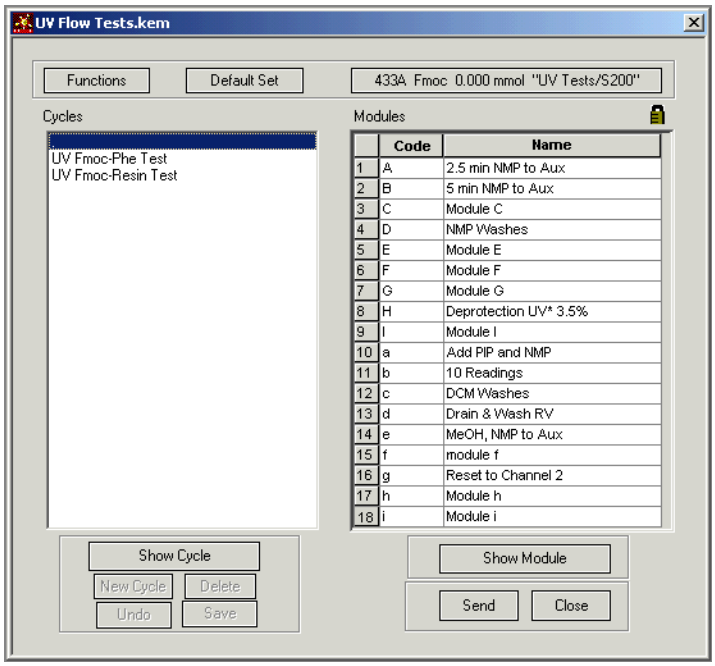


Figure C-1 UV Flow Test chemistry

Modules A and B

Modules A and B are used to deliver NMP into a tared cartridge using the Aux A waste-port connector.

- Module A provides a 2.5-minute test of the NMP flow and should be used when the restrictor tubing is first being cut to a shorter length.
- Module B provides a 5-minute delivery and should be used when the restrictor tubing is approaching its proper length or whenever the NMP flow is to be verified.

Modules eabd

Modules eabd (e, a, b, and d in sequence) are used for the Fmoc-Phe Test. The Fmoc-Phe Test verifies that the NMP flow is stable and that the capture of the UV peaks is reproducible.

Modules ecDHDc

Modules ecDHDc (e,c, D, H, D, and c in sequence) are used for the Fmoc-Resin Test. The Fmoc-Resin Test verifies that the UV baseline-to-signal ratio is low and is below the default value of 3.5% for Function 134.

Note: After the NMP flow has been properly established using the Flow Tests, no changes to the NMP flow are necessary, or advisable, when running the 3 mL UV chemistries.

Module e

Module e (see Figure C-2) delivers methanol followed by NMP to the S200 detector and is used to autozero the detector. Each solvent is initially delivered as a start/stop/start flow so that bubbles can rise to the top of the flow cell and be expelled. Module e is typically run twice (or more) before starting a run to verify that the solvent baselines are stable.

Module e delivers methanol for 60 seconds in Step 7 and you manually autozero the S200 detector during the final 10 seconds of the step. Module e delivers NMP for 60 seconds in Step 13, when a slightly higher 301-nm UV absorbance than that of methanol is seen during the final seconds of NMP delivery (see Figure 3-4 on page 3-8).

UV FastMoc 0.10, S200.kem **MeOH, NMP to Aux**

Function	Number	Name	Time	Add Time	Elapsed
1	1	Wait	1	0	1
2	135	Monitoring reset	2	0	1
3	78	Pressurize manifold	10	0	11
4	19	Flush bottom valve block with MeOH to waste	2	0	13
5	101	MeOH to Aux	60	0	91
6	1	Wait	3	0	31
7	101	MeOH to Aux	15	0	108
8	14	Flush bottom valve block with NMP to waste	2	0	93
9	118	Flush bottom valve block with NMP to aux. Waste	15	0	108
10	1	Wait	3	0	111
11	118	Flush bottom valve block with NMP to aux. waste	15	0	126
12	1	Wait	3	0	129
13	118	Flush bottom valve block with NMP to aux. waste	60	0	189

Figure C-2 Module e

Note: The UV Flow Tests chemistry is installed as a locked file and should be maintained as a locked file.

UV FastMoc 0.10 mmole UV Chemistry Modules

The UV FastMoc™ 0.10 S200.kem chemistry uses 16 of 18 available modules and defines 18 cycles (Figure C-3). The predefined chemistry file is locked. Modules B, b, and g, unique to the UV chemistries, are described below.

Note: You can make changes to the predefined chemistry file by first selecting **File** > **Save As** and saving it under a different name.

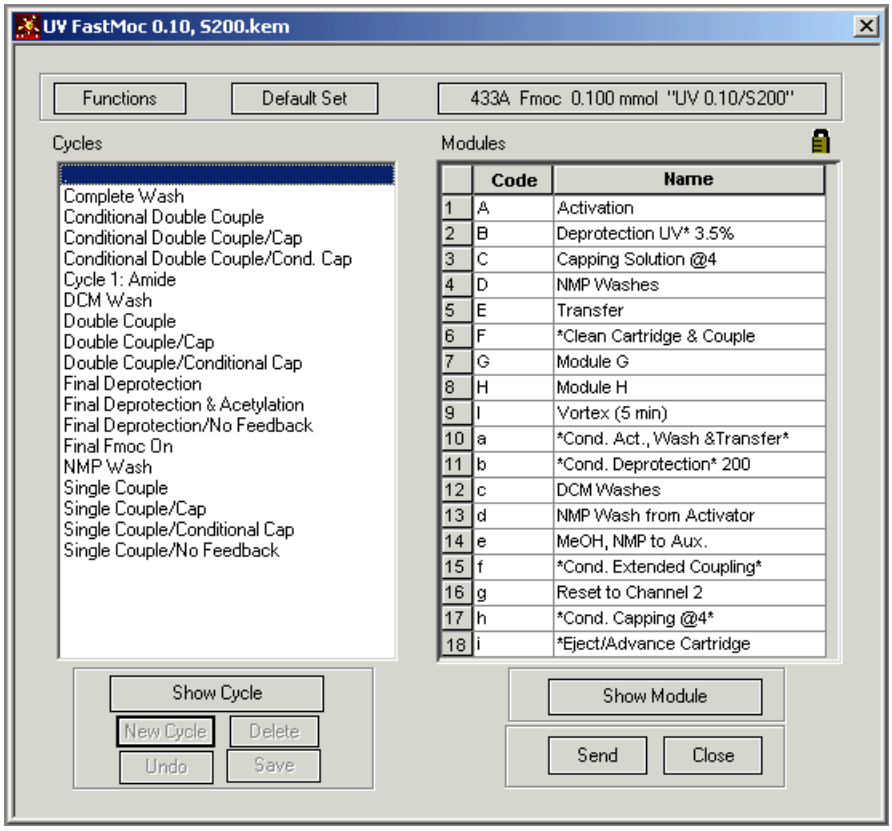


Figure C-3 UV FastMoc 0.10 S200 chemistry

Module B, First Deprotection Peak

Module B has 83 steps that list the sequence of functions and their time (in seconds) of operation. The following steps (shown in Figure C-4 on page C-6 and Figure C-5 on page C-8) are noteworthy:

- Step 4 directs the 433A instrument to collect monitoring data from Channel 2, where the S200 UV detector is connected.
- Steps 5 through 11 direct NMP washing of the resin.
- Steps 13through19 direct the deliveries of NMP and Piperidine to the Reaction Vessel and are the beginning of the first deprotection of the peptide resin. (The deprotection runs for 2 minutes, Steps 17 through 31.)
- Steps 31through 37 run the UV plug through the flow cell of the S200 detector. These steps generate the first UV peak for a given residue.

- Steps 40 through 79 are the Conditional Monitoring Loop.

UV FastMoc, S200.kem **Deprotection UV* 3.5%**

Function	Number	Name	Time	Add Time	Elapsed
1	1	Wait	1	0	1
2	14	Flush bottom valve block with NMP to waste	2	0	3
3	118	Flush bottom valve block with NMP to Aux waste	20	0	23
4	135	Monitor reset	2	0	23
5	56	Deliver NMP to reaction vessel	4	1	27
6	2	Vortex reaction vessel on	5	0	32
7	40	Mix reaction vessel	2	0	34
8	3	Vortex reaction vessel off	1	0	35
9	42	Drain reaction vessel to waste	8	1	43
10	50	Flow NMP through reaction vessel to waste	5	0	48
11	42	Drain reaction vessel to waste	8	1	56
12	14	Flush bottom valve block with NMP to waste	2	0	58
13	56	Deliver NMP to reaction vessel	5	0	63
14	40	Mix reaction vessel	2	0	65
15	79	Pressurize Pip	10	0	75
16	16	Flush bottom valve block with Pip to waste	2	0	77
17	51	Deliver Pip to reaction vessel	5	0	82
18	56	Deliver NMP to reaction vessel	3	0	85
19	40	Mix reaction vessel	1	0	86
20	2	Vortex reaction vessel on	1	0	87
21	78	Pressurize manifold	10	0	97
22	19	Flush bottom valve block with MeOH to waste	2	0	99
23	101	MeOH to Aux	20	0	119
24	1	Wait	3	0	122
25	14	Flush bottom valve block with NMP to waste	2	0	124
26	151	Toggle user function on	108	0	124

UV FastMoc, S200.kem			Deprotection UV* 3.5%		
Function	**Number**	**Name**	**Time**	**Add Time**	**Elapsed**
27	1	Wait	70	0	194
28	3	Vortex reaction vessel off	1	0	195
29	113	Toggle all off	1	1	195
30	1	Wait	2	0	197
31	42	Drain reaction vessel to waste	3	0	200
32	151	Toggle user function on	108	0	200
33	1	Wait	5	0	205
34	129	Monitor first peak	1	0	205
35	1	Wait	25	0	230
36	131	Monitor stop	1	0	230
37	132	Read monitor peak	1	0	230
38	1	Wait	15	0	245
39	113	Toggle all off	1	0	245

Figure C-4 Module B, Steps 1 through 39, the first deprotection peak.

Module B, Conditional Monitoring Loop

Steps 40 to 83 contain the Conditional Monitoring Loop: Functions 133 to 134.

UV FastMoc 0.10, S200.kem **Deprotection UV* 3.5%**

Function	Number	Name	Time	Add Time	Elapsed
40	**133**	**Begin loop monitoring**	**3**	**0**	**245**
41	42	Drain reaction vessel to waste	8	1	783
42	98	Begin loop UPPER	2	0	783
43	56	Deliver NMP to reaction vessel	5	1	822
44	2	Vortex reaction vessel on	5	0	827
45	40	Mix reaction vessel	2	0	829
46	3	Vortex reaction vessel off	1	0	830
47	42	Drain reaction vessel to waste	8	1	838
48	50	Flow NMP through reaction vessel to waste	5	1	843
49	42	Drain reaction vessel to waste	8	1	851
50	99	End loop UPPER	1	0	851
51	14	Flush bottom valve block with NMP to waste	2	0	853
52	56	Deliver NMP to reaction vessel	5	0	858
53	40	Mix reaction vessel	2	0	860
54	79	Pressurize Pip	10	0	870
55	16	Flush bottom valve block with Pip to waste	2	0	872
56	51	Deliver Pip to reaction vessel	5	0	877
57	56	Deliver NMP to reaction vessel	3	0	880
58	40	Mix reaction vessel	1	0	881
59	2	Vortex reaction vessel on	1	0	882
60	78	Pressurize manifold	10	0	892
61	19	Flush bottom valve block with MeOH to waste	2	0	894
62	101	MeOH to Aux	20	0	914
63	1	Wait	3	0	917
64	14	Flush bottom valve block with NMP to waste	2	0	919
65	151	Toggle user function on	108	0	919
66	1	Wait	70	0	989

UV FastMoc 0.10, S200.kem			Deprotection UV* 3.5%		
Function Number		**Name**	**Time**	**Add Time**	**Elapsed**
67	3	Vortex reaction vessel off	1	0	990
68	113	Toggle all off	1	0	990
69	1	Wait	2	0	992
70	42	Drain reaction vessel to waste	3	0	995
71	151	Toggle user function on	108	0	995
72	1	Wait	5	0	1000
73	129	Monitor first peak	1	0	1000
74	1	Wait	25	0	1025
75	131	Monitor stop	1	0	1025
76	132	Read monitor peak	1	0	1025
77	1	Wait	15	0	1040
78	113	Toggle all off	1	0	1040
79	**134**	**End loop monitoring**	**35**	**0**	**1040**
80	40	Mix reaction vessel	2	0	1042
81	2	Vortrex reaction vessel on	1	0	1043
82	14	Flush bottom valve block with NMP to waste	2	0	1045
83	118	Flush bottom valve block with NMP to Aux waste	20	0	1065

Figure C-5 Module B, Steps 40-83. The conditional monitoring loop: functions 133 to 134.

Step 40 (Function 133) specifies that (less than) three loops are allowed to reach a UV baseline that is <3.5% (Step 79, Function 134 = 35) of the first deprotection peak. The Function 133-to-Function 134 Loop fails if the <3.5% condition is not achieved in fewer than three loops. If the Function 133-to-Function 134 Loop fails, Module b continues the deprotections. If Module B fails, a conditional flag is set and is read by Function 137 at the beginning of Module b.

Module b

Step 1 (Function 137) directs Module b to run if, and only if, Module B fails (this is the meaning of the term "Conditional Deprotection"). Steps 3 through 41 represent the Conditional Deprotection Loop. Step 3 specifies a maximum of six loops to reach a UV baseline value <200 units (Function 145 = 20). Steps 15 through 30 bracket the deprotection time of 10 minutes per loop. See Figure C-6 below.

UV FastMoc 0.10, S200.kem ***Cond. Deprotection* 200**

Function	Number	Name	Time	Add Time	Elapsed
1	137	Do module if condition not met	2	0	0
2	3	Vortex reaction vessel off	1	0	1
3	**98**	**Begin loop UPPER**	**6**	**0**	**1**
4	42	Drain reaction vessel to waste	8	1	3509
5	50	Flow NMP through reaction vessel to waste	5	0	3514
6	56	Deliver NMP to reaction vessel	5	1	3519
7	2	Vortex reaction vessel on	4	0	3523
8	3	Vortex reaction vessel off	1	0	3524
9	42	Drain reaction vessel to waste	8	1	3532
10	14	Flush bottom valve block with NMP	2	0	3534
11	56	Deliver NMP to reaction vessel	5	0	3539
12	40	Mix reaction vessel	2	0	3541
13	79	Pressurize Pip	10	0	3551
14	16	Flush bottom valve block with Pip to waste	2	0	3553
15	51	Deliver Pip to reaction vessel	5	0	3558
16	56	Deliver NMP to reaction vessel	3	0	3561
17	40	Mix reaction vessel	1	0	3562
18	2	Vortex reaction vessel on	1	0	3563
19	1	Wait	460	0	4023
20	78	Pressurize manifold	10	0	4033
21	19	Flush bottom valve block with MeOH to waste	2	0	4035
22	101	MeOH to Aux	20	0	4055
23	1	Wait	3	0	4058
24	14	Flush bottom valve block with NMP to waste	2	0	4060

UV FastMoc 0.10, S200.kem ***Cond. Deprotection* 200**

Function Number		Name	Time	Add Time	Elapsed
25	151	Toggler user function on	108	0	4060
26	1	Wait	90	0	4150
27	3	Vortex reaction vessel off	1	0	4151
28	113	Toggle all off	1	0	4151
29	1	Wait	2	0	4153
30	42	Drain reaction vessel to waste	3	0	4156
31	151	Toggler user function on	108	0	4156
32	1	Wait	5	0	4161
33	129	Monitor first peak	1	0	4161
34	1	Wait	25	0	4186
35	131	Monitor stop	1	0	4186
36	132	Read monitor peak	1	0	4186
37	1	Wait	15	0	4201
38	113	Toggle all off	1	0	4201
39	145	Test X greater than peak	20	0	4201
40	147	Do module if test false	1	0	4201
41	**99**	**End loop UPPER**	**1**	**0**	**4201**
42	14	Flush bottom valve block with NMP to waste	2	0	4203
43	118	Flush bottom valve block with NMP to Aux waste	20	0	4223

Figure C-6 Module b, conditional deprotection.

Note: The target in Module b is based on an absorbance value, not on a percentage value as is the case in Module B. The time-value of Function 145 specifies the target baseline absorbance level. The default setting for Function 145 is based on standard UV baseline values observed over an extended period of time. The UV baseline value typically observed depends on the quality of the solvents and reagents in use on the 433A instrument. If you need to change the value of Function 145, review the Function 145 discussion in Appendix A, "About UV Monitoring."

Module g Conditional Chemistry

Function 135 (see Figure C-7) in Module g directs the 433A instrument to acquire monitoring data using Channel 2, connected to the S200 UV detector. Activating Function 135 also clears any conditional flags that may have been previously set.

UV FastMoc 0.10, S200.kem **Reset to Channel 2**

Function Number		Name	Time	Add Time	Elapsed
1	1	Wait	1	0	1
2	135	Monitoring reset	2	0	1

Figure C-7 Module g.

The default UV cycles are conditional, that is, the list of modules for single coupling is:

BbADEFfhd

where modules b, f, and h run only if Module B fails. However, Module F, the coupling module, runs only if Module B passes. These two paths are controlled by Function 137 or Function 136, respectively, as Step 1 in a module.

The Cycle 1 Amide (see Figure C-8) uses Module g to eliminate the possibility of extended coupling (Module f) in the first cycle of an amide resin. The Cycle 1 deprotection of an amide resin should never proceed to a failure of Module B (thus setting the conditional flag). In the remote chance that Module B could fail due to defective resin or reagents, Module g clears the flag and permits Module F to carry out a standard coupling.

UV FastMoc 0.10, S200.kem **Cycle 1: Amide**

1 Cartridge Single Couple No Resin Sample

Module Code	Module Name
e	MeOH, NMPto Aux
c	DCM Washes
D	NMP Washes
B	Deprotection UV* 3.5%
g	Reset to Channel 2
A	Activation
D	NMP Washes
E	Transfer
F	*Clean Cartridge & Couple
d	NMP Wash from Activator

Figure C-8 Cycle 1: Amide.

Millimole-Scale Chemistries

D

This appendix covers:

The 0.25 mmole Scale UV Chemistry

The organization of the 0.25 mmole modules and cycles follows the pattern of the 0.10 mmole modules, except for longer delivery times of NMP, Piperidine, or DCM. The 40 mL Reaction Vessel requires a larger volume of solvent than the 8 mL Reaction Vessel. The values of the monitoring Functions 133, 134, and 145 in Modules B and b are the same in both scales of the chemistry.

The 1.0 mmole Scale UV Chemistry

The large quantity of resin and solvents necessary for the 1.0 mmole scale of synthesis requires adjustments in the UV operating parameters, both on the S200 detector and in the modules.

In contrast to the 0.10 and 0.25 mmole scales, which by default run conditional cycles, the 1.0 mmole chemistry runs nonconditional cycles exclusively.

Conditional chemistry helps to clarify sequence-dependent synthesis problems that should be discovered and resolved at the 0.10 or 0.25 mmole scale. Discovering sequence-dependent problems at the 1.0 mmole scale is too costly and time-consuming to be practicle. Any step taken to solve a sequence problem at 0.10 mmoles, such as the use of a pseudoproline derivative, can be incorporated into a 1.0 mmole synthesis.

The high concentration of Fmoc-derivatives at the 1.0 mmole scale requires that you manually increase the absorbance range of the S200 detector to 0.030 A before you start a synthesis. Function 134 in Module B of the 1.0 mmole chemistry is increased to 7.5% by default.

S200 UV Detector Flow Connections E

This appendix covers:

Flow Connections

The flow connections between the ABI 433A Peptide Synthesizer and the Series 200 UV Detector are shown in Figure E-1. The red tube connects the Aux Port 9 from the 433A valve block to the input of the flow cell (lower side port). The yellow (restriction) tubing connects to the exit (top) port of the flow cell. The blue tubing connects to the Aux A waste port.

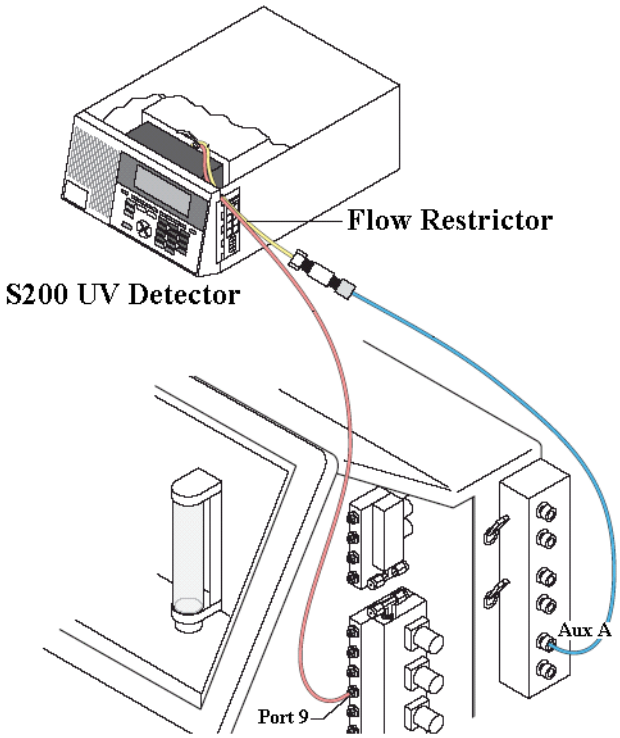


Figure E-1 ABI™ 433A Peptide Synthesizer/S200 UV detector connections.

The connector for the yellow-to-blue tubes can be opened in order to change the length of the restrictor tube when necessary for adjusting the NMP flow to the detector. Before atempting to change the length of the restrictor tube, verify that the NMP flow to the 433A instrument is within the specified range. If the NMP flow to the Aux A port is out of specification, determine whether adjustments to Wait 1 or Wait 2 are sufficient to control the detection of the UV peak . (See Chapter 4, "Troubleshooting," to troubleshoot Wait times.)

Part Numbers for the UV Tubing

Item	Part Number
Yellow (Flow Restrictor	4332156
Red	4332157
Blue	4332158
UV Tubing Kit (contains yellow, red, and blue tubing)	4332159

iScience. To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible.

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

07/2004

Applied Biosystems

Part Number 4352299 Rev. A