

Procedures for Use of Symphony Peptide Synthesizer

1. General Overview

1.1 Capabilities

- Synthesis of 1-12 peptides by solid phase peptide synthesis (Fmoc- or Boc- chemistry)
- Cleavage of peptides from resin with premixed cleavage cocktail
- Synthesis of nonnatural polyamides (β -peptides, peptoids, flu-labeled, biotinylated peptides)

1.2 Chemistry

- Standard practice in our lab utilizes the Fmoc-based method, with HBTU as the activating agent in the *absence* of HOBT (we are capable of using HOBT in our couplings, but the standard Symphony protocol omits it; this amounts to a choice of the individual user, and in fact, the presence of HOBT is known to minimize epimerization at the α -carbon of reacting amino acids). N-terminal Fmoc cleavages are with 20 % piperidine in DMF. Any unreacted amines can be capped with 5 % Ac_2O and 5 % N-methylmorpholine in DMF, though this step has become optional. It has been observed that the capping solution is not always rinsed out of the reaction vessel and can then interfere with subsequent deprotection and coupling steps. If you will be washing the sides of the reaction vessels manually between steps, there is no issue with the capping step. However, several syntheses have been successfully completed without the use of the capping step.
- For most peptides, the standard resin cleavage cocktail contains 83% TFA, with scavengers of triisopropylsilane (2.5%), ethanedithiol (2.5%), thioanisole (4%), phenol (4%), and water (4%). This reagent is similar to "Reagent K", the standard cleavage reagent used in Fmoc-based synthesis. Other resin cleavage cocktails with less toxic and malodorous reagents can also be used. See Section 3.1 for more details on choosing the appropriate resin cleavage cocktail.
- Our peptides are almost always synthesized on a 25 μmol scale. When more peptide is required, we typically use more than one reaction vessel.
- For information on the chemistry of peptide synthesis, see one of the following for the basics:
 - Novabiochem catalog – the back of this catalog contains an excellent tutorial (available online at <http://www.emdbiosciences.com/html/NBC/peptideres.htm>)
 - "Fmoc Solid Phase Peptide Synthesis. A Practical Approach", by W. C. Chan and P. D. White, Oxford University Press (Excellent basic text covering a wide range of solid phase peptide synthesis topics; the information in the Novabiochem catalog borrows heavily from Chan and White.)

2. Setting up your synthesis

2.1 Preparations prior to approaching synthesizer czar

- Decide what you want to make:
 - 1) Desired C-terminus (amide, acid, other); for C-terminal acids, an appropriate pre-loaded Wang resin needs to be ordered if we do not have it already
 - 2) Desired N-terminus (amine, acetyl, biotin, flu, other)
 - 3) Any unnatural amino acids? If so, order them.

2.2 Approach synthesizer czar

- Determine scheduling of instrument time; czar will try to optimize efficiency by having multiple people use the instrument simultaneously.
- Czar will instruct you when and with whom you will be working to set up the synthesis

2.3 Initial instrument setup

- Enter each peptide sequence into instrument separately; each will be a separate file
 - Click 'P' in top left of window (**Figure 1** – all figures at end of document)
 - Set Reagent Data File to desired setting ('standard' for α -peptides) (**Figure 2**)
 - Enter sequence, from N-C, using the keyboard (one letter codes) or by clicking sequentially on the buttons to the right of the screen
 - Set the C-terminus to either 'Acid' or 'Amide' (top of screen); if set on 'Acid', the instrument will *ignore* the C-terminal amino acid in your sequence, since this amino acid is assumed to be pre-loaded on a Wang resin
 - Set the chemistry for each step to the desired cycle (a 'cycle' is the complete list of events that will occur during the addition of one amino acid to your growing peptide)
 - As you entered your sequence, each amino acid was assigned the cycle that is displayed in the 'default' box in the top left of the screen. To change this setting, change the 'set' box to the desired cycle. Next, you must highlight the amino acid(s) you wish to set to this cycle, and then click 'Set'. The desired chemistry for each amino acid will be listed in the box above and to the right of the sequence.
 - Cycles are named according to what they will do; i.e., "deprotect2cplcap" will 1) remove Fmoc from the N-terminus, 2) couple the next amino acid, 3) couple the next amino acid again (double coupling), and 3) cap the unreacted sites with an acetyl group (See the chemistry tutorial for a detailed description of these events.)
 - The first amino acid in your sequence must use a cycle that first 'swells' the resin (not necessary if you are using TGR resin).
 - Custom cycles can be programmed; consult the czar if this is desired
 - Save your peptide by clicking on the disk icon on the toolbar; if necessary, create a new folder named according to your initials

2.4 Calculations

- Once each user has entered and saved all peptide sequences/chemistries, you will need to determine how much of each amino acid and HBTU to weigh, how much N-methylmorpholine (NMM) and acetic anhydride (Ac₂O) to measure out, and how much N,N-dimethylformamide (DMF) to dissolve each of these reagents in prior to putting them on the instrument, in addition to how much 20% piperidine in dimethylformamide (DMF) you will need to prepare.
- From the "Calculations" pull-down menu, select "Calculations", then "Open", and enter each peptide you will be synthesizing into the list (**Figure 3**).
- The "Calculations" menu is tabbed, such that the calculations can be viewed by clicking the appropriate tabs. In the screen detailing the amino acid masses/volumes (**Figure 4**), make sure there is enough of each amino acid available (next to the balance in room 303). In the screen for solvents and solutions (**Figure 5**), several alterations will be necessary:
 - 1) Solvents 5 and 6 are not required for synthesis; for clarity, you can set these to '0'.
 - 2) Note the amount of solvent 1 necessary for synthesis. The Czar should ensure there is sufficient DMF for completion of the synthesis.
 - 3) It is standard practice to overestimate the amounts of bottles 2, 3, and 4. Bottle 2 (20% piperidine in DMF) can be reused from previous syntheses, but make sure there is more than plenty (25% excess) available on the instrument.
 - 4) Set the necessary volumes of bottles 3 and 4 to between 10 and 20% excess above the required amount. This will ensure smooth completion of the synthesis. To set a new amount, enter the desired volume in the window and hit 'enter'.
 - 5) To properly set the values in the bottom section of the window, you must enter the new value for volume of bottle 3 again in the center window indicating activator (bottle 3) volume. This window does not automatically update. By entering the new volume for bottle 3, the entire bottom window should update the appropriate values. The numbers in this window indicate mass of required HBTU* and volume of required NMM, dissolved to the total volume in DMF; this entire window is solely for the preparation of bottle 3. ****When preparing the solution, add DMF first, followed by the NMM and HBTU. Do not add the HBTU directly to neat NMM**.** *Excess HBTU can lead to resin capping via guanidine formation. *The concentration of the activator solution (bottle 3) should never exceed the concentration of the amino acid solutions, which is entered to the right of the amino acid amounts in the calculations page.*

2.5 Making solutions and preparing the Symphony for launch

Note: Stocks of DMF and all other necessary reagents are kept in the flammables cabinet of the extra hood in room 305. Amino acids and coupling reagents are in the desiccators in room 303. Resins are in the refrigerator in room 311.

- Once the Calculations have been printed, everyone who is making peptides should coordinate and participate in making the solutions and preparing the instrument. Solutions should be made in clean glassware and placed in the proper bottles on the instrument. Ensure that the bottles already on the instrument are clean before using.

- **It is important that a FRESH bottle of DMF is used with the bottle-top dispenser for each new synthesis. All solutions should be prepared with FRESH DMF.** What remains of the old bottle can be placed into solvent bottle 1.
- Remember that all bottles on the instrument should have been cleaned after the previous synthesis, and therefore may contain residual acetone or ethanol; ensure the bottles are dry before use.
- Reaction vessels should also be clean, dry, and free of any residual resin, and collection tubes (15 mL polypropylene Falcon tubes) should be new and clean.

2.5.1 Amino acid solutions

- Amino acids should be weighed into 50 mL polypropylene Falcon tubes with DMF added to the proper volume. Occasionally, more than 50 mL of amino acid solution is required for a synthesis; in these cases, weigh and dissolve accordingly. Prior to placing amino acid solutions on the instrument, verify that all of the amino acid has dissolved.
- When measuring DMF into the 50 mL Falcon tubes containing amino acid, take advantage of the bottle-top liquid dispenser located in the peptide synthesis hood. This dispenser is far more convenient than graduated cylinders. In addition, the graduations on the 50 mL Falcon tubes are often wildly inaccurate.
- If the synthesis requires more than ~120 mL of an amino acid solution, not all of the solution can be placed on the instrument at one time. Initially dissolve only the amount of amino acid you will place on the instrument at the start of synthesis. Determine when the instrument will run out of that particular amino acid (acid solutions are not consumed evenly throughout the synthesis, so pay close attention to the sequences being synthesized) and plan to dissolve and add more amino acid at that time.

2.5.2 Solutions for solvent bottles 1-4

- Bottles 5 and 6 (CH_2Cl_2 and the cleavage reagent, respectively) can be ignored during synthesis set-up; they are used *only* during cleavage.
- The piperidine/DMF solution (Bottle 2) can be reused if it is fairly fresh (~ 2 weeks), and the DMF canister is never emptied; simply refill as needed, recycling the older DMF. No other solutions should be reused from previous syntheses, and should already have been cleared off the instrument following the previous synthesis.
- The Ac_2O /NMM solution in DMF (Bottle 4) should consist of 5-7 % each of Ac_2O and NMM.
- The activator solution (Bottle 3, HBTU/NMM in DMF) is the most perishable solution on the instrument. ****When preparing the solution, add DMF first, followed by the NMM and HBTU. Do not add the HBTU directly to neat NMM****

2.5.3 Preparing the reaction vessels

- The appropriate resins should be placed in the reaction vessels and positioned on the instrument. We typically do not label the vessels; instead, we rely on the computer to keep things organized. It is not, however, a bad idea to label the reaction vessels, especially in large batches.

- If resin remains on the sides of the reaction vessels, it is important to wash it down to the bottom of the vessel prior to synthesis. Use a disposable glass pipette and DMF to wash the resin down the sides; use manual instrument control (section 2.6) to drain the DMF from the vessel. It is easy to break the tips off of the glass pipettes, and this tip will remain inside the vessel for the entire synthesis. If the piece is capable of sequestering liquids via capillary action, it must be removed to avoid cross contamination of the synthetic cycles; otherwise, the glass itself will not be a hindrance to the synthesis.

2.5.4 Beginning the synthesis

- Open the "RV Operations" screen (**Figure 6**) by using the "RV Operations" pull-down menu.
- Decide which RV's you intend to utilize in your synthesis and open the respective peptide files in these slots.
- Confirm that your RV's are in the proper locations on the instrument, relative to your list in the RV Operations screen.
- Open the Bottle Preparation screen (**Figure 7**) by selecting the "Tools" pull-down menu, then "Bottle Preparation". You need to prime bottles 1, 2, 3, and 4; priming will also pressurize the bottles. Next, click the 'Amino Acids' tab (**Figure 8**) and select all of the amino acid bottles you will be using. Prime these bottles.
- Begin the synthesis by clicking 'Start' for each peptide you are synthesizing.
- If there are any errors, clear them to allow the synthesis to start. It is common to be asked to re-prime or re-pressurized various bottles. The instrument is fairly intelligent; it will not allow you to begin a synthesis without the proper tools in place. If you've put the chemistry together properly, the Symphony will make sure everything is primed, pressurized, and sealed. Good luck!

**** Special note** – if at any time the synthesis is paused for a significant period of time (i.e. greater than 8 hours), you should use the manual operations mode to swell the resin for about 30 minutes

2.6 Manual control of the instrument functions

- The Symphony can be controlled manually by using the Manual Operations screen. Select the "RV Operations" pull-down menu, select "RV Operations", then click the "Manual Operations" tab on the window that opens (**Figure 9**).
- The screen lists the 12 RVs and various functions. The Operation/Solvent function allows you to select the desired function or solvent (Bottle 1/DMF, Bottle 2/Piperidine, Collect Clean, RV rinse, etc.) Select the desired function, then the desired Mix Time (how long N₂ will agitate the solution after it is delivered to the RV), whether the Drain is on/off (selecting 'on' means the RV will automatically drained after the operation; 'off' means the solution will remain in the RV after the operation is finished), how many Repetitions (Rep) of the operation will be performed, and finally, the volume of solvent/reagent to be used (1 volume = 1.25 mL).
- Once your operation is entered, click 'Start' and the operation will be performed.

2.7 Special considerations for β -peptide synthesis or including non-natural amino acids

- If you wish to manually add your amino acid to the RV at a certain point you can insert a pause into your synthesis. First, click the Start/Stop button in the Automated RV Operations window. From here, choose the Pause tab and select which residues you would like to pause and at which steps. For a β -peptide synthesis you would insert a pause for each residue before each coupling step so that you can add the β -amino acid and coupling reagents before the instrument proceeds with the coupling. The instrument will then pause the synthesis at this point until you resume it. Remember that if there is a long pause, you should first swell the resin with DMF for 20-30 minutes. You can then use the manual operations screen to enter your mixing time and resume the automated synthesis when the coupling step is completed.
- For β -peptide synthesis, it has been found necessary to add an extra deprotection step after the 4th or 5th coupling. You just need to select a different synthesis program for these amino acids that includes the additional deprotection with DBU. You will also need to put this reagent on the instrument (this will be clear when you select the β -amino acid Reagent Data File instead of the standard one used for α -amino acids).
- For precipitation of cleaved β -peptides, using a 50:50 mixture of ether:pentane seems to be more effective especially when hydrophobic residues are involved. If this does not work, concentrating the solution by rotary evaporation is also useful.

3. Completion of the synthesis

3.1 Guide to choosing appropriate cleavage cocktails

- The cleavage cocktail composition has become more of a matter of preference among users. Depending upon the amino acids in a given peptide, fewer scavengers may be required. A suggested set of cleavage cocktails based upon amino acids used is posted on the hood by the synthesizer.
- Here are some useful online references if you want to learn more about finding the appropriate cleavage cocktail:
http://www.emdbiosciences.com/SharedImages/TechnicalLiterature/6_Fmoccleavage.pdf
http://staff.science.nus.edu.sg/~syao/protocols/Peptide%20Cleavage_protocols.pdf

3.2 Cleaving peptide from the resin

- Cleavage of peptide from your resin is independent of the synthesis set-up.
- Analogously to beginning the synthesis with the RV Operations menu, the Cleavage program will be chosen in the RV Operations menu by selecting the desired program on the right side of the screen.
- You must select a desired Cleavage program for each peptide. The two typical programs and their purpose are:
 - 1) "deprotectcapcleave", which removes the N-terminal Fmoc group, caps the peptide N-terminus with an acetyl group, and subsequently cleaves the resin with the TFA cocktail

that has been placed on the instrument (Bottle 6). (Note that the N-terminus is acetylated.)

- 2) "deprotectcleave", which removes the N-terminal Fmoc group and subsequently cleaves the resin with the TFA cocktail that has been placed on the instrument (Bottle 6). (Note that the N-terminus is a free amine.)
- The instrument will prompt you to select a time at which to perform cleavage. Your choices are:
 - 1) Now – the cleavage will begin immediately, assuming the synthesis is complete and Bottle 6 is charged with cleavage cocktail.
 - 2) At the end of synthesis - the cleavage will commence immediately following the completion of the synthesis.
 - 3) At a scheduled time - the cleavage will begin at the time you instruct the instrument to begin. This choice is very convenient, but carries an important risk; if the synthesis pauses due to an error that does not affect the cleavage program (not very likely, but it is conceivable), the cleavage *can* begin prior to the completion of synthesis!
 - Your peptide, following cleavage, will be deposited in the collection tubes (15 mL polypropylene tubes) as a solution of crude peptide in TFA/scavengers.
 - If you are cleaving your peptide(s) independently of the rest of the group, you will need to pause their syntheses in order to prime the solvent bottle. This is true for any other time you want to manipulate the solvent bottles during a synthesis. Also, pay attention to the important clean up steps in the next section.

****How much cleavage solution to use:**

There is a certain dead volume (~20mL) that is required to prime the solvent. Each peptide will require about 6mL of cleavage solution. Therefore:

- For 1 peptide – use 25mL of cleavage solution
- For 2 peptides – use 32mL of cleavage solution
- For 5 peptides – use 50mL of cleavage solution
- For all 12 peptides – use 100mL of cleavage solution

3.3 After cleavage... precipitation of the peptide from TFA solution

- Once the synthesis is completed and the cleaved peptide/TFA solution is deposited in the collection tubes (left side of the instrument in the vented cabinet), you will need to precipitate the peptide from the TFA solution. The most important thing to remember during this step is to *never* dispose of any of the solutions until you are sure your peptide has precipitate properly.
- The TFA solution is extremely hazardous; burns occur almost immediately upon contact. Wear nitrile (blue) gloves, safety glasses, and a lab coat while handling this solution. Additionally, when thiol scavengers are used, the stench is overwhelming. Minimize the time this solution is outside of a well-vented area. The stench is quite 'sticky'; until you have experience it, you may not believe it, but simply by contact with the vapors of this solution,

you can carry this odor with you for a quite annoying amount of time. For all of these reasons, do not forget to give this solution the respect it demands.

- In preparation for the precipitation of a peptide, cool two 50 mL polypropylene tubes containing 40 mL Et₂O each to -78° C (sink the capped, filled tubes into the dry ice chest for at least 20 minutes). Use the blue tubes available at Kline Stockroom; the caps seal much better than the orange tubes. Also, confirm that you are using polypropylene tubes; polystyrene will dissolve in the Et₂O.
- Carefully pour the crude peptide/TFA mixture into one of the cold Et₂O tubes. Ideally, you will observe cloudiness in the solution.

****Important note about the precipitation of peptides****

There is the potential for the release of CO₂ gas when you pour the crude peptide/TFA mixture into the cold Et₂O tube. If you see a strong amount of bubbling that persists when you shake the tube, you should split the solution into two Falcon tubes before putting it into the centrifuge to allow enough room for any residual pressure to be released. It is a good idea to shake the tubes and vent them to ensure that you do not have this issue.

- Immediately cap the tube and centrifuge (use the swinging-arm centrifuge in the hot room deli case) at 3 krpm for 5 minutes. If you are only precipitating one peptide, you will need a suitable balance tube.
- Return to the hood and observe the centrifuged tubes; our typical peptides, at a 25µmol scale, should result in a milky white to off-white solid in the bottom of the tube that nearly fills the 'conical' area of the tube. If the peptide failed to precipitate, the solution must be concentrated by rotary evaporation; the resulting residue can either be purified or another attempt at precipitation can be made.
- Pour off the supernatant Et₂O into a waste container (do not discard until you've identified your peptide; in some cases your peptide yield may be low and more peptide can be recovered from the supernatant), being careful not to pour the peptide 'pellet' out.
- Resuspend the peptide 'pellet' in the 2nd batch of cold Et₂O by pouring the chilled Et₂O into the centrifuged tube containing the pellet. Cap tightly and shake vigorously to ensure the peptide is distributed in the Et₂O. You may need to vent the tube periodically; the Et₂O sometimes builds up pressure during this process.
- Repeat the centrifuging process, decant the supernatant, and dry the crude peptide pellet with a gently stream of N₂ (a pipette tip or glass disposable pipette attached to a Tygon tube works well). The pellet should crack in several places, like a dried desert.
- Once the peptide is dried to the point that it is in several small pieces, with some powder peptide visible, it can be resuspended in a small amount of water/acetonitrile (typically 1:1) and lyophilized before it is stored at -20°C or purified by HPLC.

****Important note about the cleavage process****

If you cleave your peptide(s) before the rest of the group, you need to perform the following cleaning operations as soon as possible (at least within 12 hours of the completion of the cleavage step). These steps can conveniently be completed while you are precipitating your peptides.

- 1) Remove reaction vessels from instrument and replace with white flow tubes

- 2) Clean the reaction vessels using acetone and water (for details see cleaning check list)
- 3) Place new 15mL Falcon tubes in the appropriate collection positions and click “Rinse” in the automated operations screen for each reaction vessel (if this option is not visible, switch to manual operations and select “collect clean” from the pulldown menu for each reaction vessel)
- 4) In the manual operations screen, perform “RV clean” for each position used.

4. Post-Synthesis clean up of the Symphony

- After synthesis and cleavage is complete, the instrument must be cleaned and prepared for the next user(s) – a cleaning checklist should be obtained from the drawer below the computer; more details for the steps on this checklist follow:
- Remove the reaction vessels from the instrument and replace them with the white flow tubes
- Clean the reaction vessels (stench!!!) using an acetone squirt bottle and water if necessary while holding the vessel over an appropriate waste bottle in the hood. Clean the vessels inside and out, flush solvent through *both* ends of the vessel/frit, and make sure all resin is rinsed from the vessels. The caps should also be rinsed. When finished, dry the vessels with N₂ or air. The vessels should be free of odor and the frits should be white. Place them in the drawer under the Symphony computer.
- On the instrument, place clean 15 mL conical tubes in 'collect' positions that were used in the synthesis. In the 'automated operations' screen, click 'rinse' to the left of any RV position that was used. This will clean the lines between the RV and the collect tubes. Note that solvent will be deposited in the collect tubes during this function. (This can also be accomplished by using 'collect clean' in the 'manual operations' screen.) The instrument will prompt you if any necessary bottles are not pressurized or primed. Once finished, the tubes where rinse solvent was deposited should be removed from the instrument, poured into waste, and put back on the instrument (further cleaning will deposit *more* solvent in these tubes).
- Next, in the 'manual operations' screen, perform 'RV clean' for each RV position used in the synthesis. The instrument will prompt you if any necessary bottles are not pressurized or primed.
- In the 'bottle preparation' window (accessed in the 'setup' pulldown menu), backflush all bottles with N₂.
- Remove bottles 3, 4, and 6 from the instrument, wipe the lines and inlet tubes, pour the solvent in waste, and put the bottles back on the instrument (do not clean the bottles at this point).
- Remove all amino acid bottles that were used, pour any remaining solvent into waste and put them back on the instrument (do not clean the bottles at this point).
- Retrieve the ethanol wash bottle (4 L) from under the hood (the bottle should have at least 1 L of ethanol in it; if not, fill the bottle using ethanol from one of the 20 L containers in the lab. Remove the inlet line from bottle 2 (20% piperidine in DMF), wipe it clean, and place it in the ethanol 4 L bottle. Bottle 2 can stay in its place, with the Ethanol wash bottle sitting on top of the waste carboys.
- In the 'bottle preparation' window, prime bottles 1, 2, and 5.
- Perform 'system solvent flush'. This is accessed through the 'tools' menu, followed by accessing the 'cleaning' menu. The instrument will prompt you if any necessary bottles are

not pressurized or primed, and to remind you to place collect tubes and rinse tubes in all positions.

- When the system solvent flush is complete, select 'system N₂ flush' from the 'bottle preparations' window.
- Remove all collect tubes from the instrument and replace with clean tubes.
- Remove all amino acid bottles from the instrument, pour solvents into waste, rinse with acetone, dry with N₂ or air, and place on the instrument in the proper positions.
- Remove bottles 3, 4, and 6 from the instrument, wipe the lines and inlet tubes, wash each bottle with acetone, dry with N₂ or air, and replace on the instrument.
- Take the inlet line from the ethanol wash bottle, wipe it, and replace it into bottle 2 (20% piperidine in DMF). Place the ethanol bottle back into storage under the hood. If it is nearly empty, please refill it using one of the 20 L ethanol containers in the lab.
- Notify the czar that the Symphony is now squeaky clean. The czar will check DMF and CH₂Cl₂ levels (replenishing as necessary) and drain waste if necessary.

5. Maintenance Notes (pertaining mainly to synthesizer czar)

Contact for service: Peter Bergwall, Manager, Service and Tech. Support (PTI)

Phone: 781-935-1942

Fax: 781-935-1945

email: peter.bergwall@pti-instruments.com

- Standard cleaning of the instrument is outlined in the Cleaning section.
- The most important periodic maintenance need on the Symphony is the Reagent Line Clean. Perform this step every 4-6 weeks, depending on instrument usage. This step helps prevent blockages due to amino acid crystallization.
- The N₂ tank will run out occasionally; replace with a new tank from the stockroom. Tanks are located across from the stockroom, and you will need a Yale I.D. to enter. Obtain a new tank (fill out the check-out form on the appropriate clipboard) and place the old tank in the 'empty tank' corral (fill out the check-in form on the appropriate clipboard). When filling in the forms, you will use the tank I.D. number on the small bar-code sticker near the neck of the tank. To change the N₂ tank, you must first disconnect the empty tank from the instrument and vent the line. Next, you need to vent the solvent bottles and reagent bottles. Once this is complete, you can replace the empty tank with the new one (use Teflon tape for a good seal). Turn on the tank and repressurize all solvent and reagent bottles. You should check after an hour or so to see that the gauge on the left reads between 70-80psi and adjust this as needed.
- The waste tanks are emptied into Yale EHS carboys by using the plastic siphon. When finished, the siphon should be wiped dry, wrapped in a lab diaper, and placed at the back of the hood.
- The frits of the reaction vessels should be fired every 6 months. They can also be cleaned with NaOH to remove silanizing solution that is causing them to clog.
- The frits for the amino acid bottles and solvent bottles should be replaced every 6 months
- It is important to silanize the RVs every few syntheses using SigmaCote to prevent resin from sticking to the sides (if this is noticed, silanize before next synthesis)

For long term shut down of the instrument:

- 1) Perform system solvent flush
- 2) Turn off Symphony, computer, and N₂ supply tank
- 3) Cool down the pneumatic pump for 10 minutes by disconnecting the "vacuum" and "air in" lines. Plug the male connectors into separate female connectors (located with Symphony supplies). After 10 minutes you can unplug the pneumatic pump (at the base of the instrument).

To adjust the air pressure (if not reading air pressure on computer):

Check pressure indicator on back on instrument (should read 80-90psi). If it is lower than 80psi, remove the back panel and adjust the regulator on the lower left.

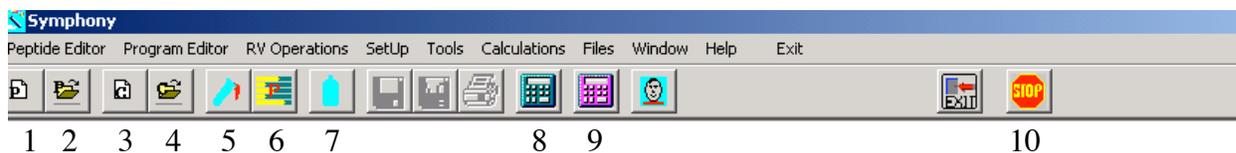
For solvent tank 1 leakage:

Remove the fitting on the left by pulling down on the outside part and then pulling the whole fitting up. Try securing the fitting again. If this does not solve the problem, try tightening the upper-most screw on the fitting. To replace the fitting, cut the tubing close to the top of the old

fitting with a razor blade or wire cutters. Remove the old fitting. Run the tubing through the top of the new fitting and tighten the upper-most screw. Replace the fitting on the solvent tank.

Figures

Figure 1 – Toolbar



- 1: New peptide file
- 2: Open peptide file
- 3: New synthesis/cleavage program
- 4: Open synthesis/cleavage program
- 5: RV operations screen
- 6: Synthesis progress screen
- 7: Solvent/reagent bottle prime/pressurize
- 8: Data calculations
- 9: System calculator
- 10: Emergency stop

Figure 2 – New peptide file window (sequence editor)

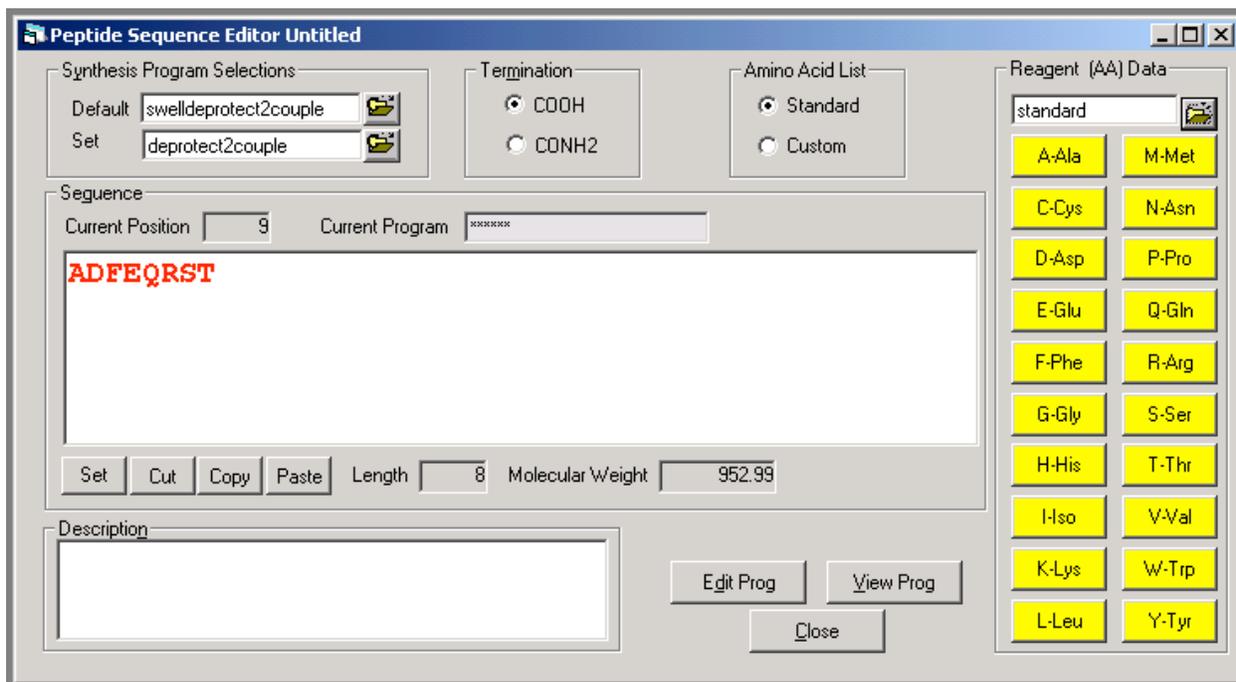


Figure 3 – Calculations window 1 (peptides tab)

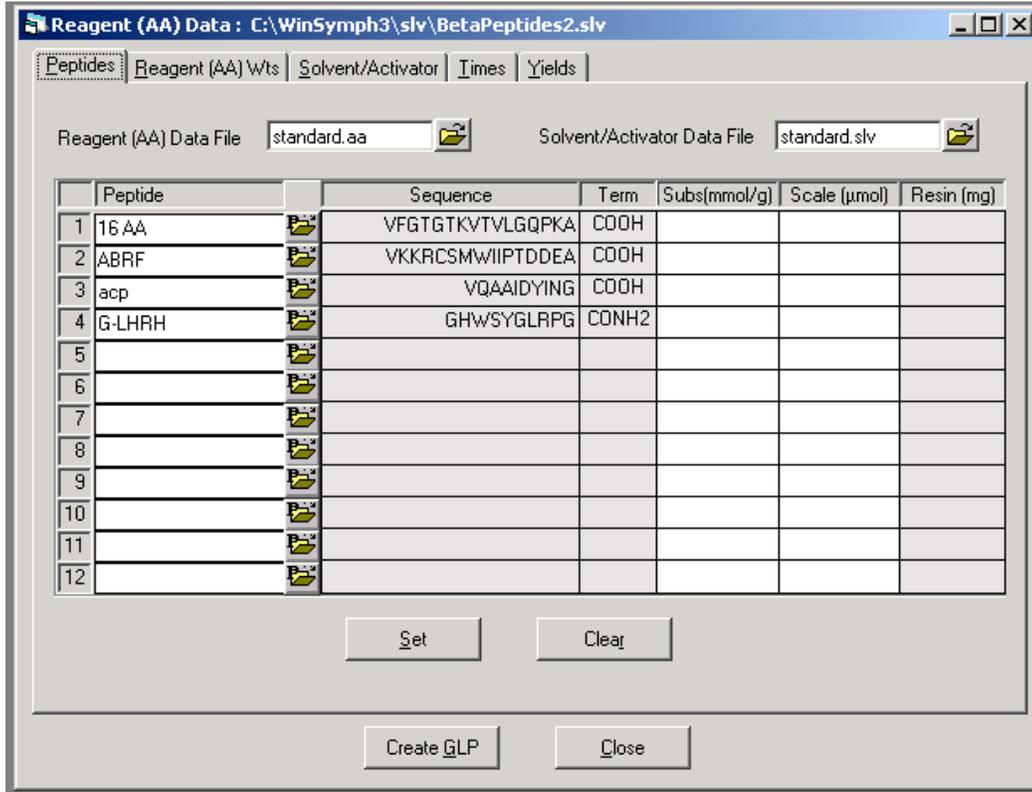


Figure 4 – Calculations window 2 (reagent/AA tab)

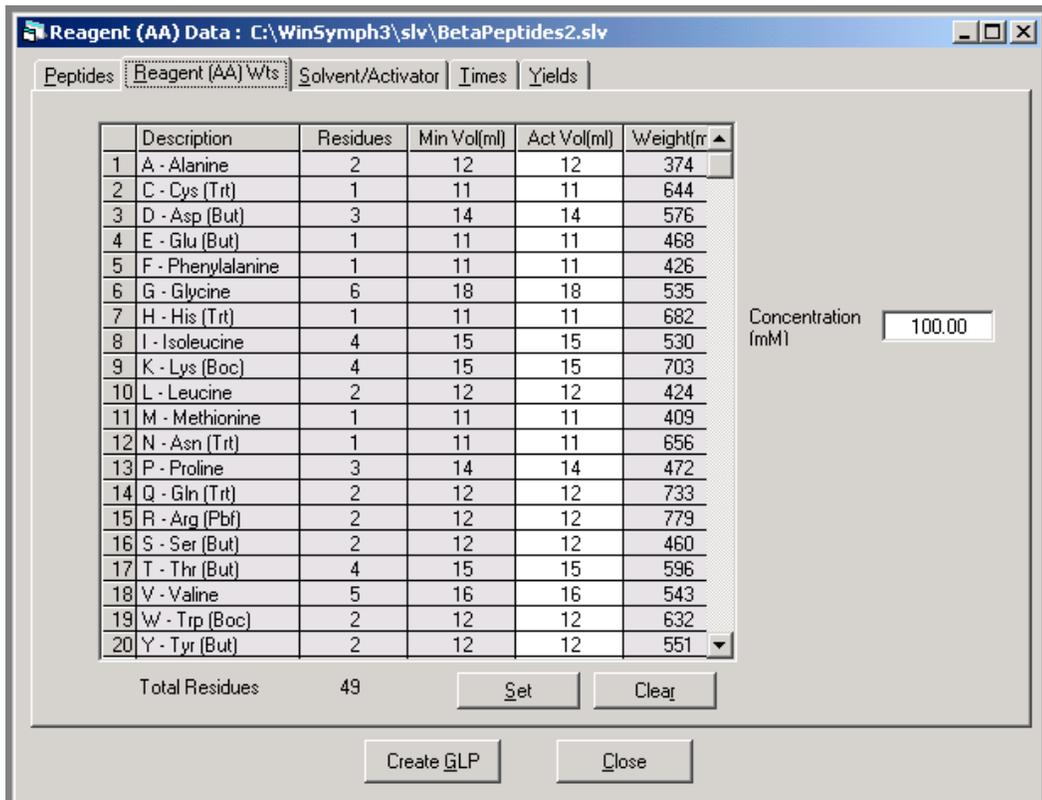


Figure 5 – Calculations window 3 (solvent/activator tab)

Reagent (AA) Data : C:\WinSymph3\slv\standard.slv

Peptides | Reagent (AA) Wts | **Solvent/Activator** | Times | Yields

Solvent	Description	Min Vol (ml)	Act Vol (ml)
SOLV 1	DMF	1382	1382
SOLV 2	20% Piperidine/DMF	132	132
SOLV 3	Activator/NMM/DMF	71	71
SOLV 4	5%Ac2O/5%NMM/DMF	10	10
SOLV 5	Methylene Chloride	10	10
SOLV 6	Cleave Cocktail	10	10

Actual Volume (ml)

Description	Conc (mM)	Vol (ml)	Weight (g)
NMM	400.00	3	2.883
HBTU	100.00	0	2.693
PyBop	100.00	0	3.140
	100.00	0	.000
	100.00	0	.000

Create GLP | Close

Figure 6 – RV operations screen

Reaction Vessel Operations

Automated Operations | Manual Operations

Run	RV	Peptide	Length	AA#	Program	Step	Reagent	Mix Time	Rep	Action	Cleavage	CLV
Start	1							00:00:00				
Start	2							00:00:00				
Start	3							00:00:00				
Start	4							00:00:00				
Start	5							00:00:00				
Start	6							00:00:00				
Start	7							00:00:00				
Start	8							00:00:00				
Start	9							00:00:00				
Start	10							00:00:00				
Start	11							00:00:00				
Start	12							00:00:00				

Set | Start All | Start/Stop | Clear | ViewReport

Close

Figure 7 – Bottle preparation screen 1 (solvent bottles tab)

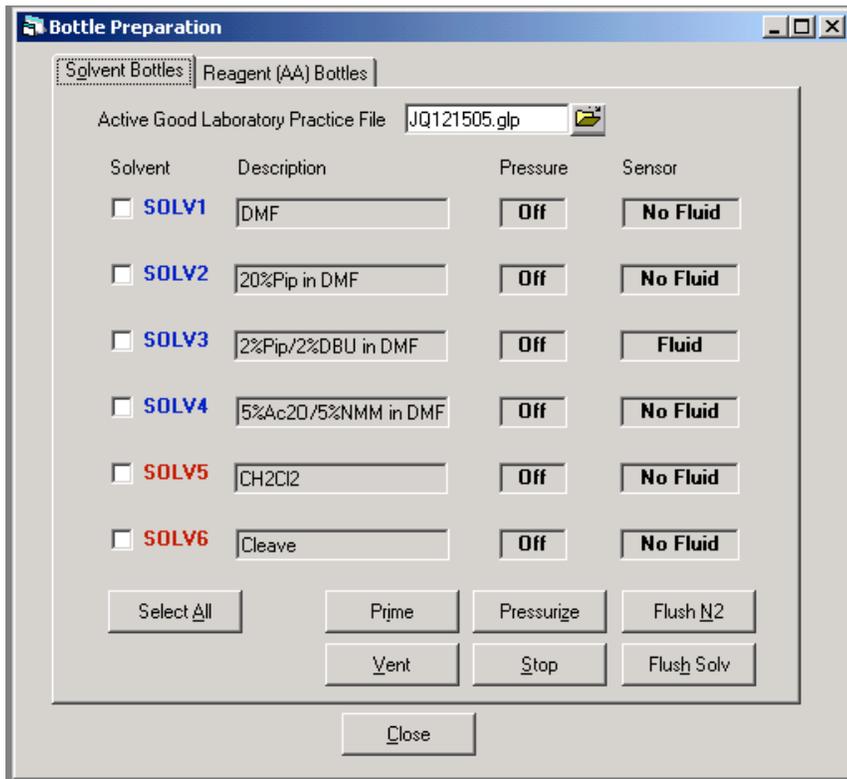


Figure 8 – Bottle preparation screen 2 (reagent/AA bottles tab)

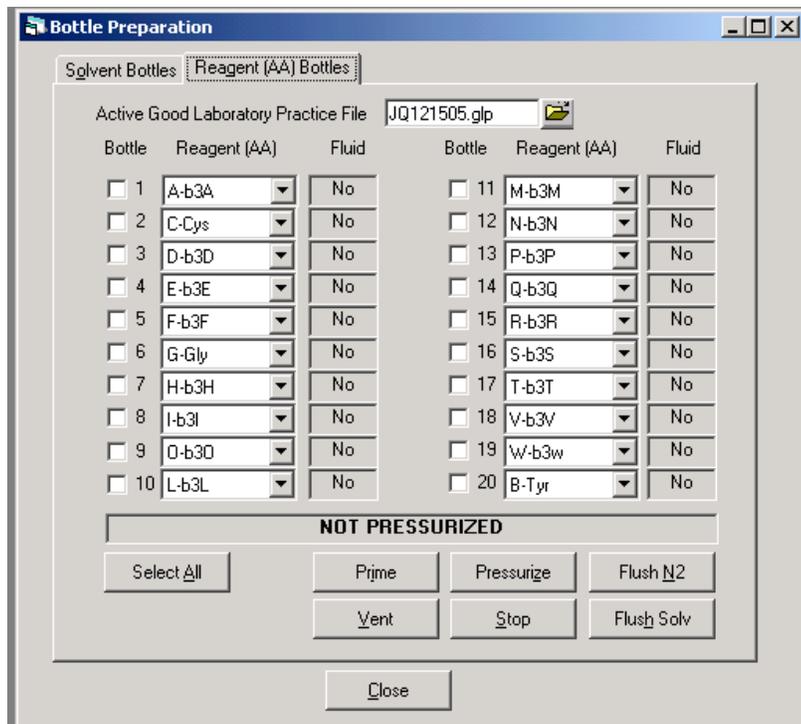


Figure 9 – Manual operations screen

The screenshot displays the 'Reaction Vessel Operations' software window. It features two tabs: 'Automated Operations' and 'Manual Operations', with the latter being the active tab. The interface contains a table with 12 rows of manual operations, each with a 'Start' button and a red indicator light. The columns are labeled: Run, RV, Operation/Solvent, Reagent (AA), Mix Time, Drain, Rep, Vol, and Action. Below the table are three buttons: 'Set', 'Pause All', and 'Clear'. At the bottom center is a 'Close' button.

Run	RV	Operation/Solvent	Reagent (AA)	Mix Time	Drain	Rep	Vol	Action
Start	1	DMF		00:00:00	ON	1	1	
Start	2	20%Pip in DMF		00:00:00	ON	1	1	
Start	3	Collect Clean		00:00:00	OFF	1	1	
Start	4	RV Clean		00:00:00	ON	1	1	
Start	5	DMF		00:00:00	ON	1	1	
Start	6	DMF		00:00:00	ON	1	1	
Start	7	DMF		00:00:00	ON	1	1	
Start	8	DMF		00:00:00	ON	1	1	
Start	9	DMF		00:00:00	ON	1	1	
Start	10	DMF		00:00:00	ON	1	1	
Start	11	DMF		00:00:00	ON	1	1	
Start	12	DMF		00:00:00	ON	1	1	

Buttons: Set, Pause All, Clear, Close

Ordering Chemicals and Supplies for the Symphony

We have standing quotations and special pricing for most of our reagents:

From American Bioanalytical

(Contact Wayne Gagnon, President/CEO, wayne@americanbio.com or 800-443-0600)

Chemical	Quantity	Catalog #	Quoted Price
NMP	4 x 4 L	AB07501-1600	\$234.40
DMF	4 x 4 L	AB00450-1600	\$115.00
NMM	1 L	AB09016-1000	\$41.28
Piperidine	1 L	AB02304-1000	\$45.36
TFA	500 g	AB02010	\$24.00
Acetic Anhydride	1L	AB02307-01000	\$48.45
HBTU	100 g	AB00883-00100	\$165.57
HOBt	100 g	AB00881-00100	\$35.00

From VWR (Novabiochem)

(Contact Dave Syvertsen, Yale Chem Dept. VWR Salesperson, 1-800-947-4270 ext. 4262)

Chemical	Quantity	Catalog & Line #	Quoted Price
Fmoc-Ala	25 g	80507-084 Line 1	\$ 28.50
Fmoc-Arg(Pbf)	25 g	80507-096 Line 16	\$299.00
Fmoc-Asn(Tr)	25 g	80507-112 Line 12	\$187.00
Fmoc-Asp(tBu)	25 g	80507-128 Line 3	\$170.00
Fmoc-Cys(Trt)	25 g	80507-148 Line 24	\$116.00
Fmoc-Gln(Tr)	25 g	80507-244 Line 15	\$187.00
Fmoc-Glu(tBu)	25 g	80507-264 Line 4	\$110.20
Fmoc-Gly	25 g	80507-276 Line 6	\$ 28.50
Fmoc-His(Tr)	25 g	80507-284 Line 7	\$133.76
Fmoc-Ile	25 g	80507-292 Line 8	\$ 28.50
Fmoc-Leu	25 g	80507-306 Line 10	\$ 28.50
Fmoc-Lys(Boc)	25 g	80507-314 Line 9	\$ 95.00
Fmoc-Met	25 g	80507-332 Line 11	\$ 28.50
Fmoc-Orn(Boc)	25 g	80507-342 Line 13	\$240.00
Fmoc-Phe	25 g	80507-366 Line 5	\$ 28.50
Fmoc-Pro	25 g	80507-376 Line 14	\$ 28.50
Fmoc-Ser(tBu)	25 g	80507-388 Line 17	\$170.00
Fmoc-Thr(tBu)	25 g	80507-406 Line 18	\$170.00
Fmoc-Trp(Boc)	25 g	80507-416 Line 20	\$278.80
Fmoc-Tyr(tBu)	25 g	80507-438 Line 21	\$104.50
Fmoc-Val	25 g	80507-446 Line 19	\$ 28.50
HBTU	25 g	80508-172 Line 22	\$ 71.40
HOBT	25 g	80508-176 Line 23	\$ 20.00
Rink amide resin	5g	80013-794	\$140.00
NovaSyn TGR resin	5g	80012-720	\$273.60
Wang resin	5g	80013-796	\$ 35.00

Most other reagents can be ordered from Sigma-Aldrich

Chemical	Quantity	Catalog #	Quoted Price
TIPS (SureSeal)	50g	233781-50G	\$95.10
TIPS	10g	233781-10G	\$25.70
Thioanisole	100mL	88470-100ML	\$30.70
1,2-Ethanedithiol	100mL	02390-100ML	\$34.20
1,8-diazabicyclo- [5.4.0]-undec-7-ene, 98% (DBU)	100g	139009-100G	\$52.40
N,N- diisopropylethylamine	100mL	550043-100ML	\$31.50
SigmaCote	100mL	SL2-100ML	\$65.10

Synthesizer Cleaning Check List

Steps 1-5 must be completed within 12hr of completion of cleavage step (see Liz if this is a problem). **Each step** must be initialed by the individual performing it

- _____ 1) Remove reaction vessels from instrument and replace with white flow tubes (make sure all have either black or white o-rings on both ends)
- _____ 2) Clean the reaction vessels using acetone and water
 - ALL** resin removed
 - Both ends of vessels flushed out
 - Caps washed completely
 - Outside of vessels washed
 - Vessels dried completely with nitrogen
 - Vessels capped and placed in drawer under computer
- _____ 3) Place new 15mL Falcon tubes (polypropylene ONLY, NOT polystyrene) in collection positions and click "Rinse" in automated operations screen (if this option is not visible, switch to manual operations and select "collect clean" from pulldown menu for each reaction vessel)
- _____ 4) In the manual operations screen perform RV clean for each position used
- _____ 5) In the bottle preparation window backflush all bottles with N₂
- _____ 6) Remove bottles 3,4,and 6 from the instrument, wipe the lines and inlet tubes to remove all reagents, pour solvent into waste, and return bottles to machine
- _____ 7) Select all the amino acid bottles and flush with N₂, then remove amino acid bottles, empty into waste and return them to the instrument
- _____ 8) Remove lines from bottle 2, rinse with EtOH, and wipe dry
- _____ 9) Place EtOH bottle from hood (labeled for cleaning) on instrument line 2 (at least 1L of solvent must be present in bottle)
- _____ 10) Perform System solvent flush (approx. 1.25hr) under tools → cleaning menu
- _____ 11) Perform Reagent line clean (approx. 2hr, ask Liz if this is necessary!)
- _____ 12) Perform system N₂ flush (approx. 20 min)
- _____ 13) Remove collection tubes and replace with new clean POLYPROPYLENE tubes
- _____ 14) Remove and clean all amino acid bottles
 - Empty bottles into waste
 - Rinse a couple of times with acetone
 - Dry with N₂ (Liz will check to ensure they are dry!)
 - Return bottles to instrument
- _____ 15) Remove and clean bottles 3,4, and 6
 - Follow procedure outlined for cleaning amino acid bottles
 - Wipe the inlet lines clean before returning bottles to the machine
- _____ 16) Remove EtOH cleaning bottle, wipe the inlet line DRY and return the line to the piperidine bottle
- _____ 17) Pick up glass and other waste and wipe any spills in the area around the computer / printer, the top of the synthesizer, and the floor in front of it
- _____ 18) Clean up everything from waste hood (graduated cylinders, pipettes, paper, paper towels, gloves, extra tubes)
- _____ 19) Latch waste funnel and replace waste bottle in the hood if it is full
- _____ 20) Refill acetone cleaning bottles

***** **Tell Liz that the machine is clean and give her this paper** *****