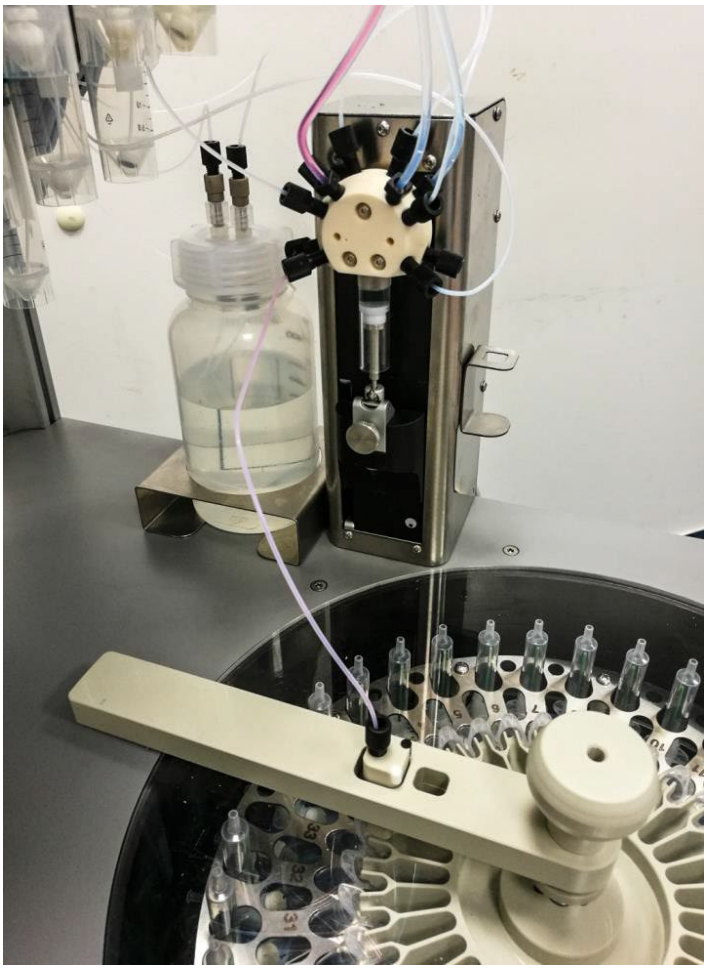
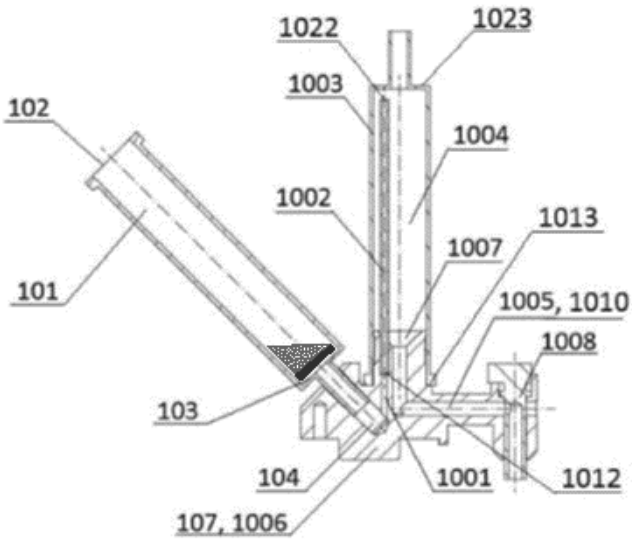
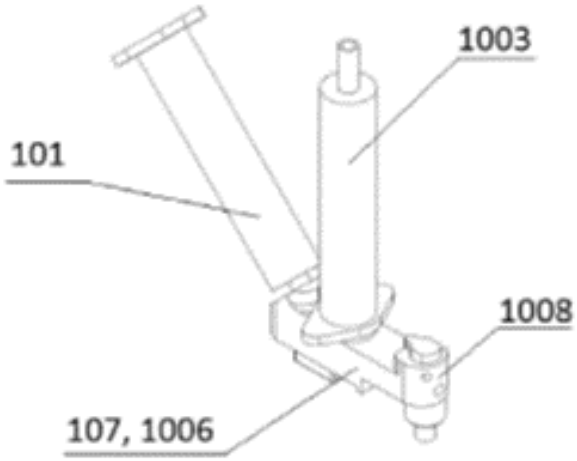
The Centrifugal Syringe Synthesizer SPYDER Mark IV

The CSS SPYDER allows the operator to run multiple peptide synthesis in parallel automatically. In any step of the synthesis, the operator may remove an individual reactor and perform a special operation requiring special conditions or utilizing very sensitive or special reactant manually. After that step, the reactor can be returned to the device for continuation of the oligomer assembly. It therefore enables the synthesis of peptides containing even very sensitive, costly or valuable monomeric units, which need special treatment and conditions.

The synthesizer was developed by Spyder Institute Praha and Institute of Organic Chemistry and Biochemistry of The Academy of Sciences of Czech Republic. It is covered by European Patent EP 17206537.7 (US-2020-0384435-A1).







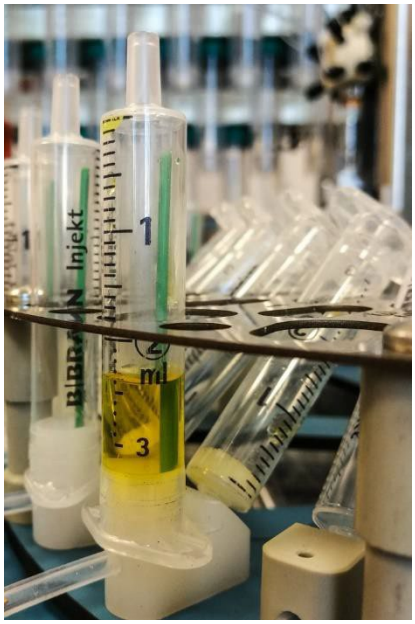
The reactors in this synthesizer are plastic syringes equipped with frit (101,102,103). The reactors and the trap outflows (1002) are positioned in the intermediary transfer container (ITC, 1004) in a way that the content of liquids in the reactor reaches a level which is vertically lower than the second end of the trap outflow (1022). The trap outflow thus operates on a siphoning principle: when the rotation speed of the centrifuge is low, the liquid in the reactor is not capable of overcoming the gravity based resistance of the trap outflow and remains fully inside the reactor. When the rotation speed of the centrifuge increases, the liquid from the reactor enters the trap outflow from the nozzle of the lower end of the reactor and continues through the trap outflow, and is removed from the reactor through the trap completely. The removed liquid is trapped in the second vertically placed syringe (ITC, 1004) and after the centrifugation stops, it flows out of the second syringe into the channel leading to waste container.

Removal of liquids using centrifugation and trap outflow is significantly more efficient than a simple aspiration of liquids.

Connection (1005) from ITC can be optionally equipped with spigots (1008). If the spigots (1008) are closed, the outflow from the reactor can be collected. (This may be used for example during final cleavage of the peptide from the resin.) Rotors with spigots are not a part of standard synthesizer setup.

Centrifugal liquid handling can be best demonstrated on the following pictures:

During the coupling the liquid is in the reactor (syringe)

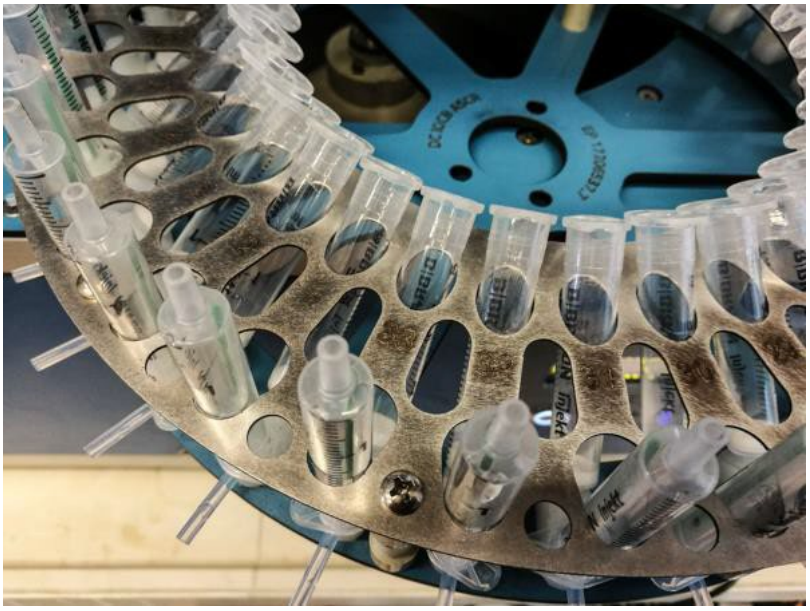


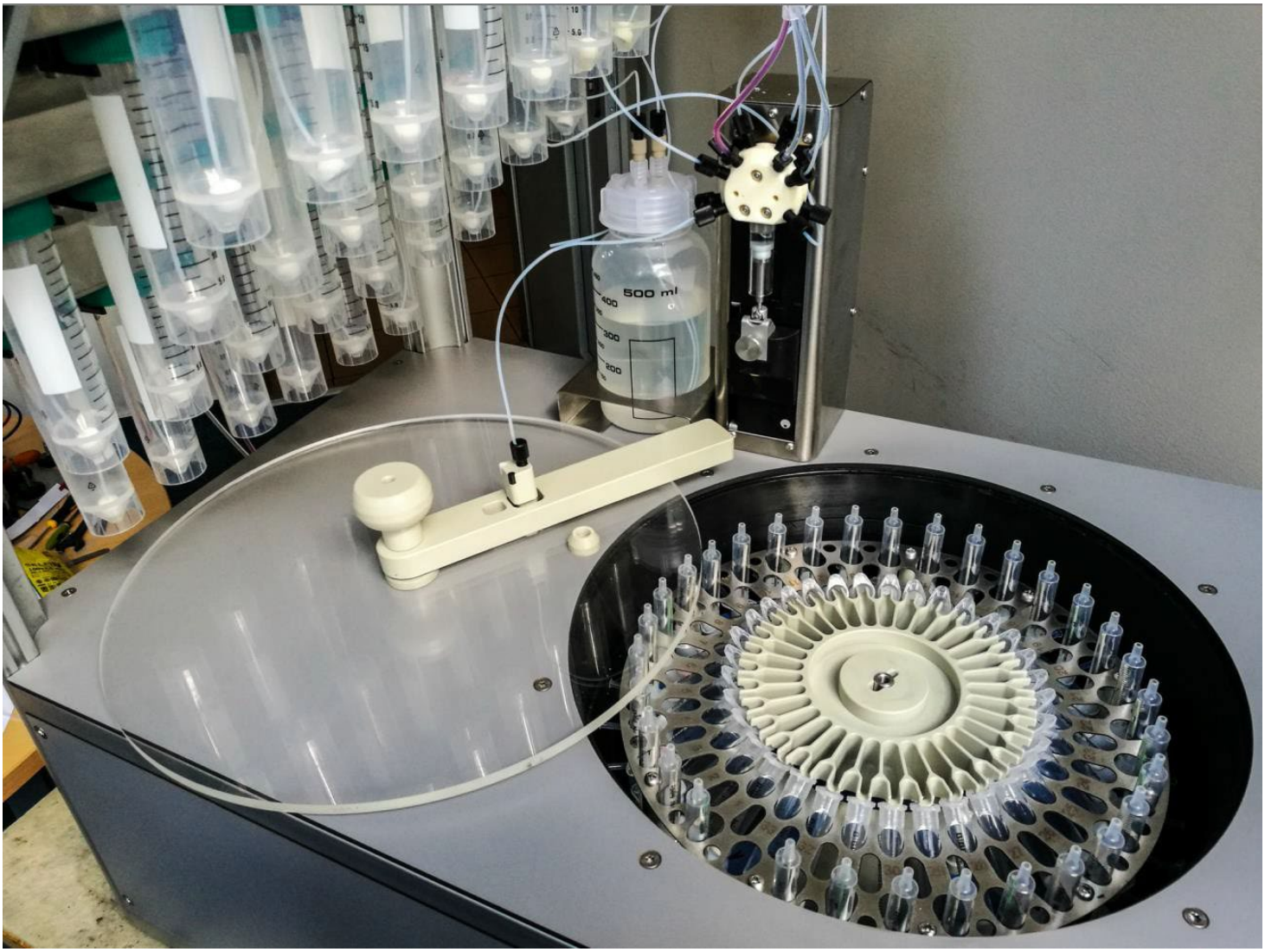
Centrifugation transfers the liquid into the intermediary container (vertical syringe)

From the intermediary container the liquid flows to the drain

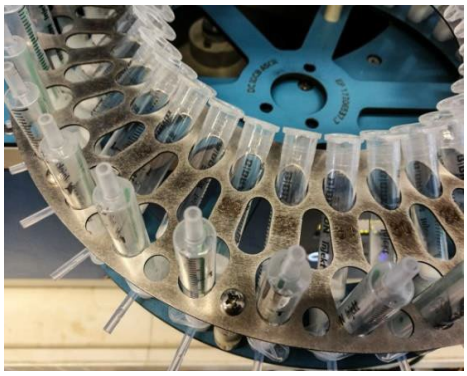
Another short centrifugation makes sure that there is no liquid in the drainage tube

Above the centrifuge rotor with reactors, a distribution insert can be positioned. It has a shape of a circular disk and is made of polypropylene. The distribution rotor can be used for preactivation of amino acids before addition to the reactors with the solid support (syringes).





How to make good peptides on Centrifugal Syringe Synthesizer SPYDER Mark IV

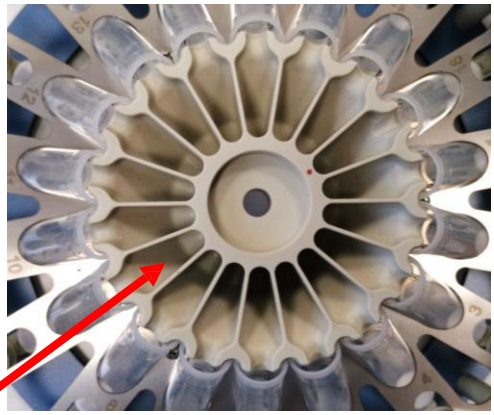


Check the inside of the centrifuge drum. You may take out the rotor and check the drum – there should be no liquid inside.

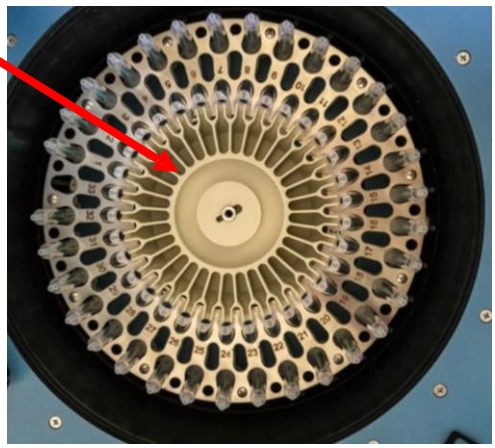
Make sure that the three pins at the center of the centrifuge are fitting in the holes of the rotor. Place the central safety insert on the metal axis and secure it with the top screw – it makes sure that the rotor sits securely on the pins of the centrifuge.

Make sure that all syringes are sitting well in the luer connection in the rotor. Don't be shy to press on them with reasonable force. The tops of all syringes should be at the same level.

Fill the syringes with equibuyoant suspension of resin (~30 mg per aliquot in DMF/DCM mixture). Alternatively you can fill the syringes with dry resin outside of centrifuge before inserting them. In this case make sure that no resin is clinging to the syringe wall. You can use the syringe piston to push the resin to the bottom. It is recommended that you use new syringes provided by the synthesizer distributor – reusing the syringes increases the chance that resin would become trapped in the scratches on the inner wall of the syringe and will not be exposed properly to the reactants. (The cost of new syringe is negligible in comparison with the cost of the need for new synthesis or extensive purification.)



In the case that you are using the distribution multichamber preactivation insert (MPI), the top screw also secures the MPI on top of the syringes. Please pay attention to placing the output channels properly on the syringe openings. The chamber #1 must be placed on top of syringe #1. On the left of the chamber #1 is the last chamber (18 or 33) which is used for the outflow for the washing procedure and in the case of 18-chamber, insert is slightly different than the other chambers (has lower wall in the direction to the syringe allowing easier outflow from this chamber preventing the overfilling) and is clearly marked with the red dot.



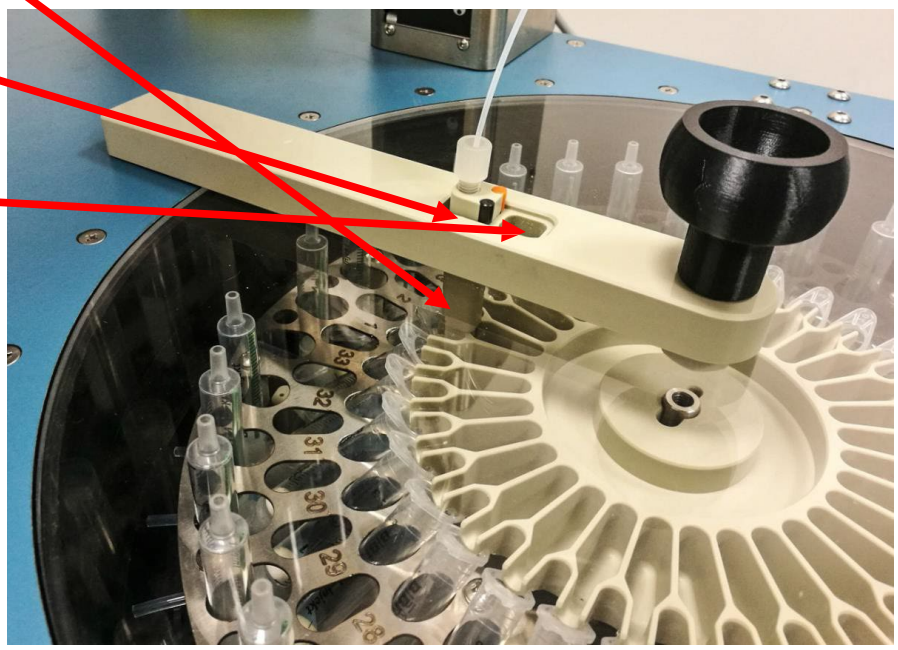
After placing the rotor in the machine, cover it with glass cover with attached nozzle. The nozzle placement depends on the rotor configuration and the mode of delivering reagents:

18 10 mL syringe rotor or
33 2.5 mL syringe rotor

Direct addition to syringes (without MPI)
or **Premix in Pump** (without MPI)
or **Addition using preactivation** (with MPI)

When the nozzle is pointing to the syringe (to the left), then you will be using **Direct Addition** to the syringes or **Premix in Pump**. When it is pointing to the right, you will be using **Addition using preactivation** (with MPI).

The nozzle should be in the left opening of the cover glass assembly for synthesis in 33 syringes rotor and in the right opening for using 18 syringes rotor. The opening without the nozzle should be covered with the safety assembly to prevent excessively low pressure inside the centrifuge when using the vacuum pump to remove the vapors from the drum.



You may first run just "Deprotection/Wash" program with 3x delivery of 0.5 ml of DMF to swell and precondition the resin. Checking inside of the drum after that before starting the synthesis makes sure that all syringes are sitting tightly in the positions and there are no leaks.

Magnet in the glass assembly assures that the centrifuge cannot be run without cover placed on top of the rotor.

Make solution of all needed amino acids (0.4 M AA in 0.4 M HOBt in DMF).
Attach the solutions in Falcon tubes to appropriate locations (you can use the color-coded Falcon tubes and Locations on the machine, if the machine is color coded).
Fill lines of 28-way distribution valve (use function “Prime lines” in Pump window using 0.8 mL as priming volume).

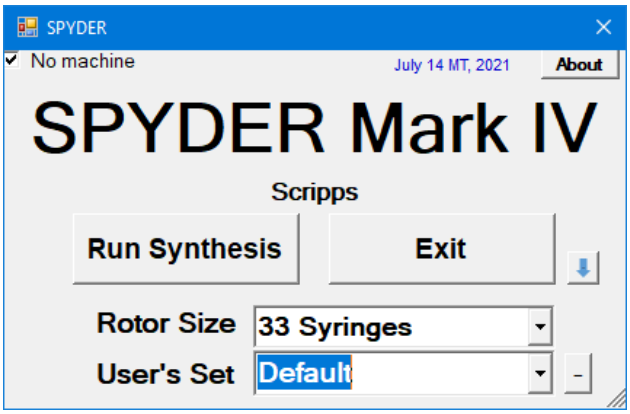
Connect all reagents and fill lines of pump (use function “Prime lines” in Pump window).

Make sure that the exhaust line is connected to vacuum pump through the waste solvent collecting container.

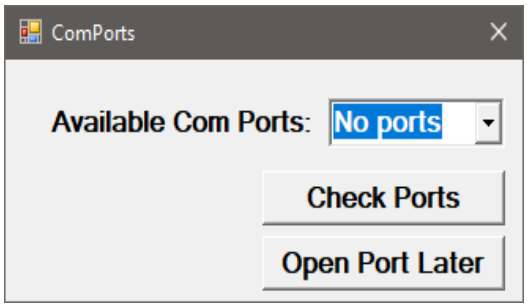
Run the machine and hope for the best…

Sample synthesis

Click the SPYDER button on the Windows main screen and this window will appear:



If you see the “No machine” checkbox in the upper left corner, then your computer is not connected to the machine and the window ComPorts will open on the left side of the SPYDER window. Check the connection (plug in the USB cable) and click “Check Ports” button. Select the port and click “Open Port” button. The ComPorts window can be activated going from the main menu to “Parameters” button and in the window which will open clicking “Show ComPorts” button.



If clicking “Check Ports” button will show “COMXX”, then you will be able to open that port. You can also choose “Open Port Later” and use the software for planning the synthesis and open the port just before the synthesis. You can also generate the synthetic protocol and save it on SD card and start the synthesis from the card without using the computer.

But for the sample synthesis let’s make sure that the machine is connected to the computer by the USB cable…

Sample synthesis

In this section you can follow the steps needed for running the simple synthesis of one peptide, just to show you the simplicity of the interaction with the machine. In the following sections you will learn how to modify every single step of machine operation.

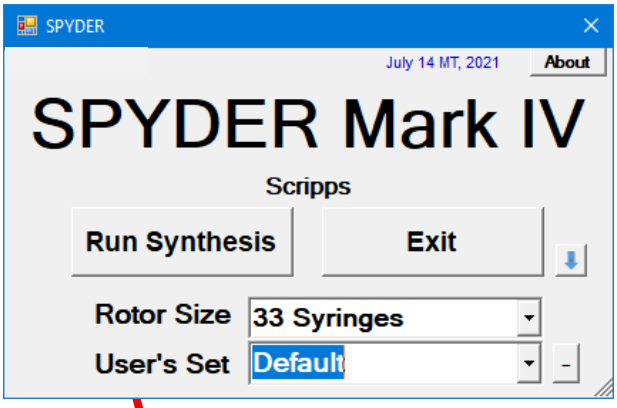
The controlling software is based on windows dedicated to individual operations which are opened and closed as needed. This allows you to keep only the information you want or need on the display and hiding those not needed away from the desktop.

Besides the interactive operation using the attached computer, you will have a choice to run the machine from a SD card inserted into the slot on the front panel without having computer connected to the synthesizer (feature implemented only in selected machines). In this case you will be protected from the issues stemming from the peculiar behavior of the Windows software which in the name of keeping everything up to date sometimes during the "non-business hours" (in the night) decides to update your computer with newest version of Windows and reboots the computer. In that case, operation of the synthesizer will be interrupted. To avoid this, you should run the system from computer with connection to the internet (wired or wireless) disabled or with update postponed (go to Settings, Update options, Pause updates – where you can postpone updating for up to 35 days). We recommend to use the computer dedicated to running only the synthesizer, so this limitation should not be difficult to handle.

Synthesis can be performed in plastic syringes with frit at the bottom. You can use any solid support you can imagine – the only concern is the size of your support particles. If they are too small to be retained by the frit, it would obviously result in failure. On the other hand, if you are using carrier of the type of modified cotton, you don't need any frit at the bottom of the syringe. The synthesis can be performed in 17 10 mL syringes or 32 3 mL syringes. Small syringes are recommended for up to 100 mg of solid support, 10 mL syringes can handle up to 400 mg of resin. However, the amount of resin depends mainly on its swelling properties – you have also to consider the fact that during the synthesis the volume of your swollen resin will grow. The limit of volume of reagents added to the syringe is 1.1 mL in the case of small syringe and 3.2 mL in the case of 10 mL syringe. The synthesizer will not let you use larger volumes, since it would probably result in not complete mixing of the resin, or in splashing the syringe content during shaking its content.

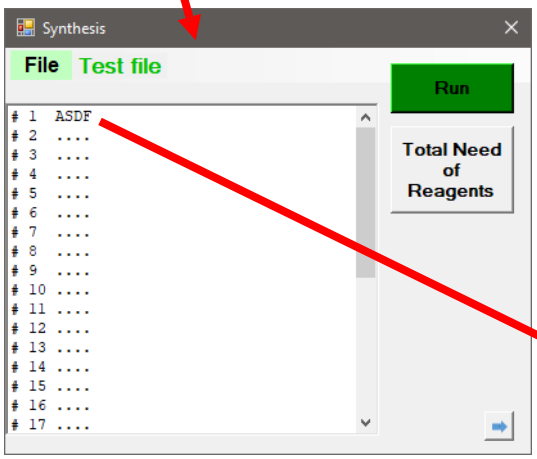
For this sample synthesis we will keep all default parameters and work with 33 syringe rotor in which only one syringe in position 1 will be equipped with 50 mg of resin. So, click on the **SPYDER** button on the desktop.

Sample synthesis

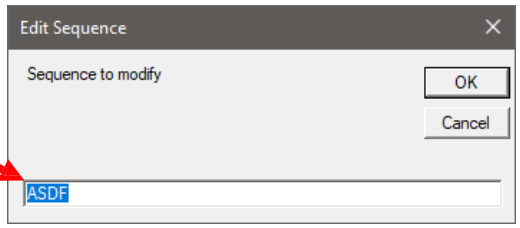


In this sample you will use rotor with 33 syringes and default parameters.

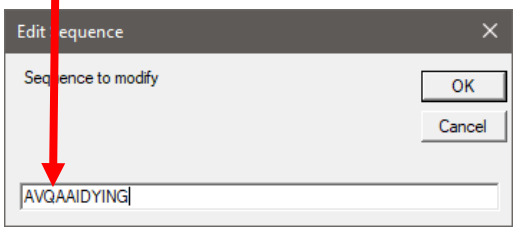
Click **"Synthesis"** button



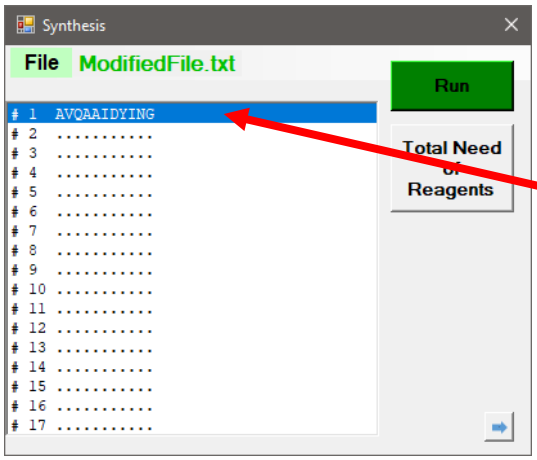
Click the sequence on line #1

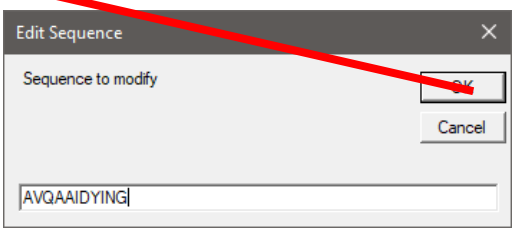


Modify the sequence for whatever sequence you want

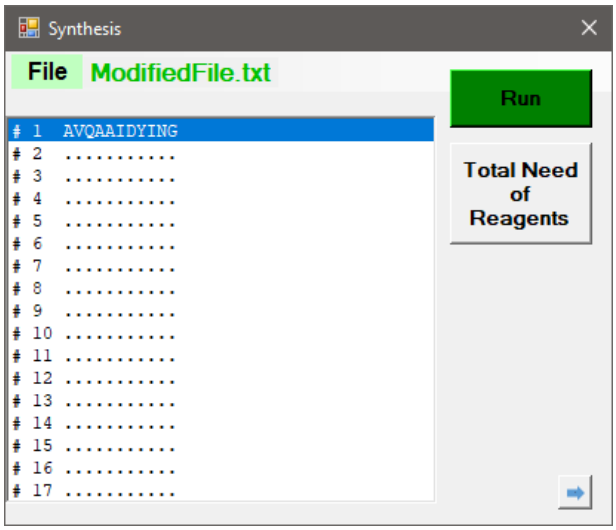


and click "OK". Modified sequence will appear on line 1

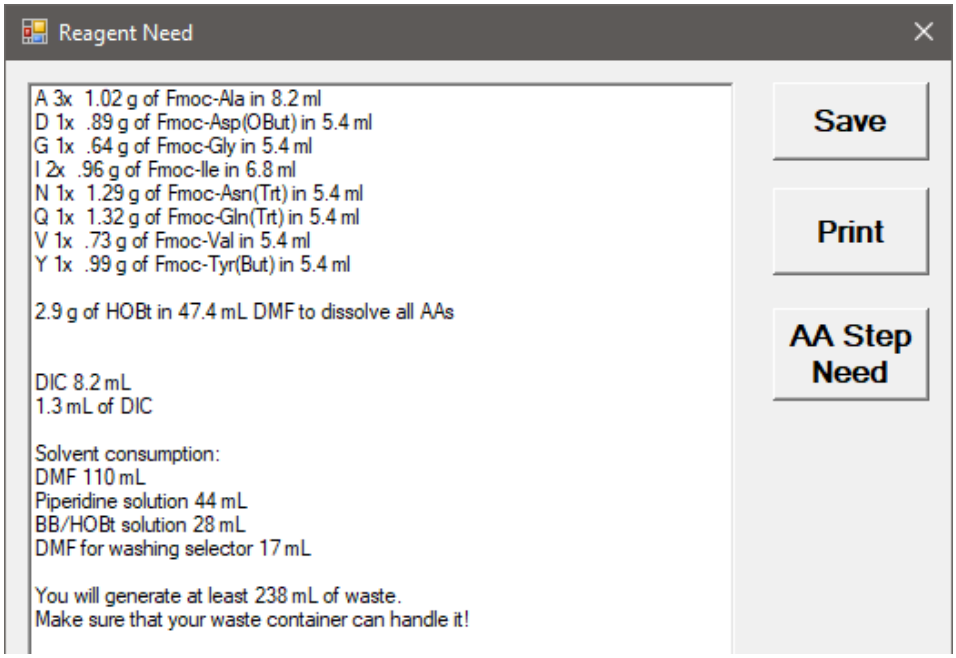




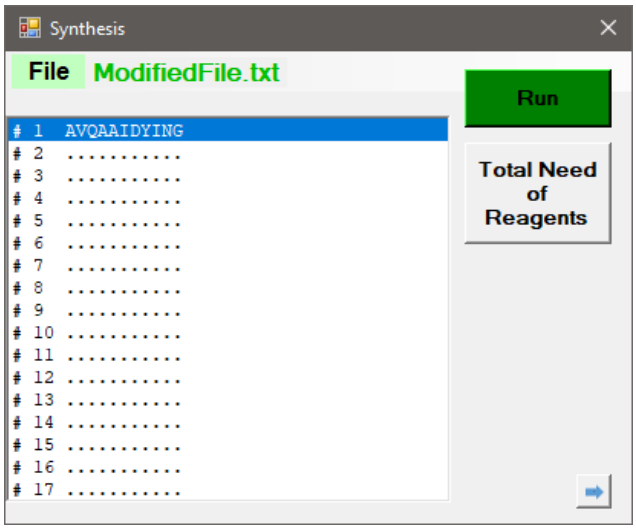
Sample synthesis



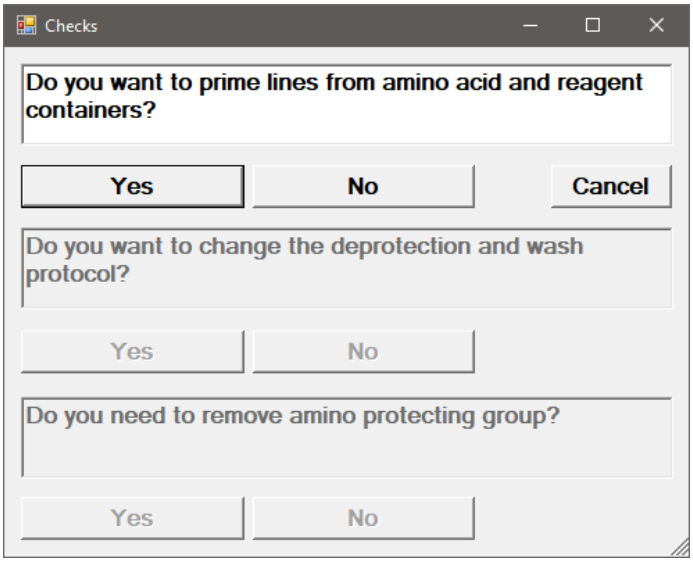
Now you click **"Total Need of Reagents",** prepare the solutions, fill the appropriate Falcon tubes and bottles and attach them to the machine. Computer uses default volumes for couplings, washings and deprotections assuming that synthetic support (50 mg) will be filled in syringe number 1.



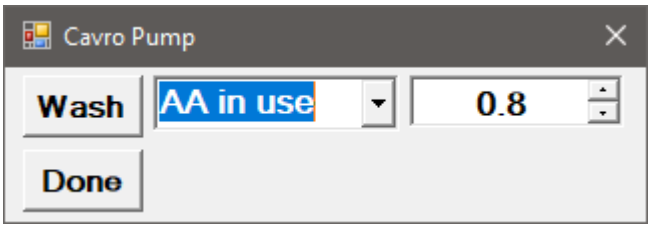
Sample synthesis

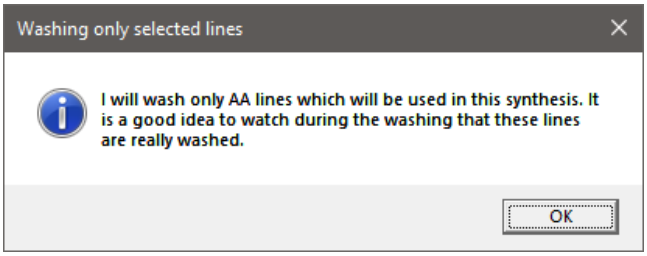


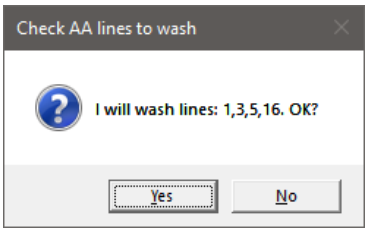
When all solutions are on the machine and resin is in the reactor (syringe #1), you can click **“Run”**

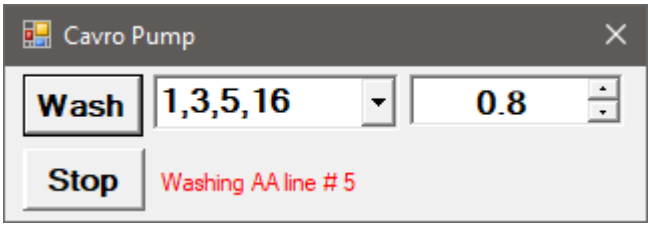


Window “Checks” will open and will guide you through necessary steps to perform before you run the synthesis. First it asks you to prime the lines with amino acids and reagents. You should make sure that all lines are filled with appropriate solutions. If you click **“No”** then the window will go to the next question. If you click **“Yes”,** the window “Cavro Pump” will open and offer you to wash the lines with amino acids which are needed for your defined peptide. So click **“Wash”** and wait for the procedure to finish. If you need to wash other reagent lines, select the appropriate line and click **“Wash”** again. When all lines are primed and ready, click **“Done”**.

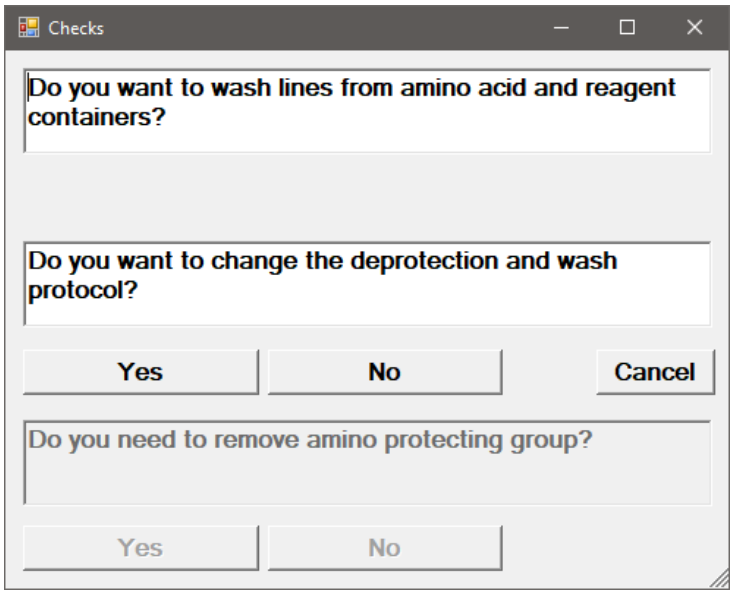




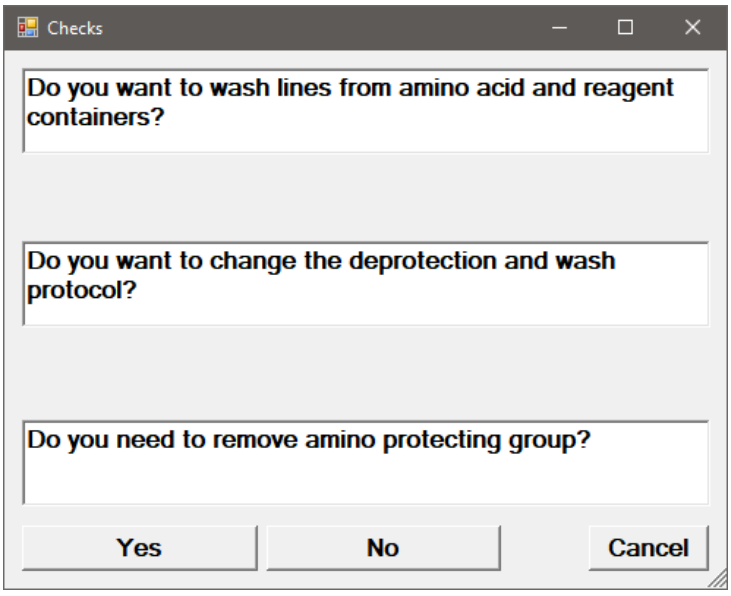




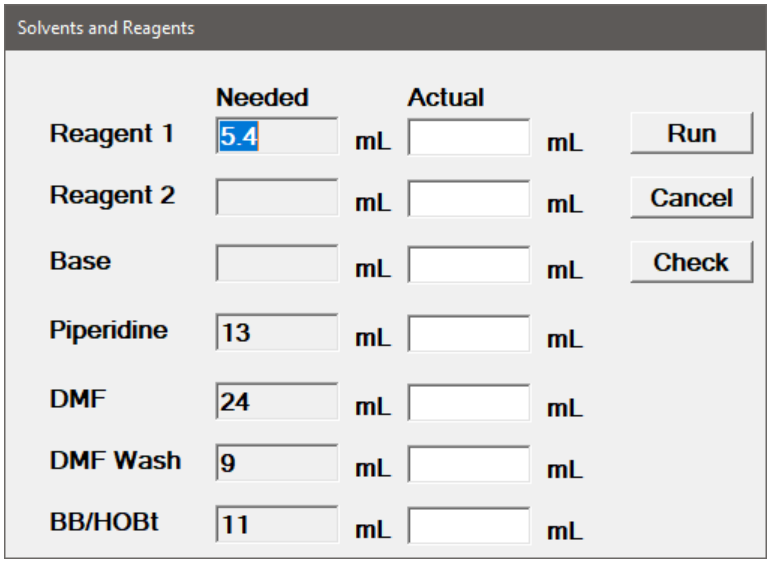
Then the next question on Checks window will let you change the wash/deprotect protocol.
But in this synthesis you will use default – press **“No”**



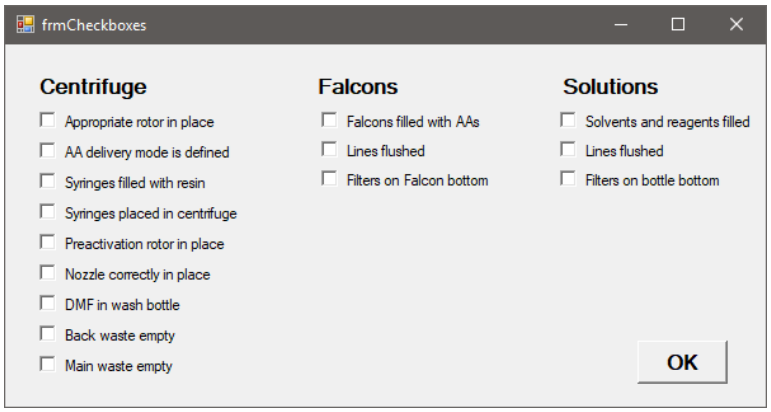
The next question on Checks window will let you choose whether you need to deprotect the solid support loaded in the syringe.
You may have Fmoc protected resin in the reactor and you need to click **“Yes”.**



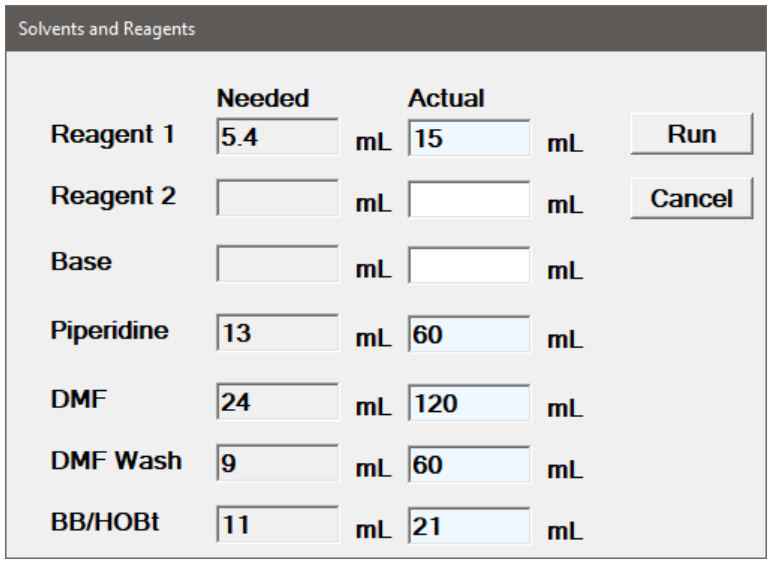
Sample synthesis



The next window ("Solvents and Reagents") will ask you about the volumes of reagents which you should have placed in the appropriate vessels. You may fill all volumes you actually have in the bottles and click **"Run",** or you can click the button **"Check".** (If you don't have enough reagents in the bottles, the machine will tell you so and will not let you go on until you have enough.)



This will bring another window ("Check Boxes") with the list of all things which have to be checked before the synthesis can be started. If you are sure that everything is correct, click **"OK"** and the window "Solvents and Reagents" will be filled with volumes which are the minimum volumes guaranteeing the successful synthesis.

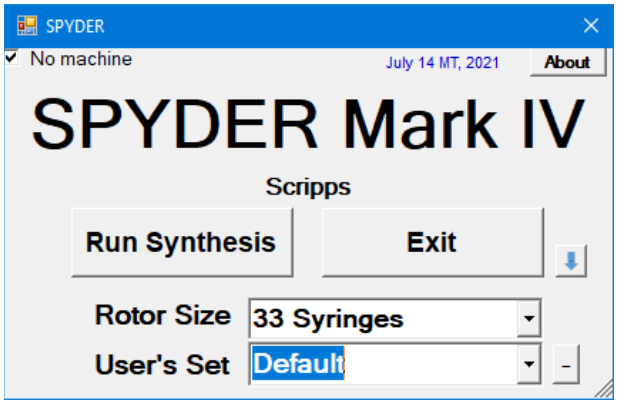


Press **"Run"** and the machine will do the rest…
You can still change your mind and cancel this run by pressing "Cancel" button.

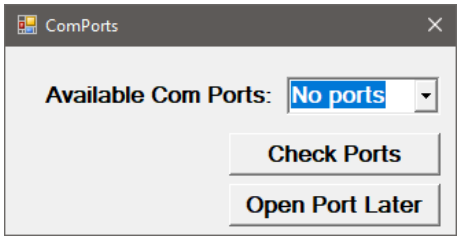
Detailed description of software functions

Click the SPYDER button on the Windows main screen

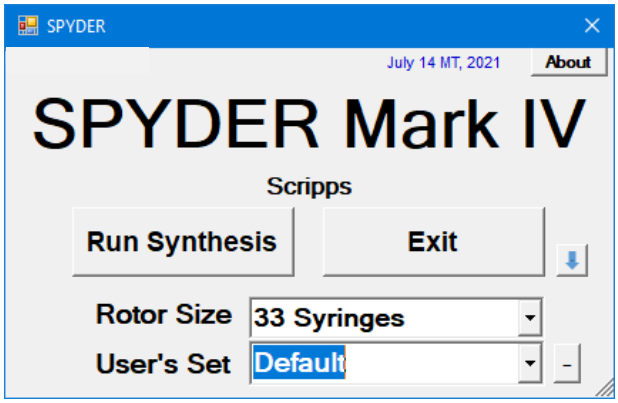
And this window will appear:



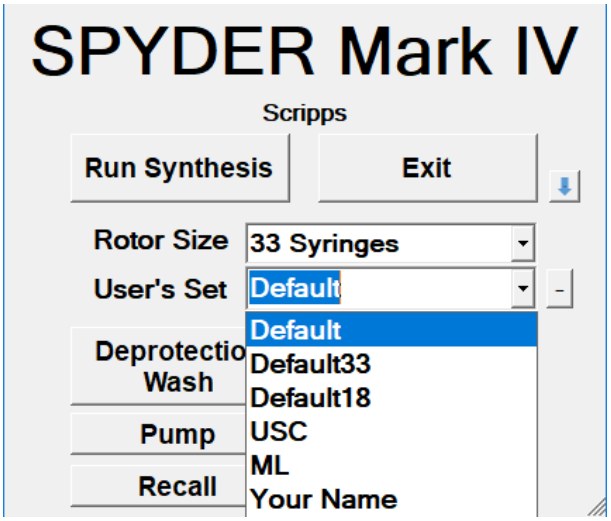
If you see the “No machine” checkbox in the upper left corner, then your computer is not connected to the machine and the window ComPorts will open on the left side of the SPYDER window. Check the connection (plug in the USB) and click **“Check Ports”** button, select the port and click **“Open Port”** button. (The ComPorts window can be activated going from the main menu to **“Parameters”** button and in the window which will open clicking **“Show ComPorts”** button – we will discuss that later.)

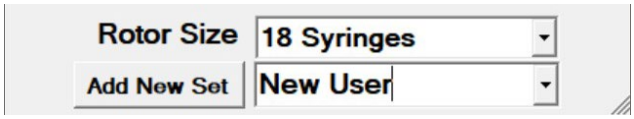


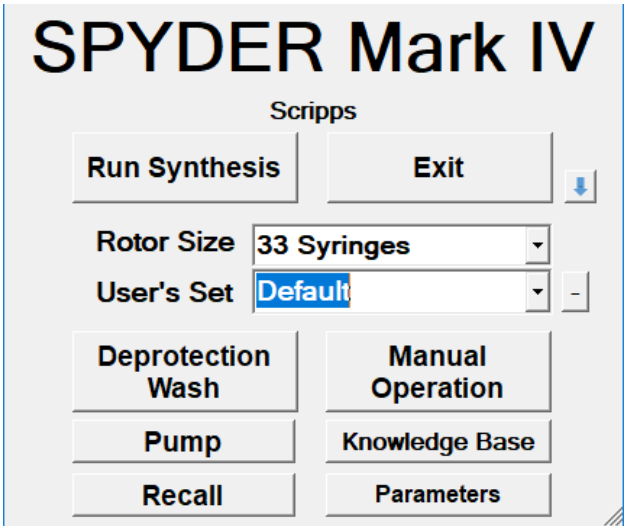
If clicking **“Check Ports”** button will show “COMXX”, then you will be able to open that port. You can also choose “Open Port Later” and use the software for planning the synthesis and open the port just before the synthesis. You can also generate the synthetic protocol and save it on SD card and start the synthesis from the card without using the computer.



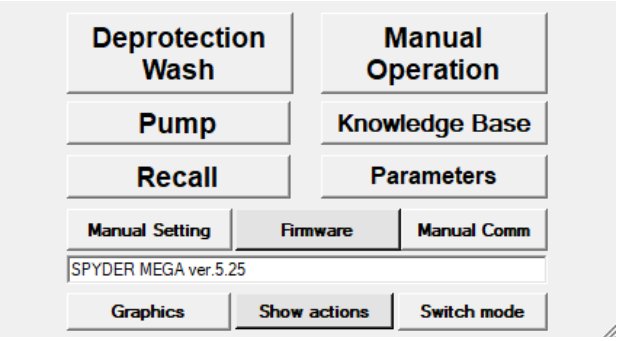
You have to select the size of the rotor 18 or 33 syringes. Syringe number 18 or 33 is dedicated to washing, so the number of peptides you can synthesize at once is only 17 or 32. You can either use the default set of parameters, or you can use parameters set (rotor size, volumes of reagents, times of reactions, etc) for different users. After you define those parameters and save them under your name, you will be able to recall them later.



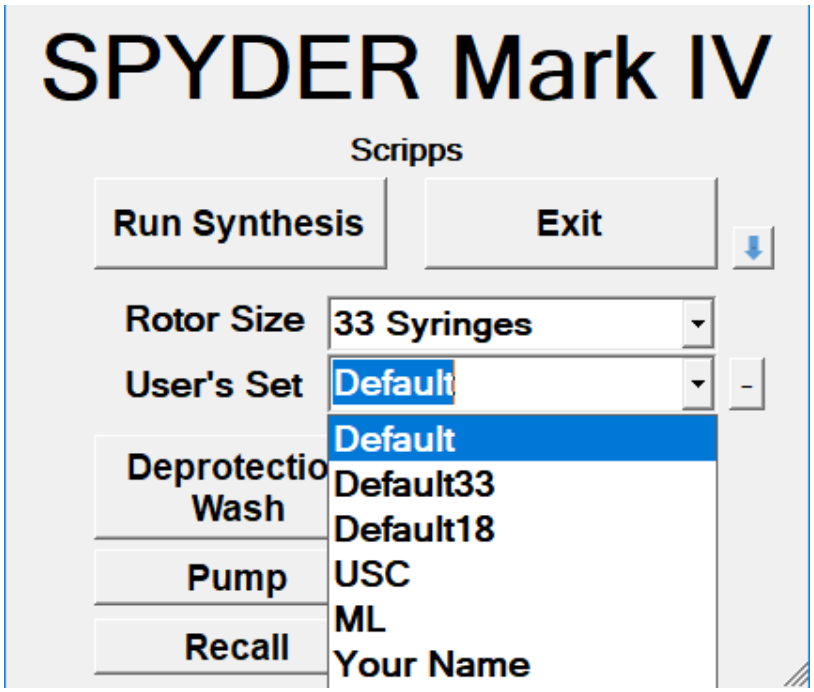




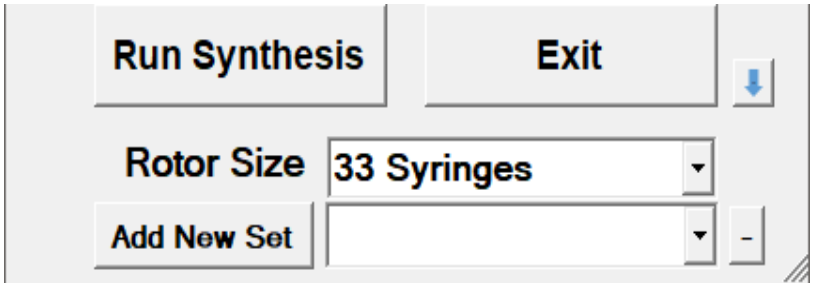
Click on the **down arrow** or click on the form will reveal additional buttons.



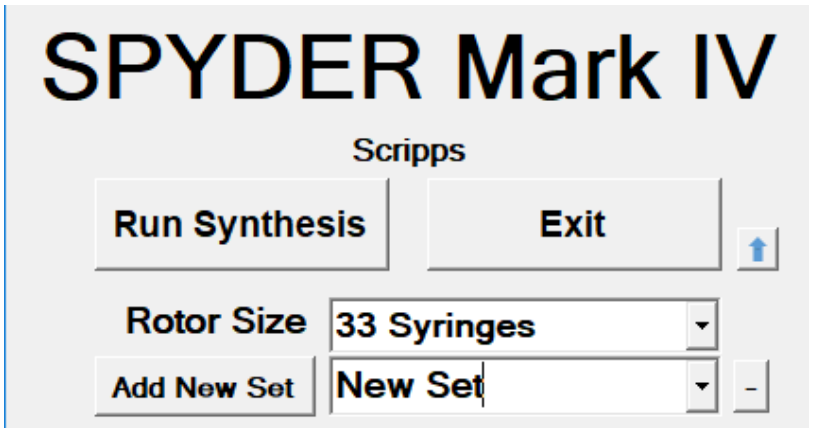
Doubleclick and pulling down on the form will reveal another buttons, which should be used only by expert user with extreme caution since it can set certain parameters which may make the machine unusable. To use these buttons, you have to know the password.



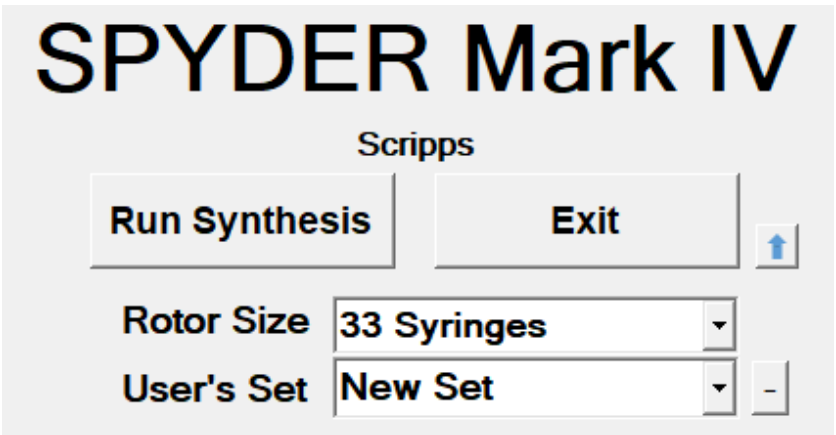
From the list of User's Sets you can select the set of parameters which you would like to use for your synthesis, or you can define your dedicated set.



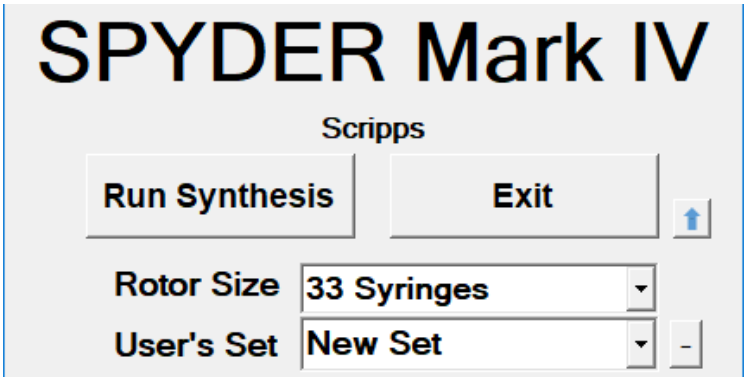
To define your set, just delete the text in the name field. The new button **"Add New Set"** will appear



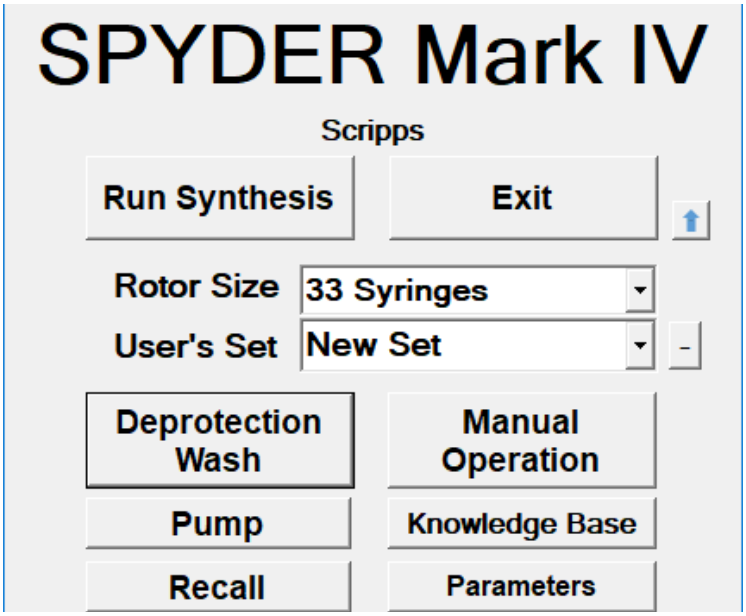
and you can type your name to it. After clicking **"Add New Set"…**



You will have your new set saved and will be able to recall it later.



To see all functions of SPYDER window, click on the **down arrow** (or anywhere on the SPYDER window)



So, now you see all options:

Run Synthesis – will prepare everything for the synthesis

Exit – obviously exits the program

Deprotection Wash – sets the protocol for deprotection and washing after coupling step

Manual Operation – let you perform all individual steps of the machine manually

Pump – will allow you to prime the lines and perform individual actions of the pump manually

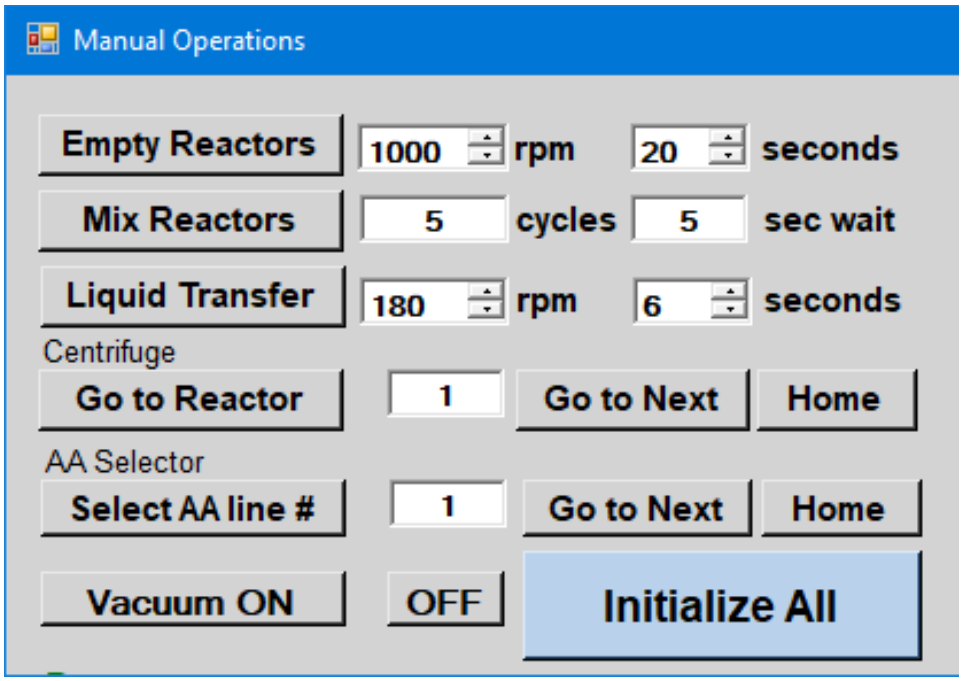
Knowledge Base – will tell you everything you need to know about peptide synthesis reagents

Recall – will recall the last operation performed on the machine

Parameters – will let you see and set the parameters of the synthesizer

Dwelling on any button with your pointer will call balloon with a little explanation what the button will do.

Let's click **"Manual Operation"** button



Empty Reactors – performs centrifugation (at 1000 rpm for 20 seconds) liquid removal from the syringes

Mix Reactors – shakes the rotor (5 cycles of shaking followed by 5 seconds waiting between cycles)

Liquid Transfer – rotates (at 180 rpm for 6 seconds) at speed not exceeding the speed needed for removal of liquids from the syringe reactors, but at speed allowing the transfer of liquid from preactivation chambers to syringes (make sure that the value of 180 is not changed for higher number)

Go to Reactor – turns the rotor to defined position (e.g. 1) placing it under the nozzle for delivering reagents

Select AA line # - Connects Falcon tube with the given amino acid to the Cavro pump port 3

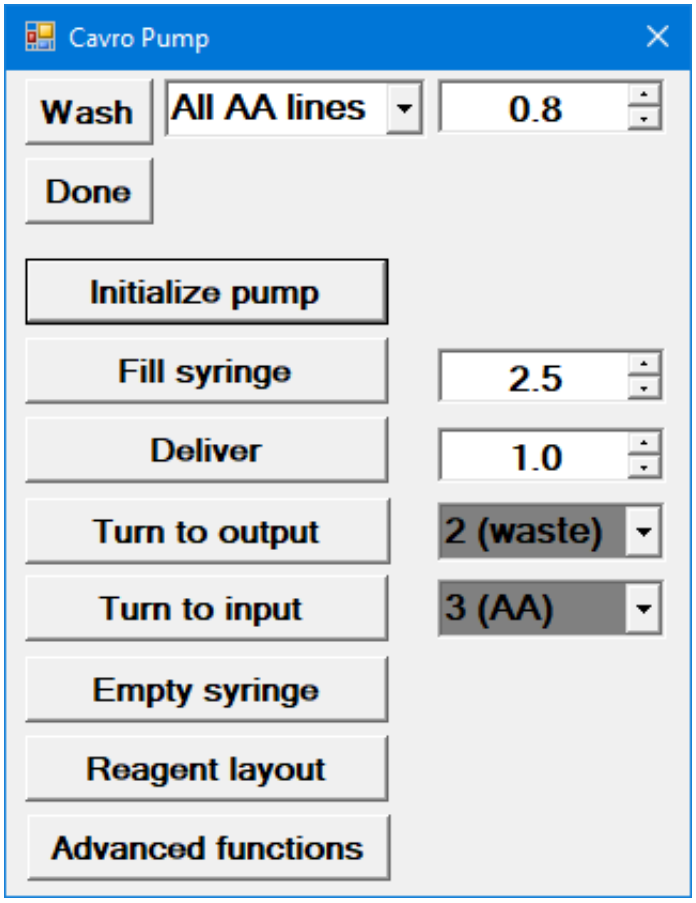
Go to Next – turns to next position (rotor or selector valve)

Home – initialize the rotor (positioning it in position 1) in case of rotor, or initializing selector valve (sending it to position 28)

Vacuum ON – switches the power on to the outlet on the back of the machine (which can be connected to vacuum pump)

OFF – switching it off

Initialize All – initializes centrifuge, selector valve and Cavro pump



Clicking **"Pump"** button will give you the following options

Wash – will wash (prime) lines defined next to the button with defined volume For washing (priming) the amino acid solutions connected through the selector valve. It uses at the minimum 0.8 mL of solution (50 uL/10cm of tubing). You can wash all AA lines, or you can specify the lines to wash by numbers separated by commas (e.g. 1,3,6,15,22). To wash reagent lines connected by wider tubing you need higher volume (10 cm of the tubing = 200 uL)

Done – closes this window

Initialize pump – expels content of the pump syringe through output #2 (waste) and establishes top position of the pump syringe

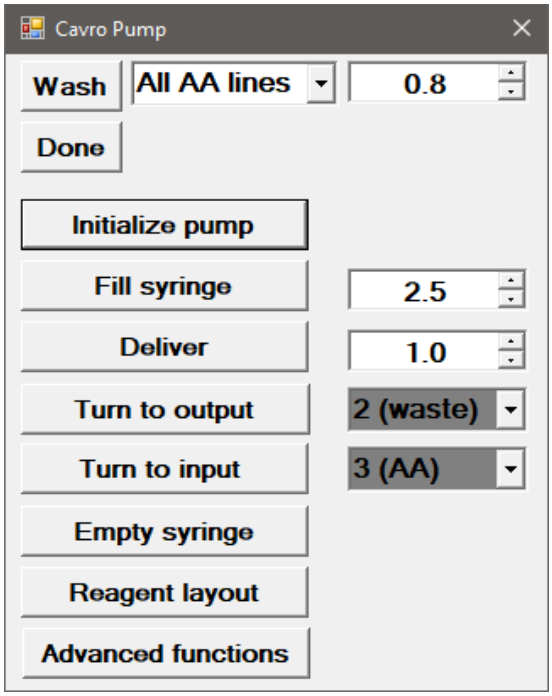
Fill syringe – sucks in the defined volume

Deliver – expels the defined volume

Turn to output – syringe valve will go to defined position (recommended positions are only position 2 – waste and position 1 – delivery nozzle). Turning it to any other positions and delivering through it creates the possibility of syringe valve crosscontamination.

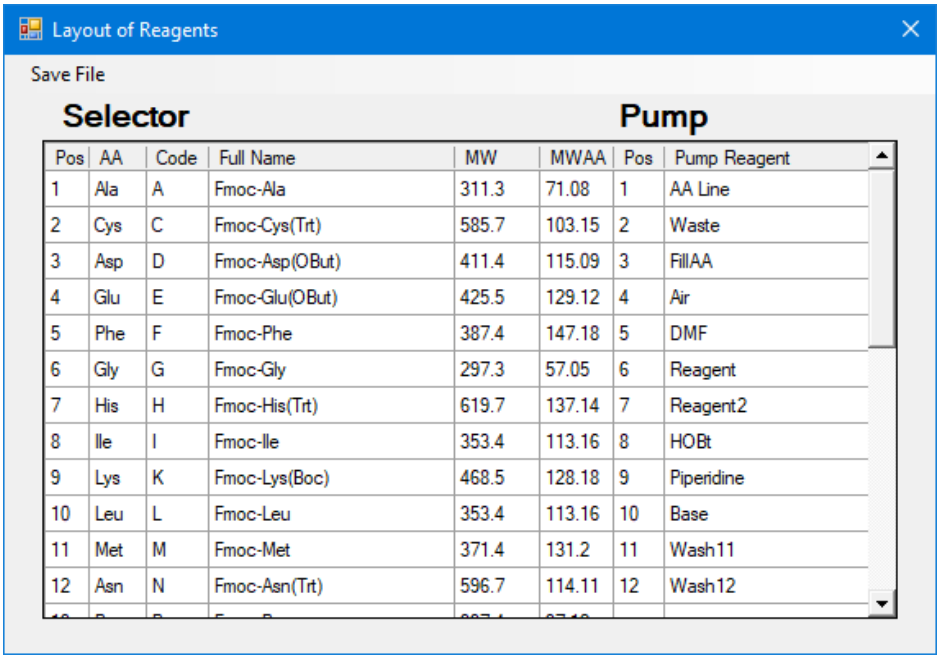
Turn to input – syringe valve will go to defined position

Empty syringe – content of the syringe will be expelled to waste (Cavro port 2)



To operate the pump manually you:
Select input port (ports on the top of pump are numbered from left to right)
Fill syringe with desired volume
Select output port
Deliver selected volume, or
Empty the syringe into the output port

Reagent layout – opens widow with list of connections and shows you layout of the amino acid containers connected to the selector valve and reagents connected to the pump. Machine recognizes only amino acids symbols in single letter notation and numbers from 1 to 7. If you want to use unnatural amino acids you have to represent them by numbers 1 to 7 and place them into positions 21 to 27 on the Falcon tubes holder.

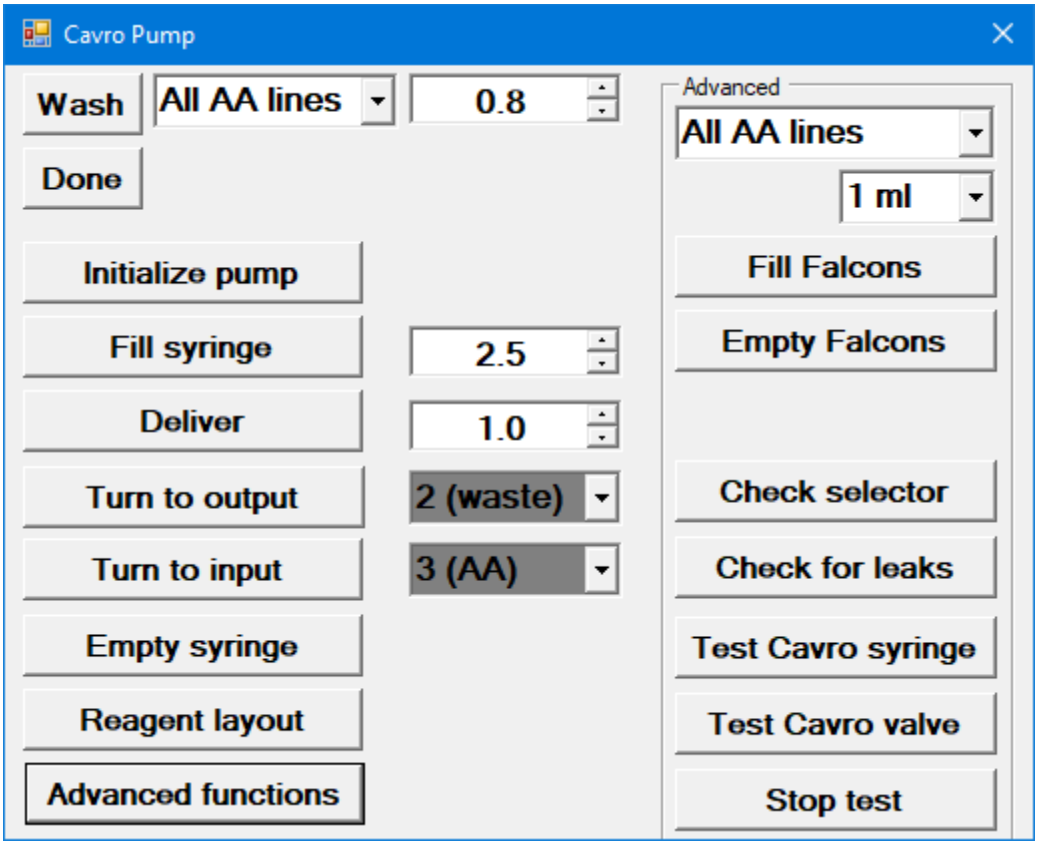


Pos	AA	Code	Full Name	MW	MWAA	Pos	Pump Reagent
1	Ala	A	Fmoc-Ala	311.3	71.08	1	AA Line
2	Cys	C	Fmoc-Cys(Trt)	585.7	103.15	2	Waste
3	Asp	D	Fmoc-Asp(OBut)	411.4	115.09	3	FillAA
4	Glu	E	Fmoc-Glu(OBut)	425.5	129.12	4	Air
5	Phe	F	Fmoc-Phe	387.4	147.18	5	DMF
6	Gly	G	Fmoc-Gly	297.3	57.05	6	Reagent
7	His	H	Fmoc-His(Trt)	619.7	137.14	7	Reagent2
8	Ile	I	Fmoc-Ile	353.4	113.16	8	HOBt
9	Lys	K	Fmoc-Lys(Boc)	468.5	128.18	9	Piperidine
10	Leu	L	Fmoc-Leu	353.4	113.16	10	Base
11	Met	M	Fmoc-Met	371.4	131.2	11	Wash11
12	Asn	N	Fmoc-Asn(Trt)	596.7	114.11	12	Wash12

Cavro pump selector ports are numbered 1 to 12 from the left (if you are facing the pump) and recognizes only the following codes: "Reagent“, "Reagent2“, "DMF“, "Base“, "Ac2O“, "HOBt“, “Oxyma”, "Piperidine“, "TFA“, "MeOH“, "FillAA“, "AA Line“, "Waste“, "DCM“, "Air“, "AcBase“, “Wash11” and “Wash12” (“WashXX” is a representation of any solvent you would connect to port 11 or 12 for the use of the final wash of the resin after synthesis – usually MeOH or DCM).

Remember that if you change the reagent layout, it would affect other users of the machine and may result in confusion since they may not expected the change and may not check the reagent layout before starting their synthesis. Therefore we do not recommend to do this change without deep consideration of consequences.

Advanced functions – see later



Advanced functions – other functions which the pump can perform automatically. Its use is allowed only with the knowledge of the password. Clicking it extends the window and shows:

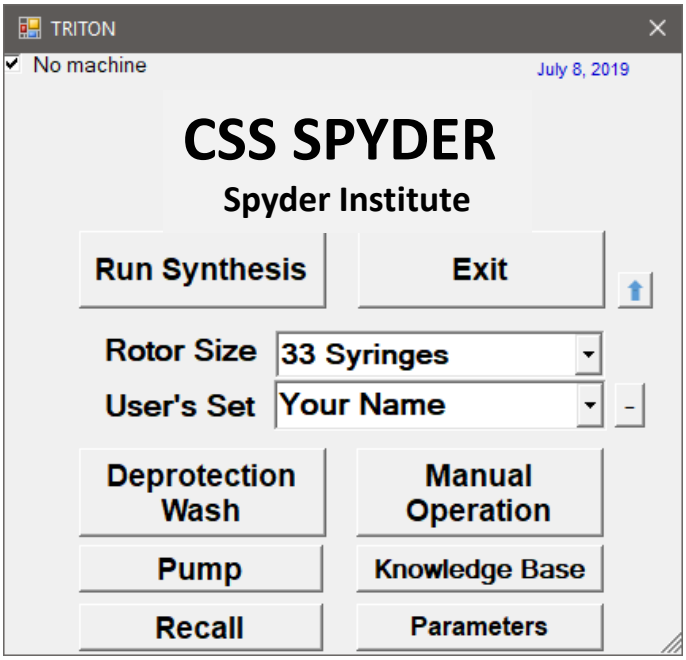
Fill Falcons – Will fill Falcon tubes defined in the rolldown field or numbers separated by commas - 1,3,4,5, - or range – 2-6 - , with volume defined above). It will use DMF from Cavro pump port #5.

Check Selector – For this test the lines have to be primed and Falcon tubes must have DMF in them. Test will select the line in the selector valve, sucks in the given volume and asks the operator to write down volume of liquid (or air gap) in the pump syringe. Recommended volume to test is at least 2 mL. The syringe should fill completely. If not, the particular line has a leak and all connections from the Falcon tube to the pump must be checked and tightened.

Test Cavro syringe – Will suck the defined volume of DMF into the Cavro syringe and expel it to the waste with increasing speed until the Cavro pump fails and has to be reinitialized. If this test fails at speed below 1200 uL/sec, Cavro pump needs maintenance.

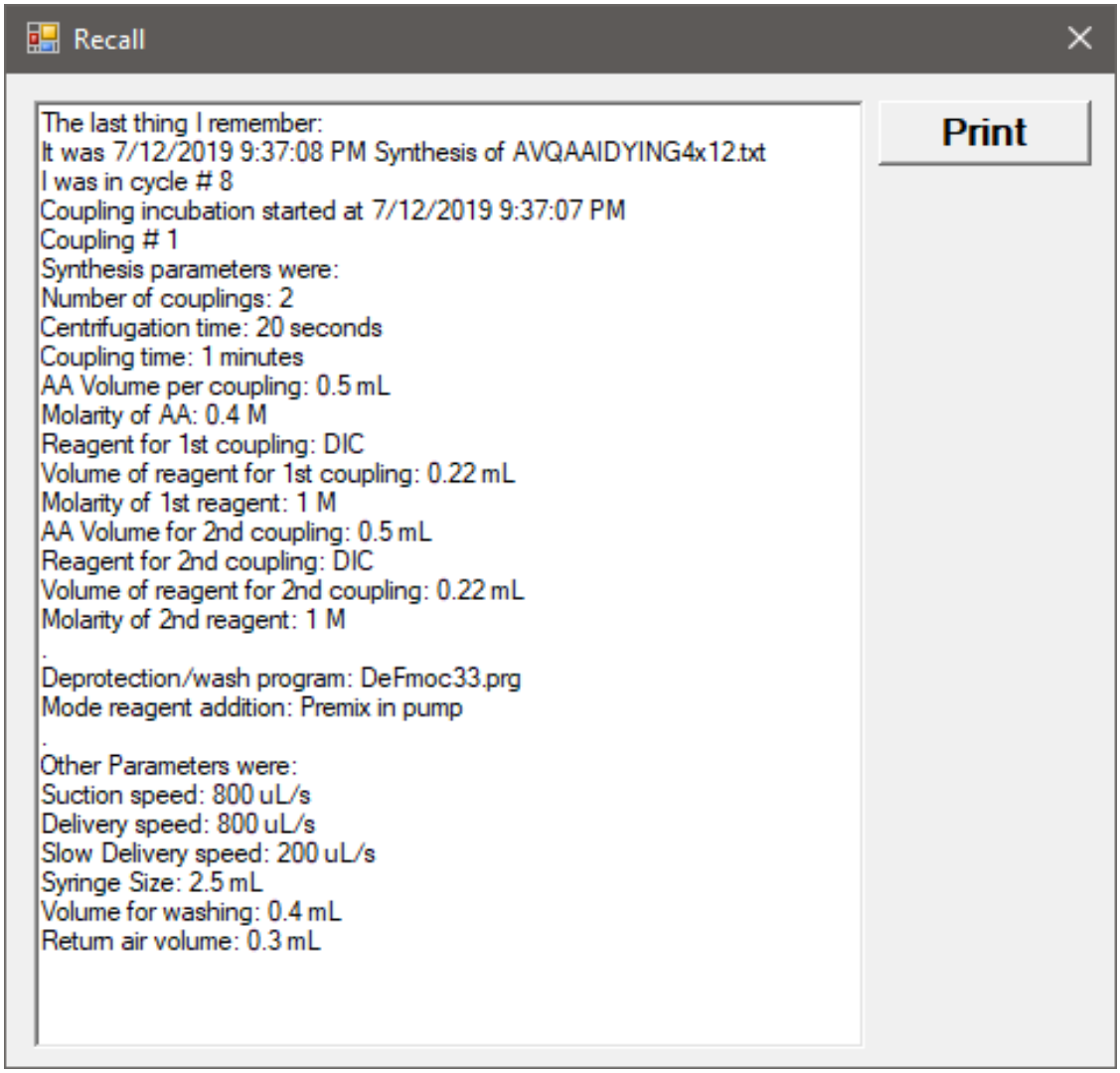
Test Cavro valve – Will turn Cavro selector valve to all positions and report the failure.

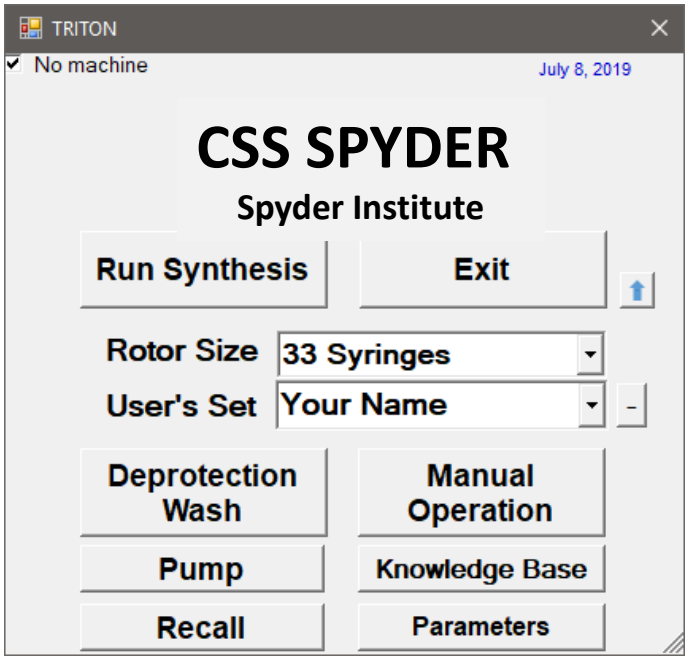
Stop test – stops test immediately



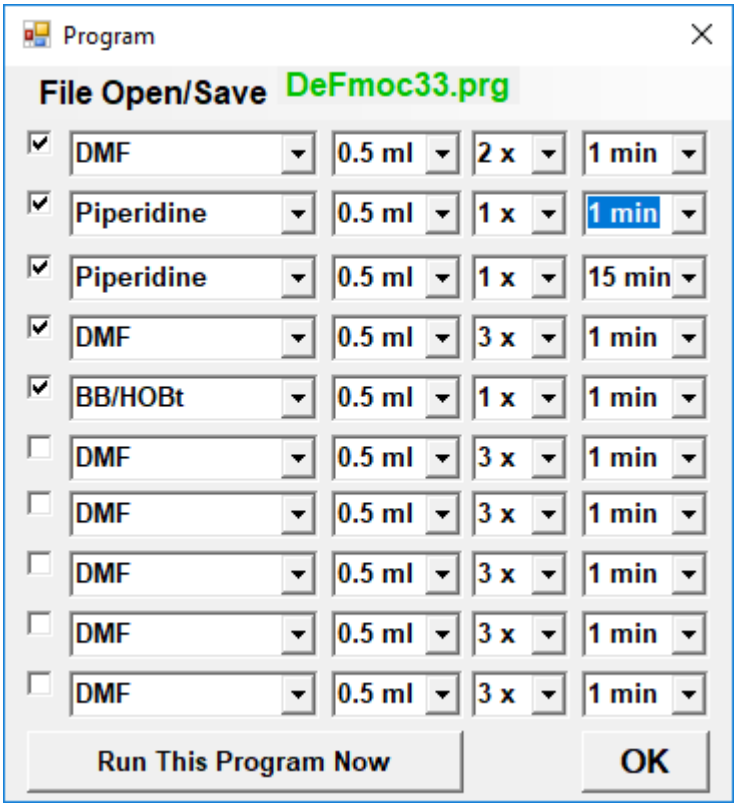
Clicking **"Recall"** button will show the information about the last known operation of the synthesizer. It is useful in the case of power failure or another human related mishap.

In addition to this last operation recall, the entire history of the synthesis performed on the machine is kept in a logfile name (date stamped) which can be found in directory SPYDER/logs. Here you can find every operation which the synthesizer performed (at the lowest level of operation) and the file is extremely large.





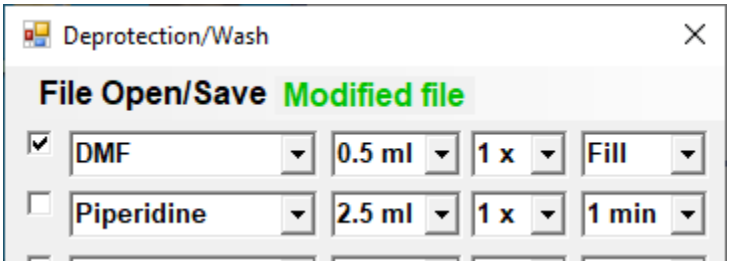
Clicking **"Deprotection Wash"** button will open window "Program" and will give you the chance to change the protocol for deprotection and washing steps.



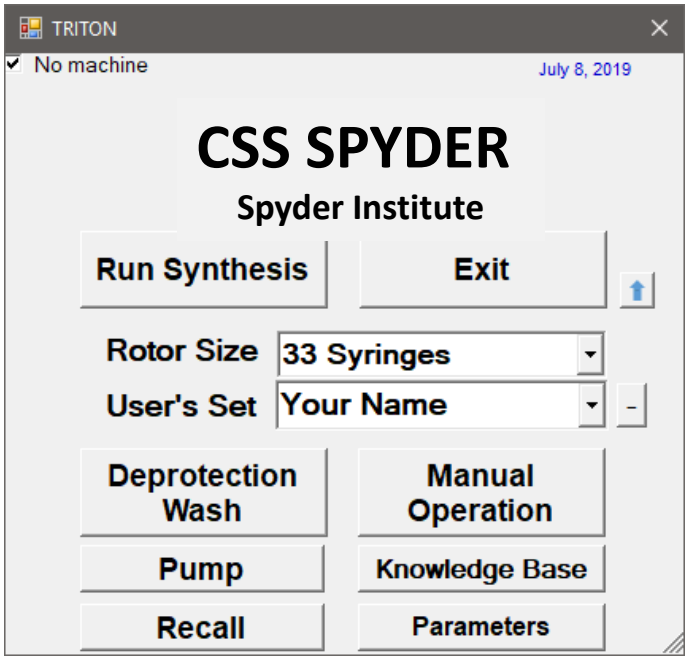
If the program listed in green at the top of the window is not what you want, click **"File Open/Save"** and find appropriate program, or change the parameters here and save the new program under new name.

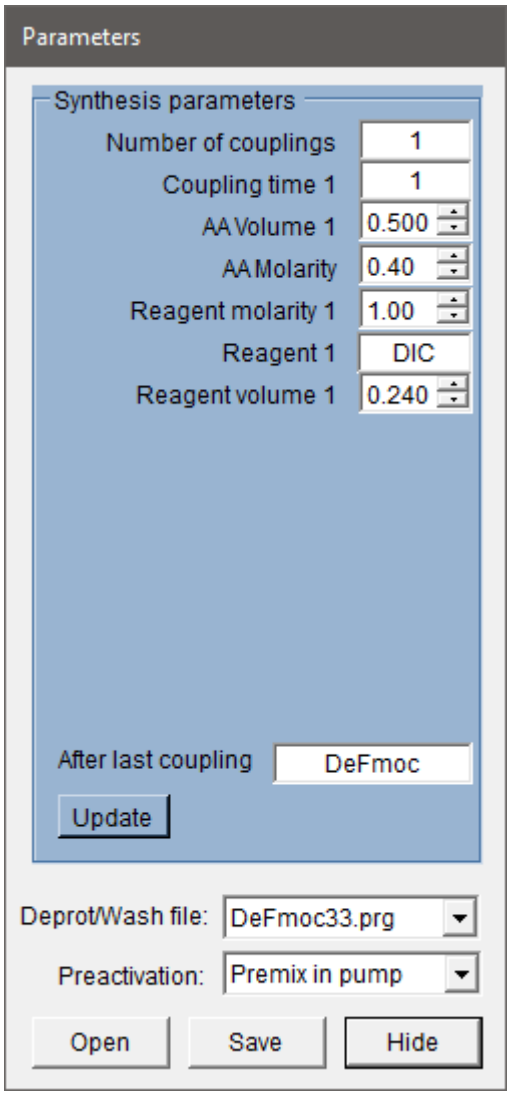
Steps with check marks checked will be performed after coupling in every step of the synthesis. To make the new protocol, just select the reagent, volume to be added, number of repetitions, and time for incubation with that reagent. Only the reagents which are defined in the rolldown selection can be recognized by the machine. (How to change the predefined selection will be shown later.)

You can run this program by clicking **"Run This Program Now"** to deprotect or prepare the resin, but it is not necessary for the synthesis because the deprotection is an option in the synthesis start step.



However, it may be useful for preswelling the resin before starting the synthesis, since some resins require long swelling times. In this case you may change the time for exposure to DMF for example to 2 x 10 min and uncheck all lines but the first one. If you select "Fill" in the rolldown time menu, the solvent will be only added, and no centrifugation will be performed.



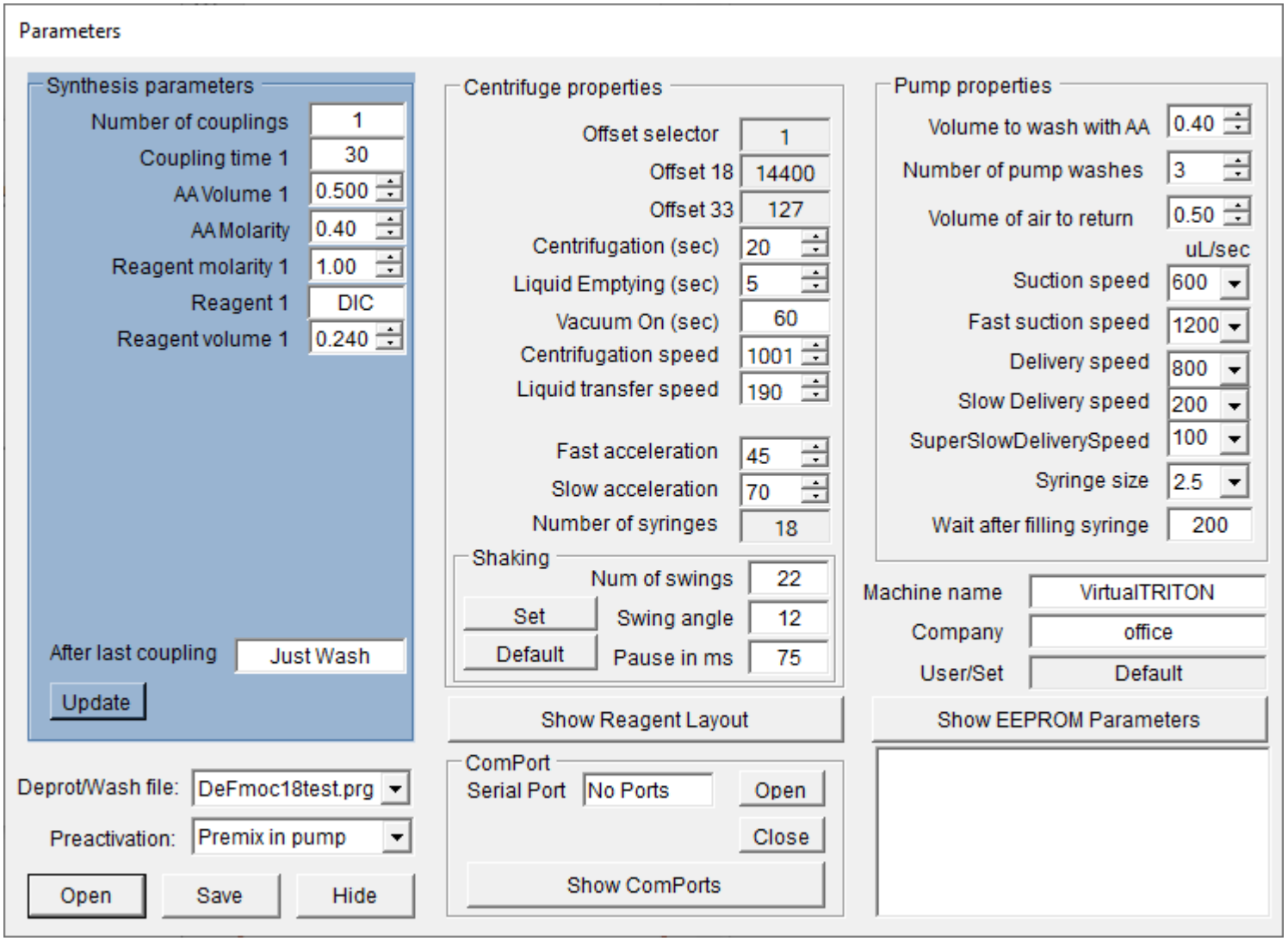


Clicking **"Parameters"** button will open window "Parameters" which will show you the reagent setup (volumes, repetitions, time, deprotection/wash protocol, action after the last step, and the mode of addition of reagents to the reactors) which was saved for the user's set defined on the SPYDER (starting) window.

These could be values defined in your set, or you can use the protocol which was saved as a default protocol (User's set "Default", "Default18", "Default33"). To update the values for the values which you selected in the Synthesis window, click **"Update"** button.

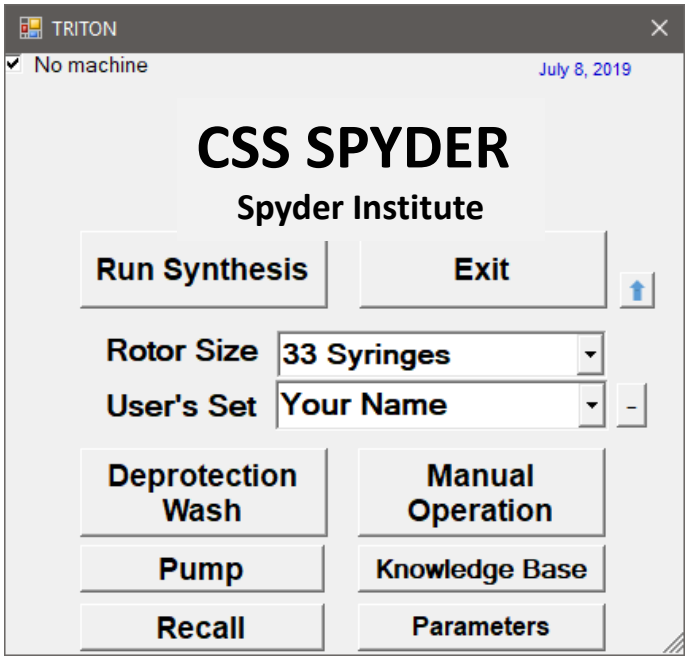
You can open your set from the file (e.g. ConfigCentr_XX.cfg) or save it under different name. However, to be recognized by the machine as a registered user set, you have to save it under the name you are using on the SPYDER starting window. During the synthesis you cannot close "Parameters" window, you can only **"Hide"** it so that it is not cluttering the desktop.

Double clicking the blue area opens additional list of parameters.

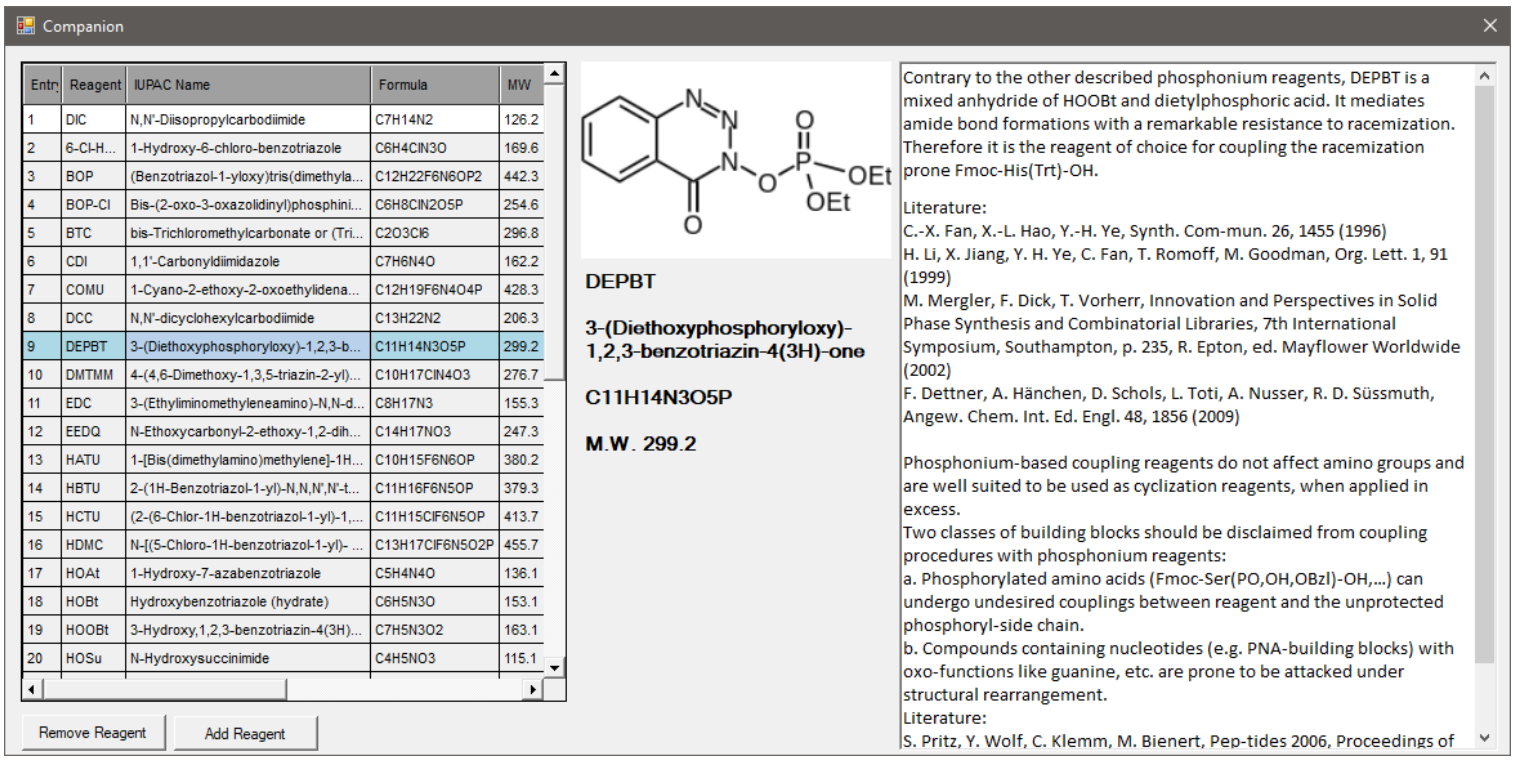


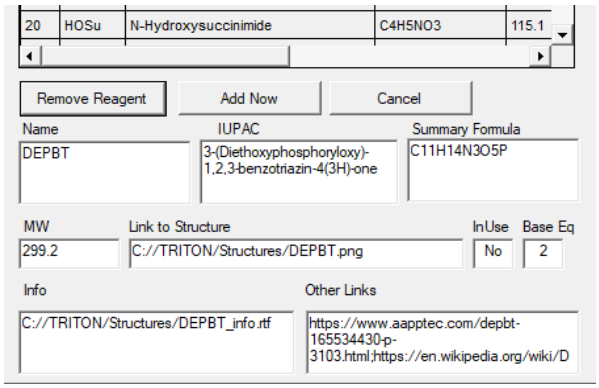
We do not recommend to change those parameters since they were optimized for the synthesizer, however, sometimes the user may “tweak” some of these parameters to have machine perform exactly as he/she wants.

Here you can change all parameters of the machine or open Com Port. Any change will be used only for the current synthetic session (after clicking **“Hide”** button), unless you save the parameters by clicking **“Save”** button. Default parameters are saved in ConfigCentr_Default.cfg file, but you can save them under any name with cfg extension. To use those parameters you would have to always open your file (e.g. ConfigCentr_XX.cfg) before running the synthesis. If you decide to use your parameters as default ones, rename your file to ConfigCentr_Default.cfg.

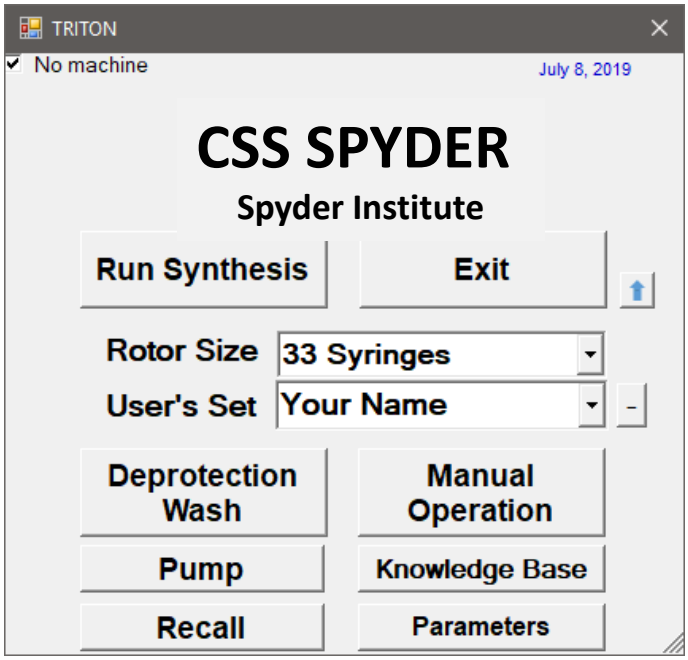


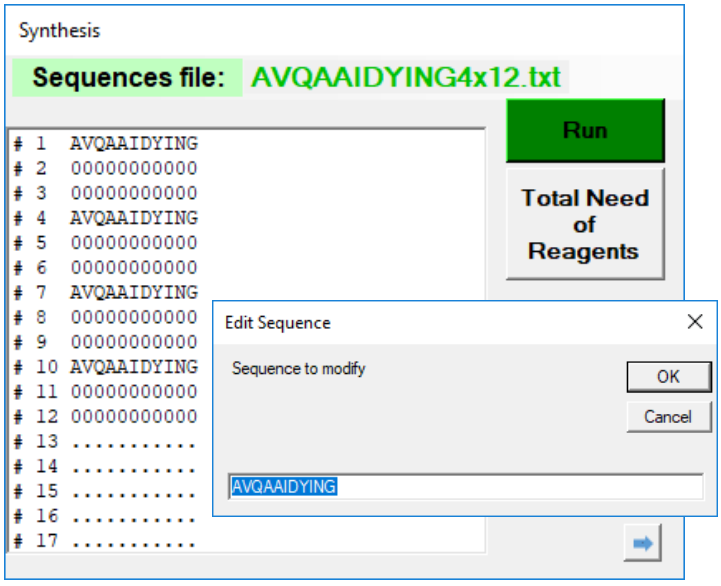
Clicking the button **"Knowledge Base"** will show the window "Companion" where you can learn about reagents for peptide chemistry. Clicking on the name will give you chemical formula, description of the reagent and literature references about its use.

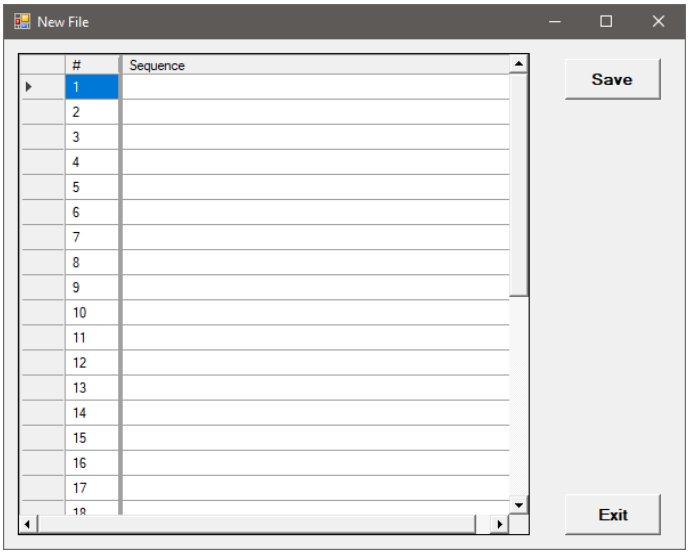




Clicking button **"Remove Reagent"** will delete the reagent from the database, clicking **"Add New"** will extend the window and let you modify the fields for your new reagent (fields are prefilled with the data for the reagent selected above to show you what is expected to be entered to the database). Most of the fields are self-explanatory – InUse means that you want to use it as a reagent which will appear in rolldown fields in synthesis planning window, Base Eq means number of equivalents of base should be added to the reaction mixture when this reagent is used.







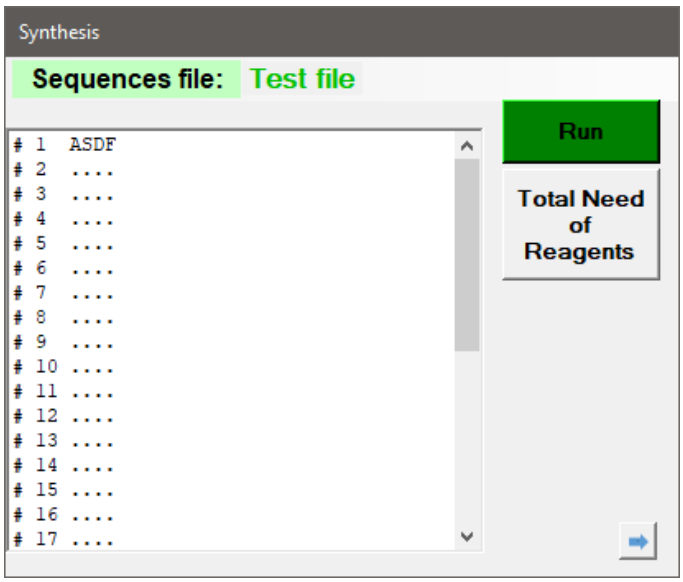
Clicking **"Run Synthesis"** button will open window "Synthesis" which will show you the list of the sequences which will be synthesized. The synthesizer understands the standard nomenclature of peptides in "one letter code". Codes can be in upper or lower case (lower case amino acid does not mean D-amino acid). In addition, for unnatural amino acids or other building blocks you can use numbers 1 to 7 for reagents filled in containers 21 to 27. So, for example, sequences YGGFL or Y1GFL or Y12FL will be recognized if properties of building blocks 1 and 2 are defined.

To define the new sequences to be synthesized, you have several options:

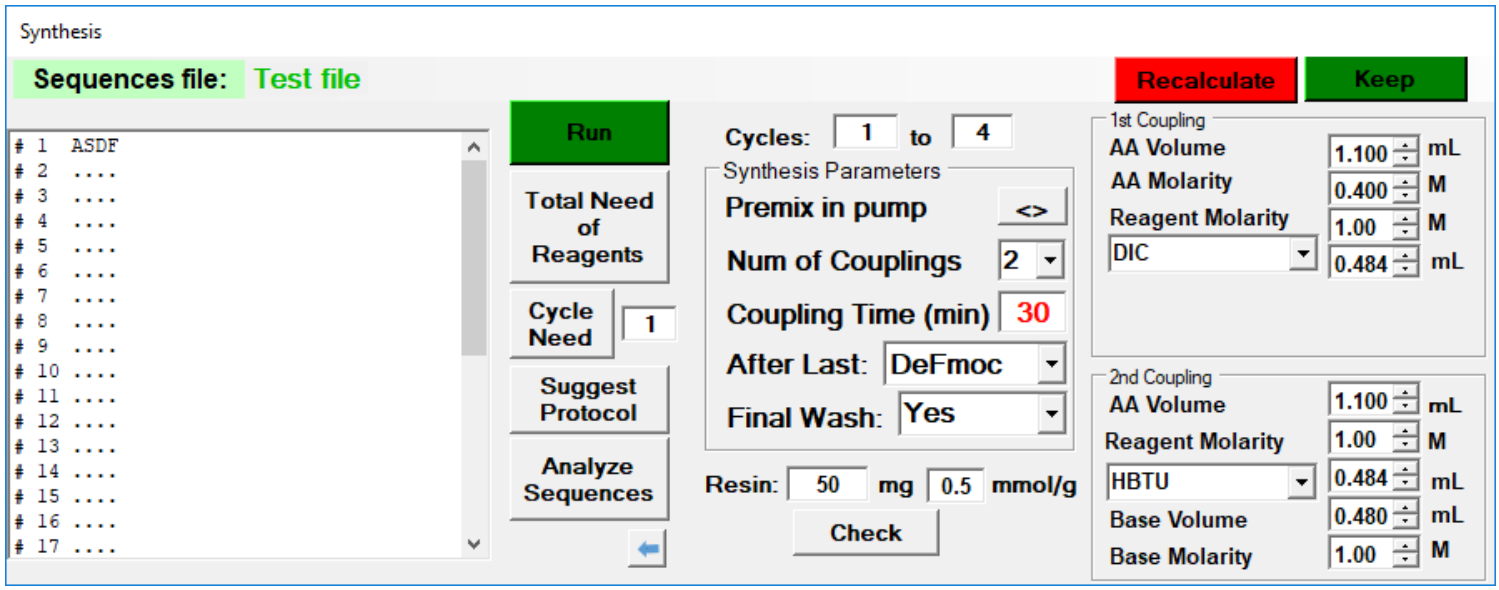
1.You can click the line in the sequences window – it will open the message box in which you can define your sequence. Then you repeat this procedure until all sequences are defined. At this point the name of the file containing the sequences will read "ModifiedFile.txt" and if you click **"Sequences file"** you can save this file under a new name for use in the future.

2.You can click **"Sequences file"** and select from the choices **"Open"** and open text file containing list of sequences. This text file can be created in Notepad, Word, Excel or any other text editor. Do not number the sequences, just use one sequence per line. If you are not planning to do the synthesis in a particular syringe reactor, just insert an empty line.

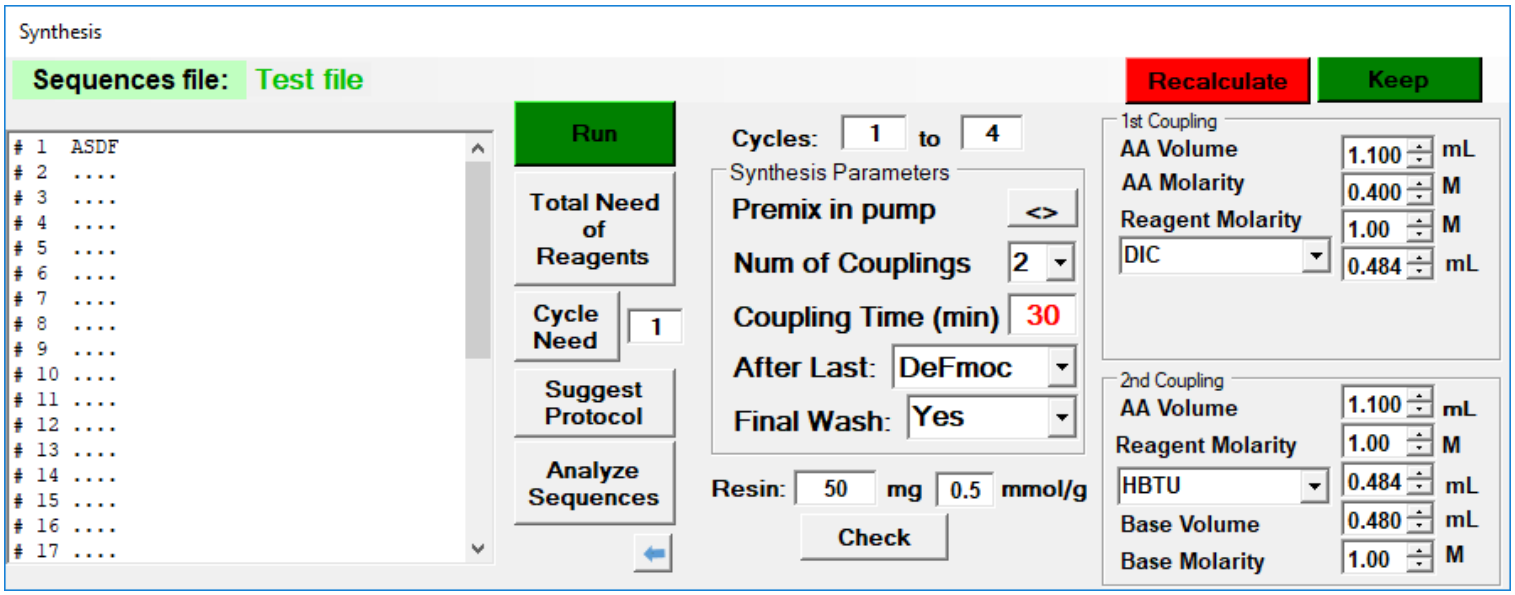
3. You can click **"Sequences file"** and select from the choices **"New"** and from the additional choices select "Sequences file". This will open the window "New File" in which you can add or edit your sequences. Do not forget to save your file at the end of editing.

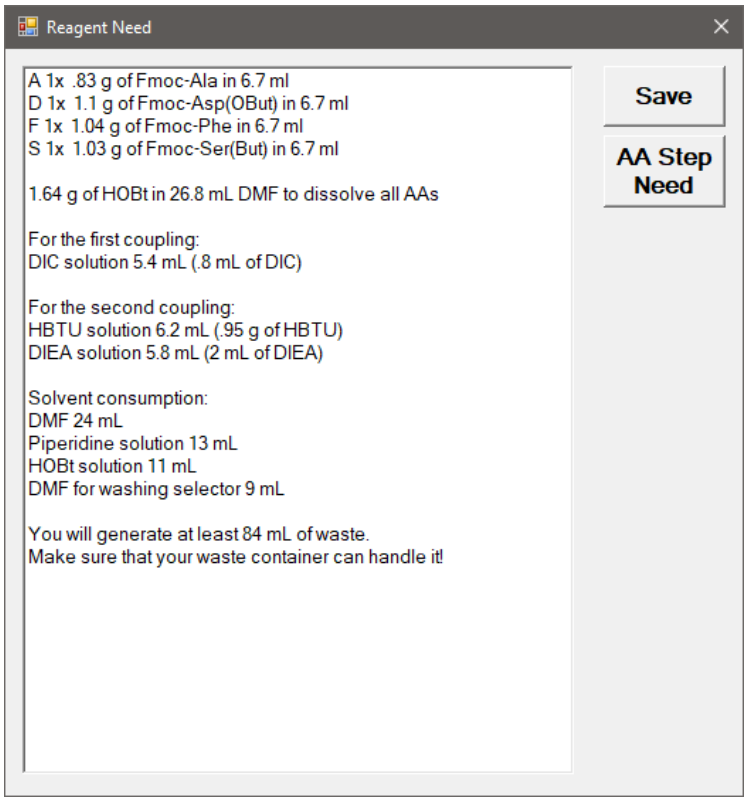


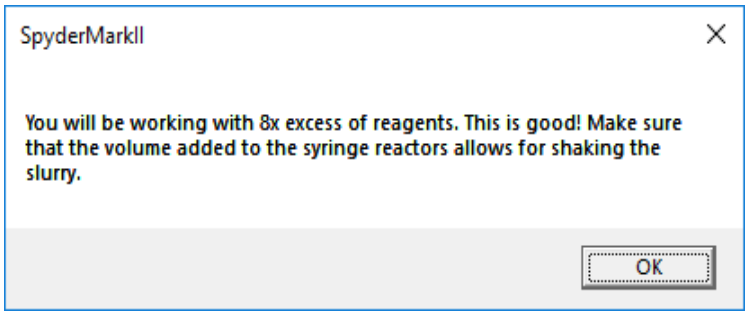
Clicking **"Right arrow"** button will extend window "Synthesis" and you will be able to modify the reagent setup (reagents, their volumes, concentrations, repetitions, coupling time, action after the last step, and the mode of addition of reagents to the reactors). The default values loaded here were taken from the values defined in your set, or the Default set if you did not load your personal set. You will also define range of the synthesis (counted from C- terminus – Cycle 1 will couple F in this example, if you would say Cycles 2 to 3, D and then S will be coupled).



Changing any value for 1st or 2nd coupling will call two buttons **"Recalculate"** and **"Keep".** If you click **"Recalculate"** it will calculate the volumes for all reagents based on the internal "best practice" algorithm. In the calculation the highest priority is given to the volume of amino acid solution, reagent volume is calculated based on that and concentrations are not recalculated (the machine can change only the volumes of added reagents). If you want to use your defined volumes without using this algorithm, click **"Keep"** and the machine will use the values defined by you.





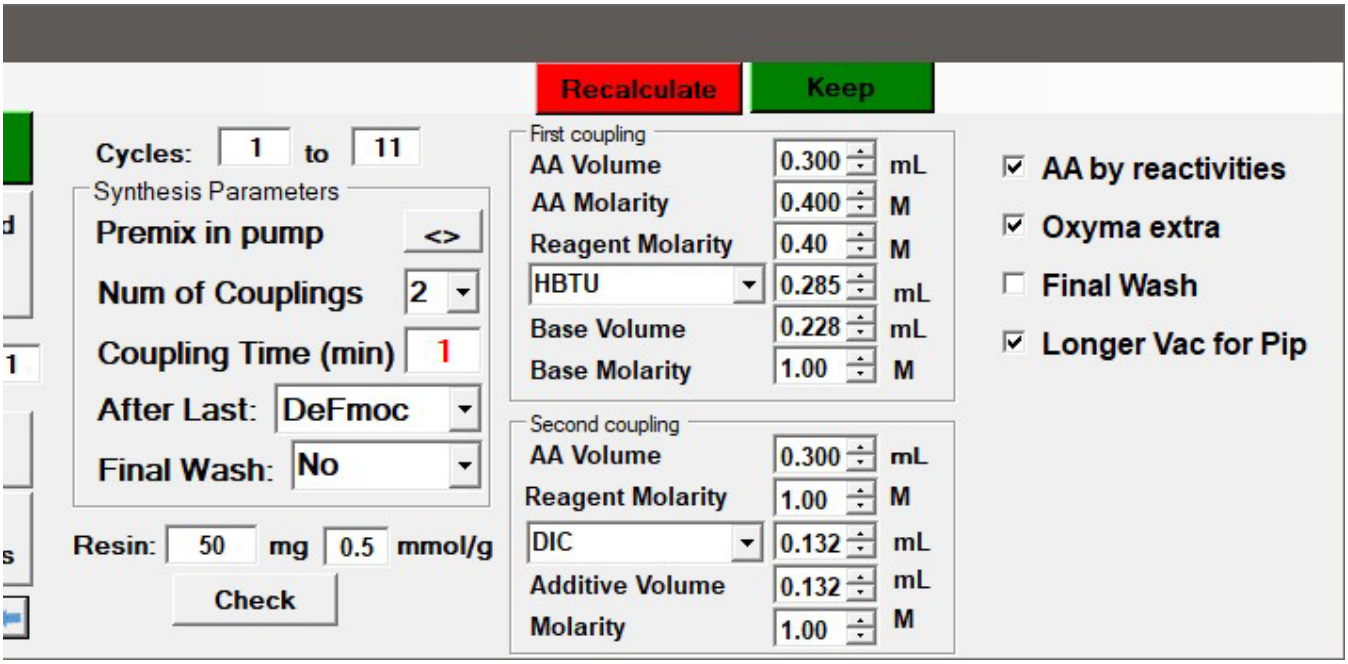


Clicking on **"Total Need of Reagents"** will show the calculation of volumes of reagents needed for the synthesis of defined sequences using the volumes defined in "Synthesis" window and in "Deprotection Wash" window. **"Cycle Need"** will show the need in only defined cycle.

A 3x .83 g of Fmoc-Ala in 6.7 ml means that A will be used three times and NOT that you should make three copies of the same solution.

What we recommend is to make the solution of HOBt (or Oxyma) in DMF (0.4M) and use this solution for dissolving all the amino acids. Calculation of the volumes considers the need to prime the reagent lines and takes into the account the fact that not all solution can be used (the dead volume). Therefore, synthesis of only one short peptide in this multiple synthesizer is relatively uneconomical.

Clicking on **"Check"** will calculate an excess of reagents which you will use and it will warn you if the excess is too low to guarantee the successful synthesis.



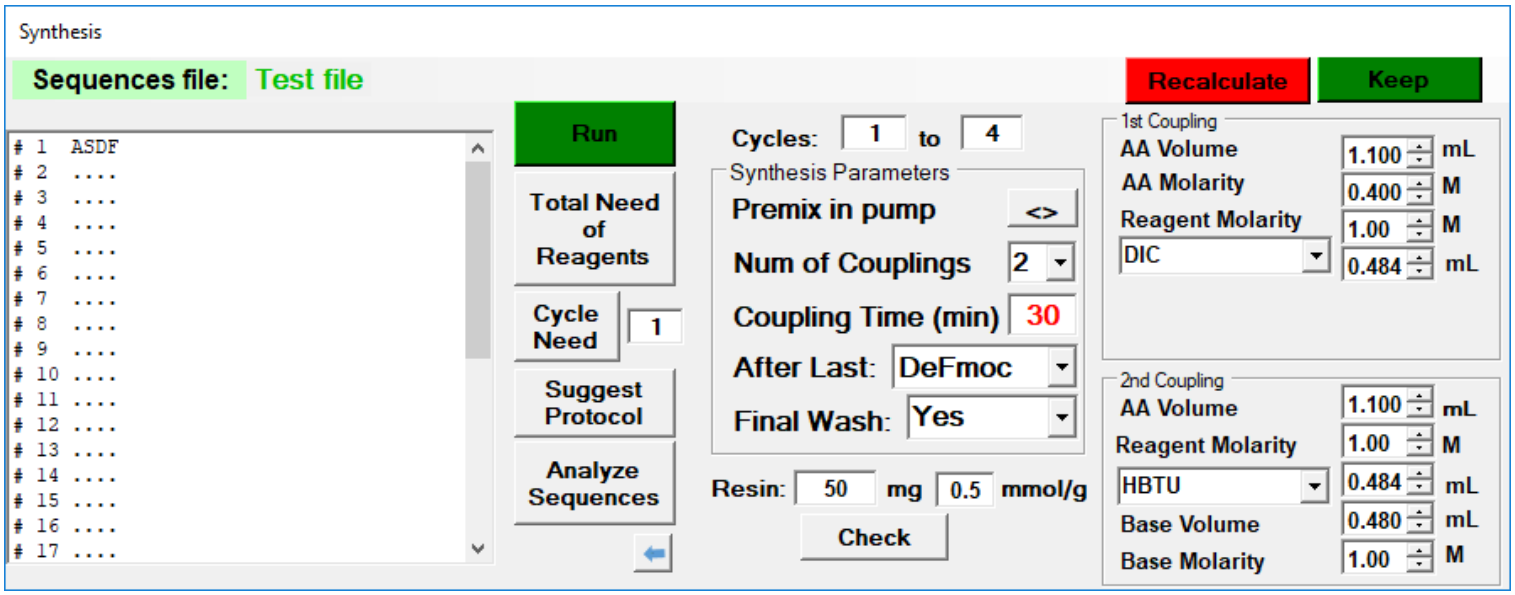
If you want to dissolve amino acids in pure DMF and add Oxyma or HOBt just prior to the coupling with DIC, you have to extend the “Synthesis” window to the right and check the checkbox “Oxyma extra”. In this case selection of DIC as the coupling reagent will let you select the volume and molarity of the additive for DIC coupling. You can save this choice in your parameter set. Additive will be delivered from the same bottle as the Oxyma or HOBt solution which you may be using for prewashing before coupling – therefore it must have the same molarity.

The check box “AA by reactivities” is giving you choice to add individual amino acids to reactors (syringes) based on the reactivities or by alphabetical order. Addition “by reactivities” gives a little edge to the slow coupling amino acids like isoleucine or valine, which are added first and will have the chance to react with solid support longer. However, this option is relevant only in the case of “Premix in pump” mode.

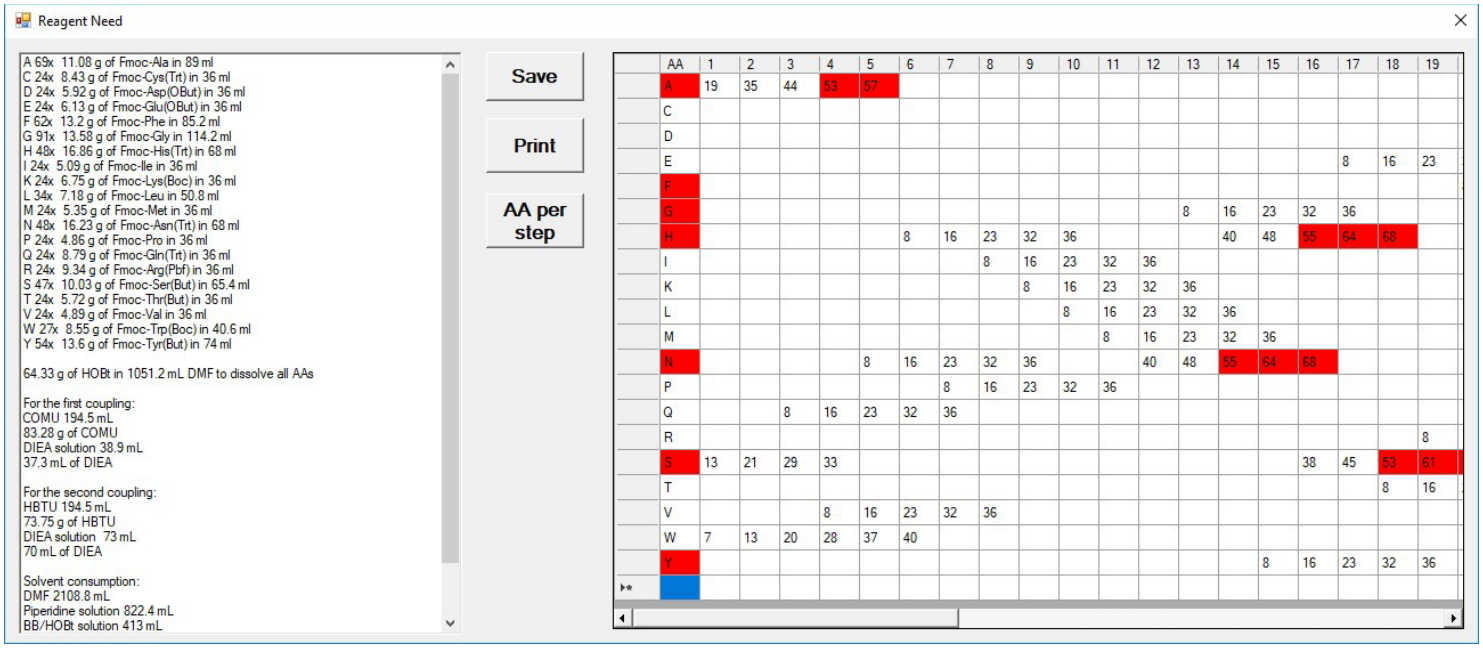
The check box “Final Wash” lets you have automatically select the choice for performing the wash of the resin after the last coupling with the solvent connected to Cavro pump port 11. If this box is checked, the synthesizer will select the program “FinalWash.prg” and perform its operations after the last step of the synthesis.

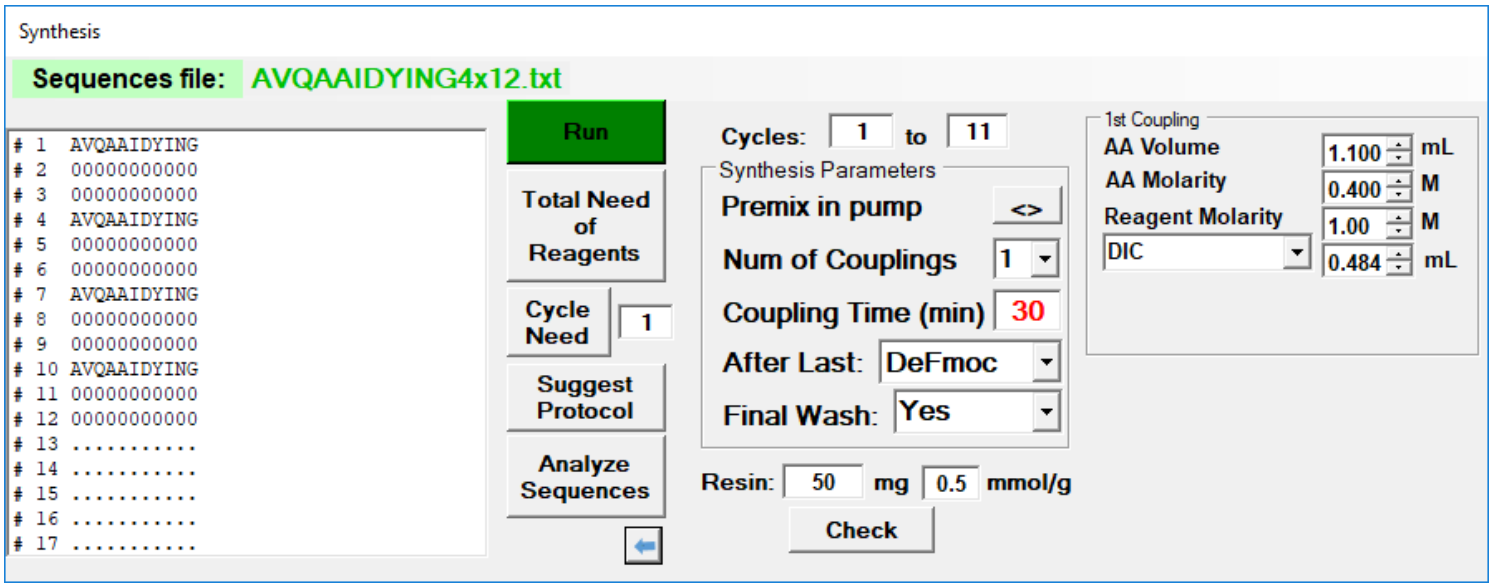
During the synthesis, the vacuum pump (optionally attached to the power outlet on the back of the synthesizer) is actuated after each centrifugation for the time defined in the parameter set – usually for 60 seconds. If you want longer pump run after piperidine involving steps, set the check box “Longer Vac for Pip”. In this case after steps involving piperidine, the pump will run longer to prevent piperidine smell escaping to the lab.

In any case, after the last step of the synthesis, the vacuum pump will be actuated for 10 minutes.

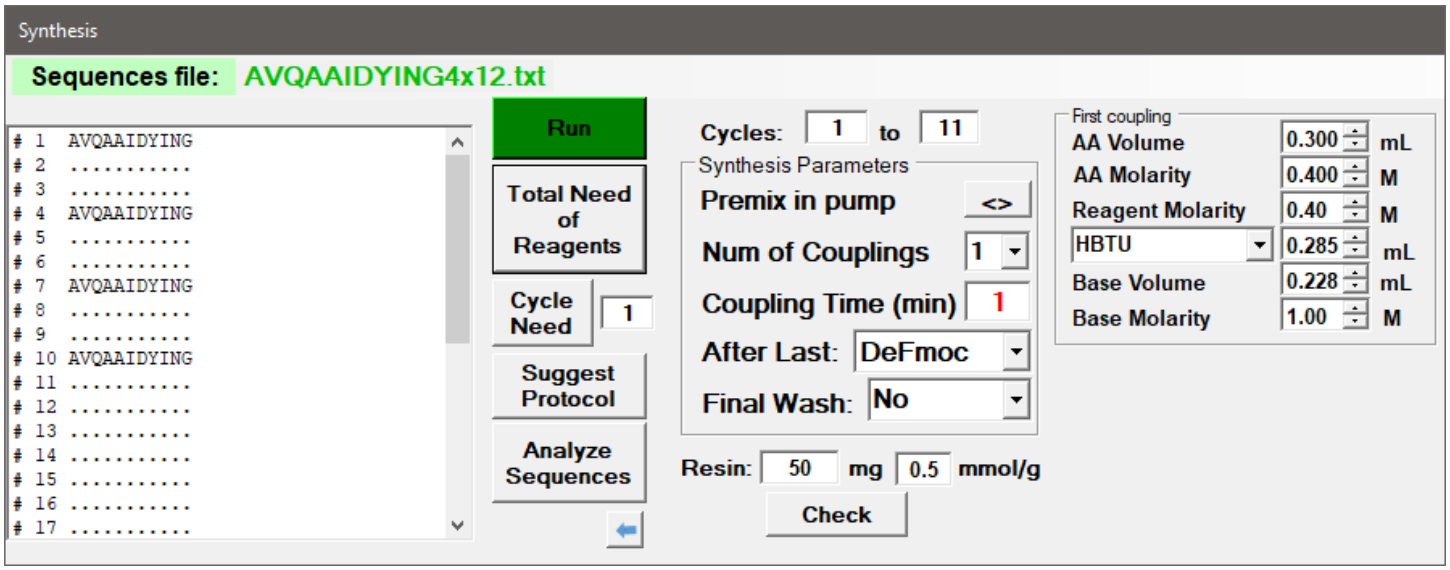


Clicking on **"Total Need of Reagents"** will open the "Reagent Need" window with the table of individual amino acid solution need for each step. If the needed amounts of amino acid solutions exceed the capacity of Falcon tubes used for their solutions storage on the machine, the spreadsheet with cumulative volumes needed for the synthesis will open (this extended spreadsheet can also be opened by clicking **"AA per step"** button). Amino acids marked in red will have to be replenished during the synthesis. In this example, alanine will have to be added before step number 4, asparagine before step number 14, histidine before the step number 16 and serine before the step 18. Phenylalanine, glycine and tyrosine will have to be added later. Alternative solution for this problem is to supply amino acid solutions from alternative containers (see later), or run the synthesis in several increments (e.g. cycles 1 to 3, 4 to 13 and 14 to 25). Amounts of solvents depend on the protocol used for deprotection and washing, so make sure that you select the appropriate protocol prior to this calculation. In the table there is also the calculated amount of waste. You should make sure that your waste container can handle it.





Selecting a sequence in the list and clicking on **"Analyze Sequences"** will present the difficulties of individual couplings in a graphical form. Amino acid represented by a blue bar should not cause any troubles in coupling, red bar represents difficult coupling and a green bar represents super easy coupling. If no sequence was selected prior to the click, difficulties in all steps will be presented only in a tabular form. Clicking on the graph brings back the tabular form.



To solve the problem of difficult couplings, you can click **"Suggest Protocol"** and the window "Suggestion" will open. Here you can define conditions for the couplings in steps which are predicted to be difficult. If you do not want the machine to fill the table for you, do the following steps:

1. Select 2 as Num of Couplings
2. Define the reagent and its concentration for the second coupling. Obviously, you cannot change concentrations of the amino acid solutions since they are delivered from the same container.
3. Then change the Num of Couplings back to 1. The definition of the second coupling conditions will disappear.
4. Click **"Suggest Protocol"** and the coupling schedule will appear.

Suggestion

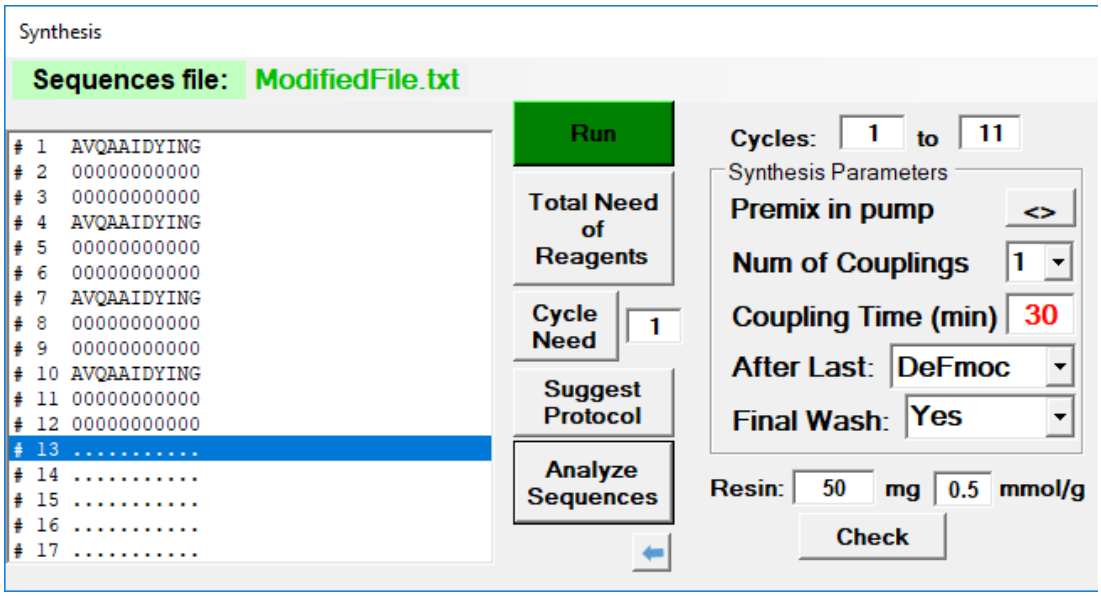
Open **Save**

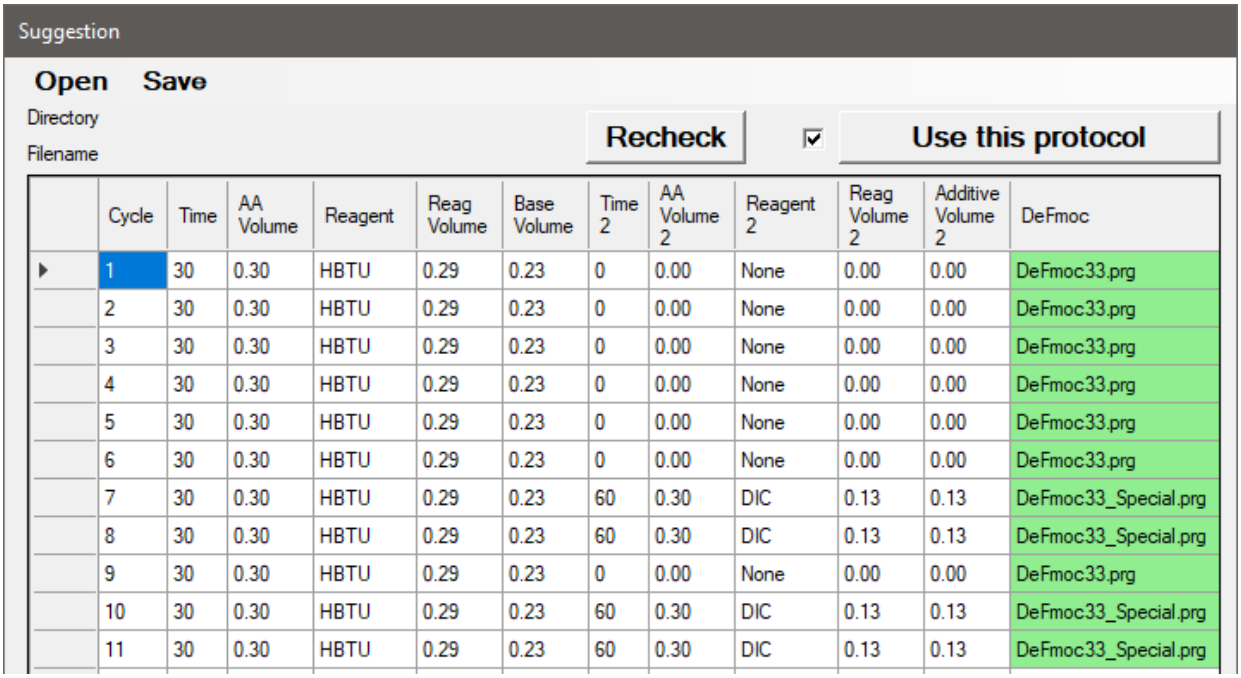
Directory

Filename

Use this protocol

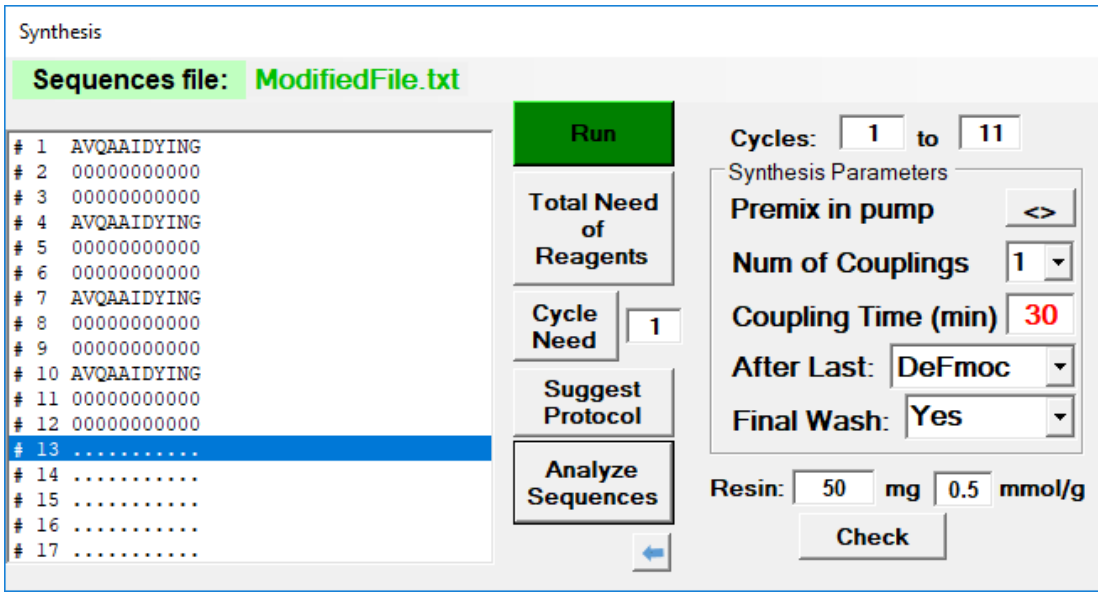
	Cycle	Time	AA Volume	Reagent	Reag Volume	Base Volume	Time 2	AA Volume 2	Reagent 2	Reag Volume 2	Additive Volume 2	DeFmoc
▶	1	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	2	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	3	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	4	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	5	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	6	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	7	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	8	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	9	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	10	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	11	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg

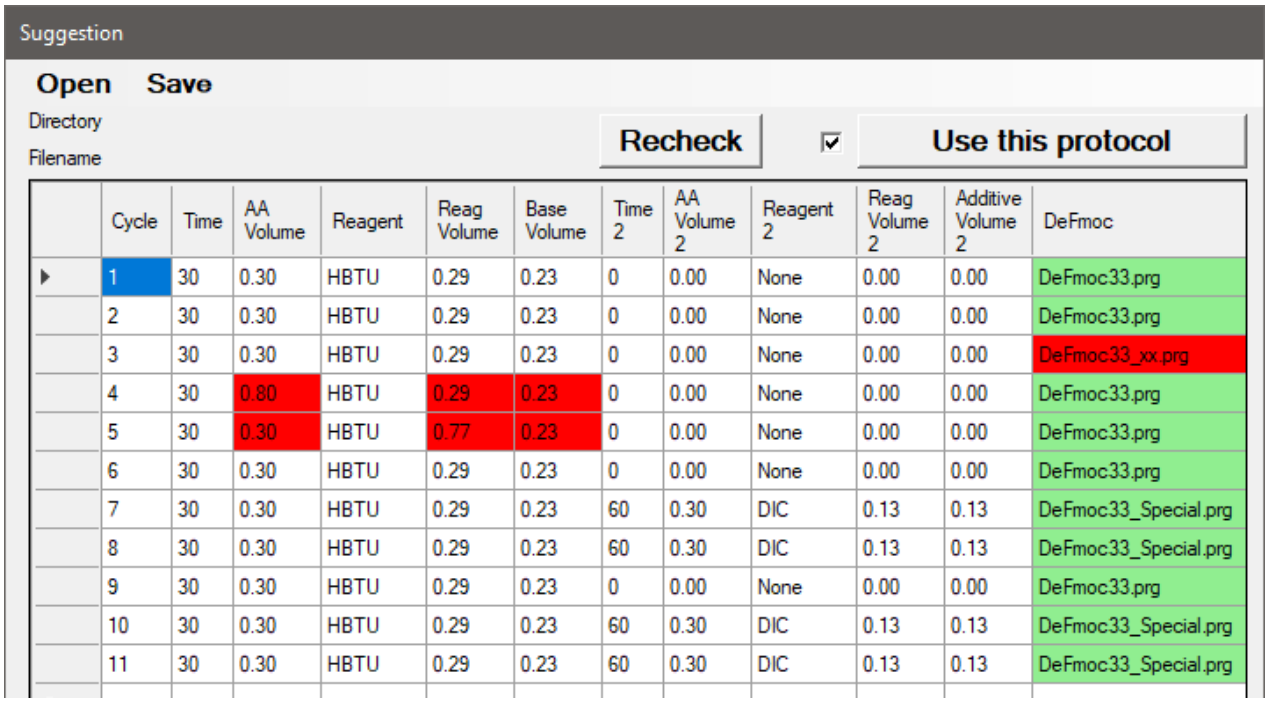




	Cycle	Time	AA Volume	Reagent	Reag Volume	Base Volume	Time 2	AA Volume 2	Reagent 2	Reag Volume 2	Additive Volume 2	DeFmoc
▶	1	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	2	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	3	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	4	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	5	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	6	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	7	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	8	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	9	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	10	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	11	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg

You can modify this table (change the coupling times and volumes of reagents) but keep in mind that the reagent for the first coupling and reagent for the second coupling must be the same in all steps. Also the volume of reagents added in each step must be smaller than 1.1 mL in the case of small reactors and 2.5 mL in the case of big reactors (syringes). You can also change the deprotection protocol (DeFmoc column) for individual steps. If you click **“Use this protocol”**, the program will check for the correct volumes of all reagents in both couplings and it will make sure that the file defined for the deprotection and washing is defined. If it recognizes that there are difficult couplings expected it will also automatically load different deprotection protocol for those steps – if such protocol exists. As a default, this protocol should be named “YourProtocol_Special.prg” (in the above example “DeFmoc33_Special.prg”) – it could use longer time for treatment with piperidine, or number of repetitions, or washing with different solvent prior to the coupling. However, you can define different deprotection and washing protocol for each step. After all your changes in this table you should click **“Recheck”** button, which will make sure that the modified volumes and protocol names are OK.



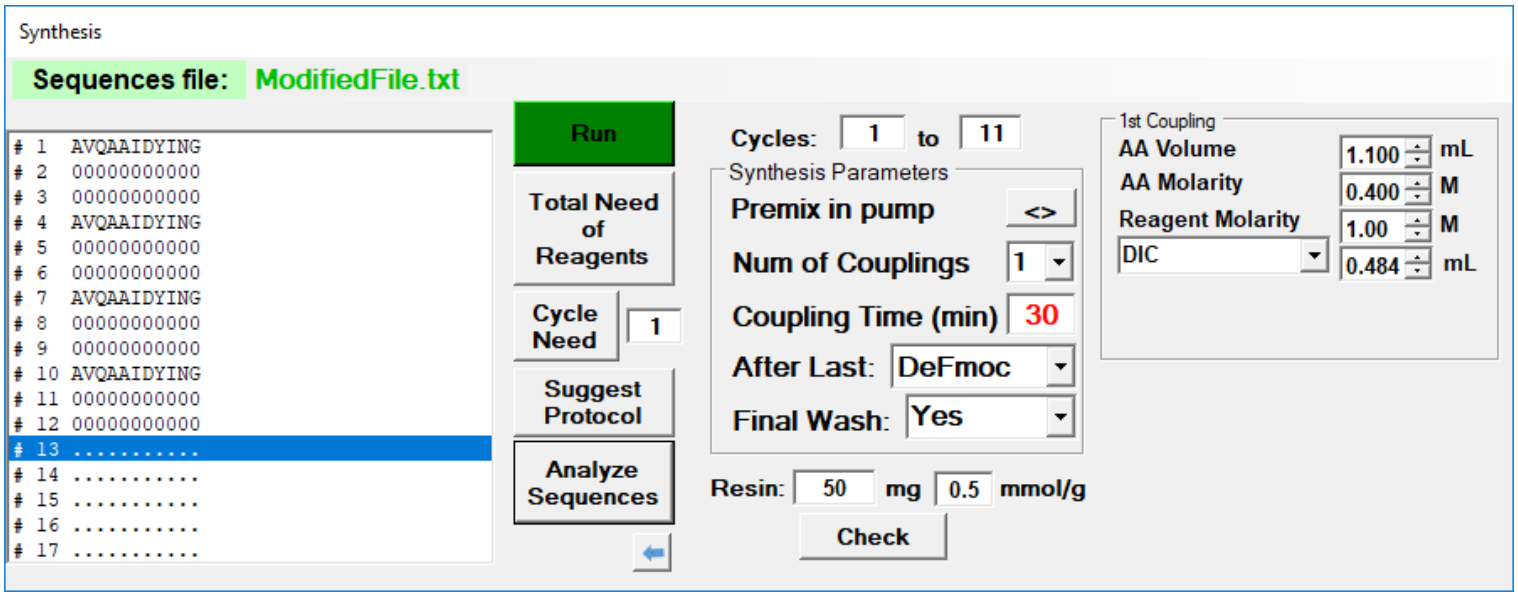


	Cycle	Time	AA Volume	Reagent	Reag Volume	Base Volume	Time 2	AA Volume 2	Reagent 2	Reag Volume 2	Additive Volume 2	DeFmoc
▶	1	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	2	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	3	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33_xx.prg
	4	30	0.80	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	5	30	0.30	HBTU	0.77	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	6	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	7	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	8	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	9	30	0.30	HBTU	0.29	0.23	0	0.00	None	0.00	0.00	DeFmoc33.prg
	10	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg
	11	30	0.30	HBTU	0.29	0.23	60	0.30	DIC	0.13	0.13	DeFmoc33_Special.prg

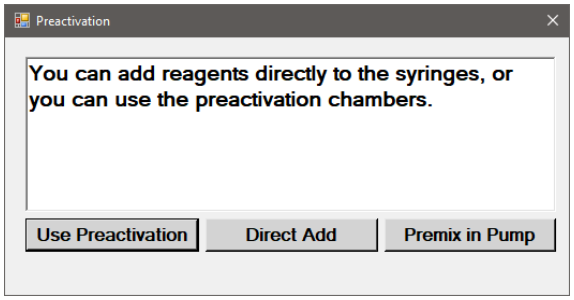
If the changes you made would result after clicking “Recheck” button in some fields being marked in red, make sure that you adjust all those fields before starting the synthesis.
If the protocol name is marked, make sure that that file exists and can be found in the subdirectory “C:/Triton/Programs/”.

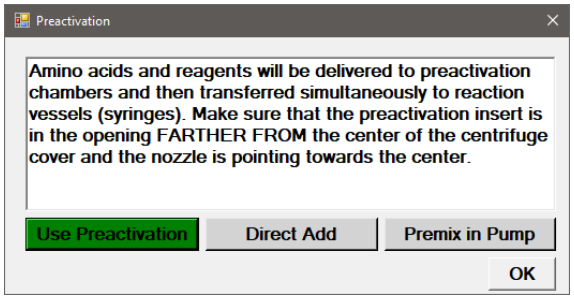
Do not start the synthesis when any of the fields in the table are marked in red!

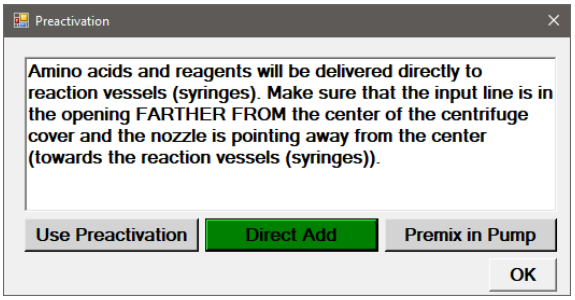
If you click “Use this protocol” again, the checkmark will disappear and this suggestion table will NOT be used. Before continuing, close this window by clicking “x” on its upper right hand corner.

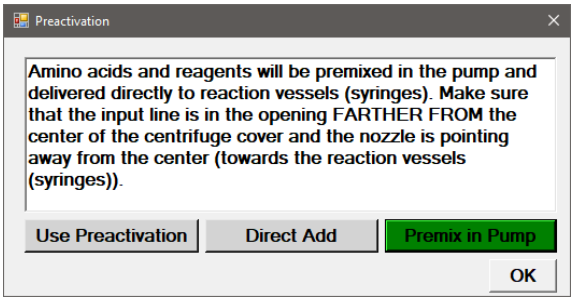


Changing the mode of addition of reagent: Click **"<>"** button and window "Preactivation" will open.



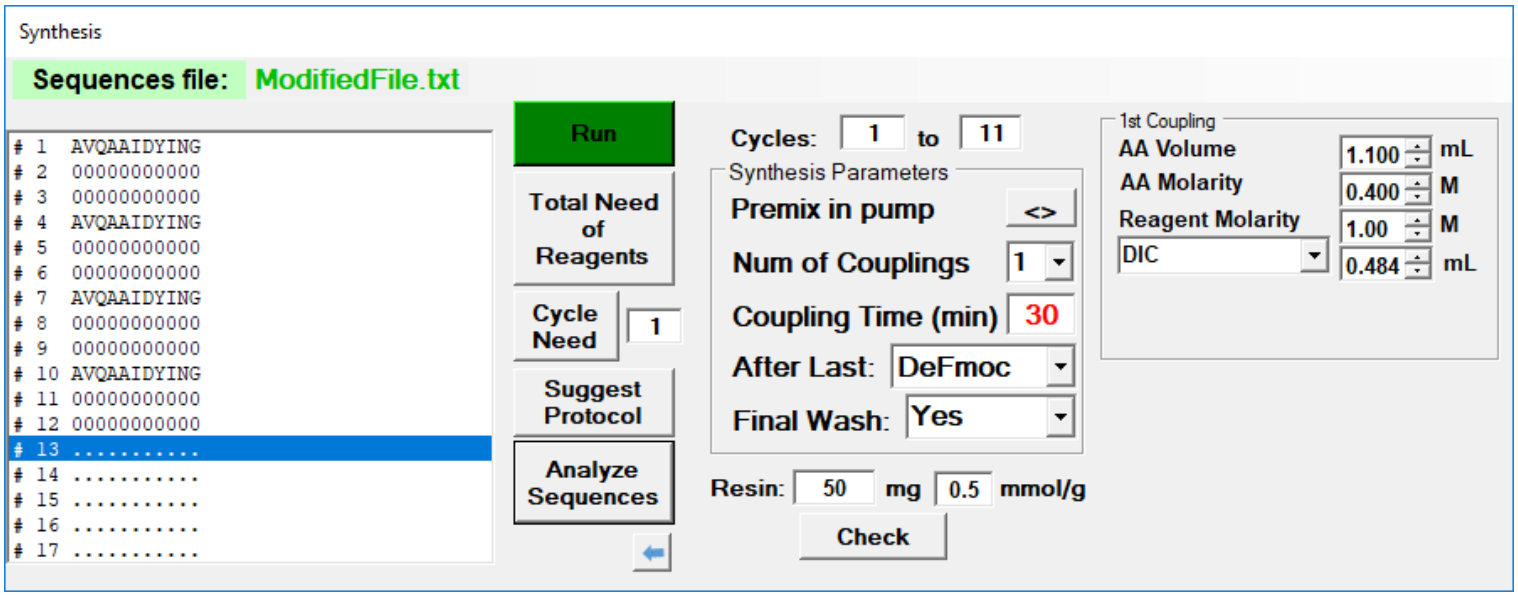




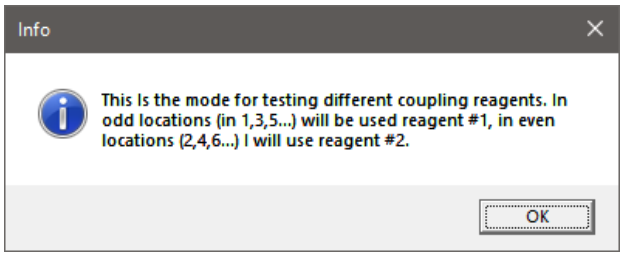


You can select three modes of addition of reagents:
1. Preactivation –amino acids and reagents are added to the distribution insert and then simultaneously transferred by slow rotation to the appropriate syringes.
2. Direct addition – amino acids are added to all syringe reactors and only after all amino acids are distributed, reagents are added
3. Premix in pump – amino acid solution, reagent and base are all sucked into the Cavro pump and then expelled to the syringe reactors.

You click the addition mode which you desire and window with description how the nozzle for delivering reagents should be placed and whether you should use the distribution insert.



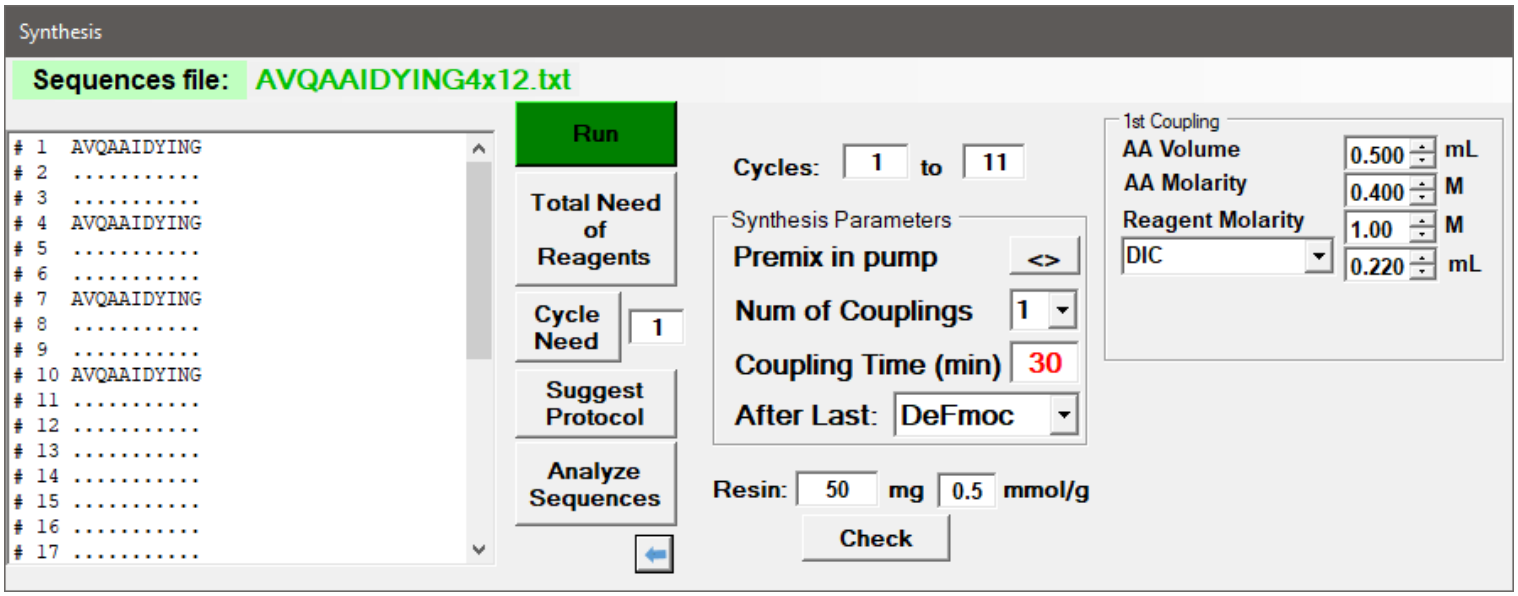
"Num of Couplings" selection rolldown has three options – 1, 2, and T. Selection T is used for testing coupling reagents. In this case the first reagent will be used for coupling in syringes placed in location with odd numbers and the second reagent will be used in even numbered syringes.

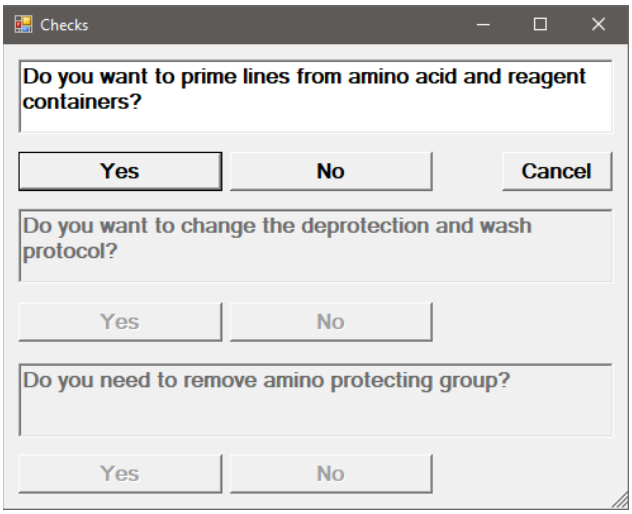


"After Last" selection rolldown has two options – DeFmoc and JustWash. If you select DeFmoc, peptide will be deprotected and washed with DMF. If you select JustWash, resin will be only washed by DMF.

"Final Wash" selection rolldown has two options – Yes and No. If you select Yes, peptide will be after the last step washed with solvent connected to Cavro port #11 (usually MeOH or DCM). If you select No, resin will not be washed and the last operation is the one defined by your Deprotection/Wash protocol. If you have HOBt wash in the protocol, it will not be used in the last step and only DMF will be used.

Changing the selection of cycles to be run: If you select only several cycles of the synthesis, always double check whether you are really talking about synthesis cycle and not number of amino acid in the peptide chain! The cycle 1 of the synthesis of the AVQAAIDYING will couple G (amino acid in position 11 of the peptide chain).

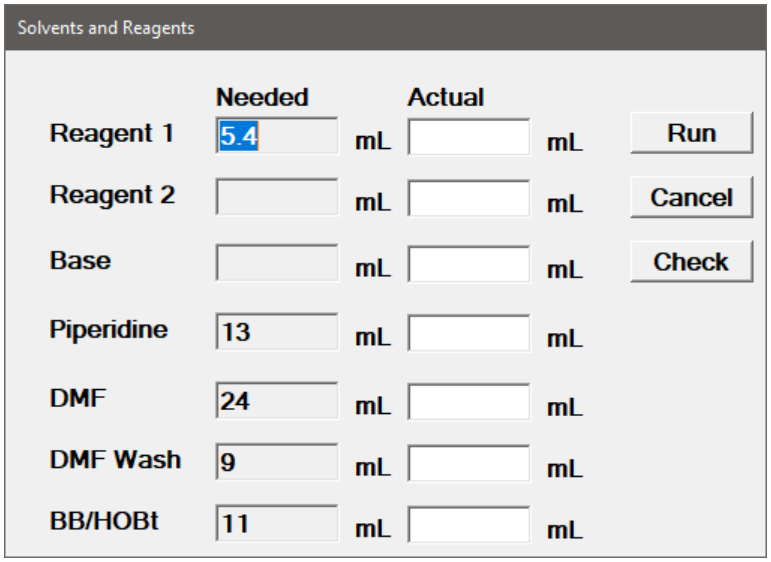




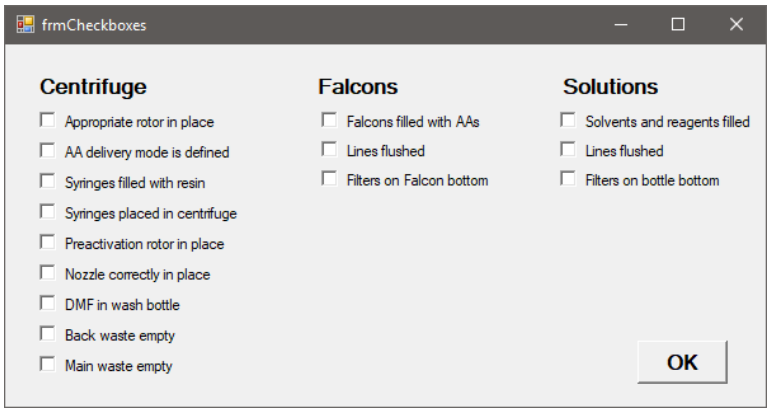
Clicking **"Run"** will start the synthesis, but first you have to answer three questions:

1. Do you want to prime lines?
2. Do you want to change deprotection protocol?
3. Do you need to remove amino protecting group?

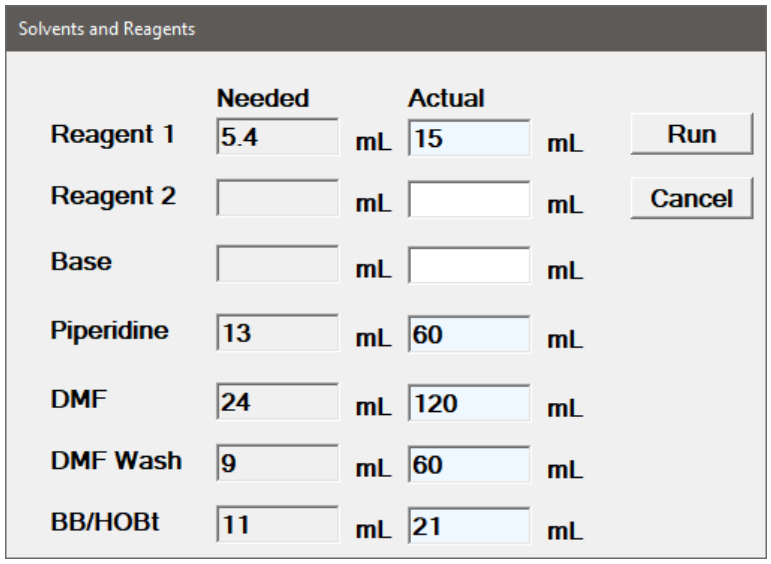
Answering Yes or No will guide you through necessary steps (see e.g. Sample synthesis – earlier in this text)



The next window ("Solvents and Reagents") will ask you about the volumes of reagents which you should have placed in the appropriate vessels. You may fill all volumes you actually have in the bottles and click **"Run",** or you can click the button **"Check".** (If you don't have enough reagents in the bottles, the machine will tell you so and will not let you go on until you have enough.)

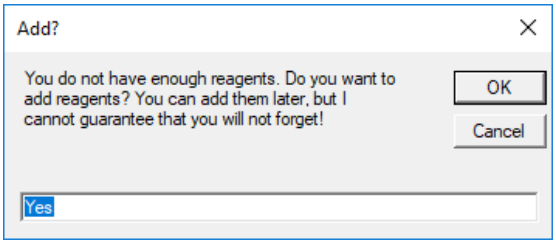


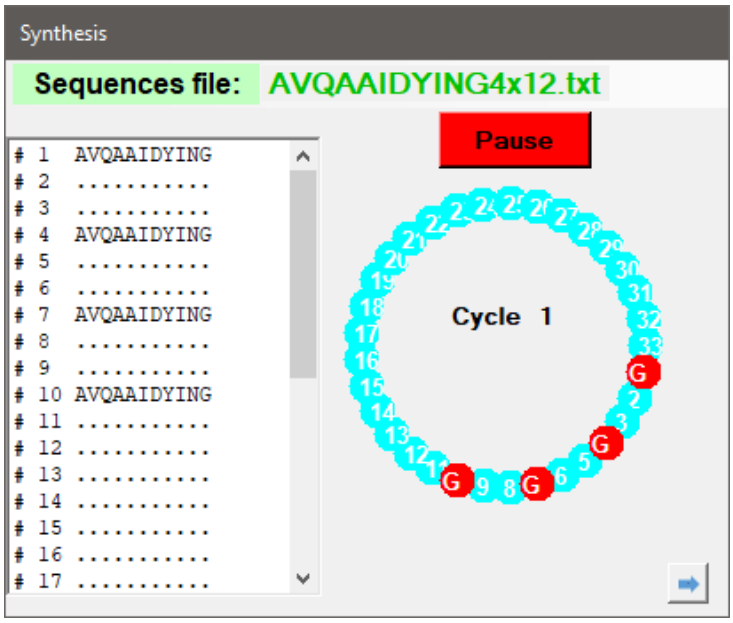
Clicking **"Check"** will bring another window ("Check Boxes") with the list of all things which have to be checked before the synthesis can be started. If you are sure that everything is correct, click **"OK"** and the window "Solvents and Reagents" will be filled with volumes which are the minimum volumes guaranteeing the successful synthesis.



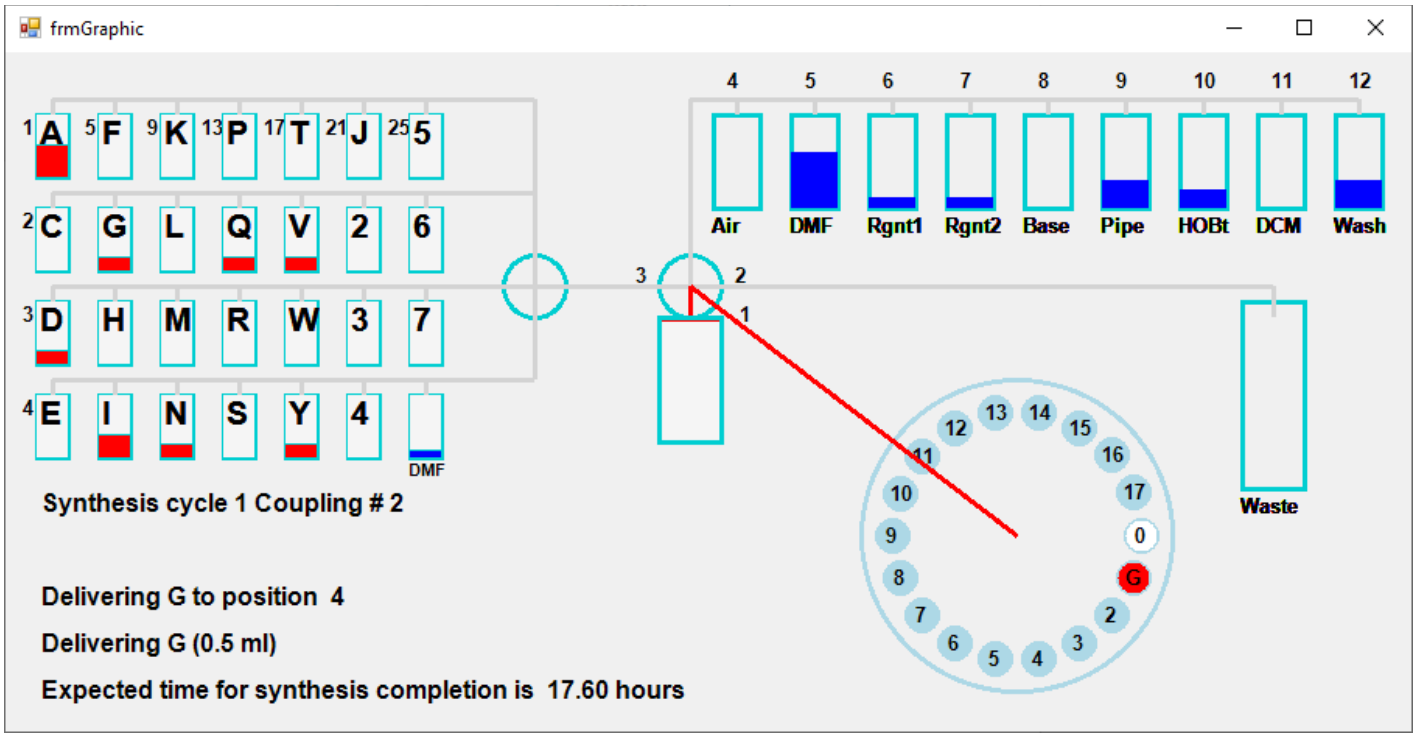
Press **"Run"** and the machine will do the rest…
You can still change your mind and cancel this run by pressing **"Cancel"** button.

If you will not fill all containers, computer will let you run the synthesis, but will be interrupting the process to remind you that you should add the reagents. We strongly recommend not to start synthesis without filling all solutions.



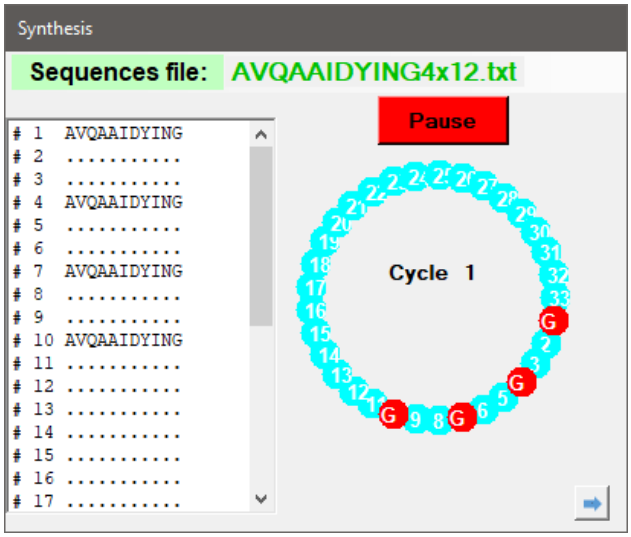


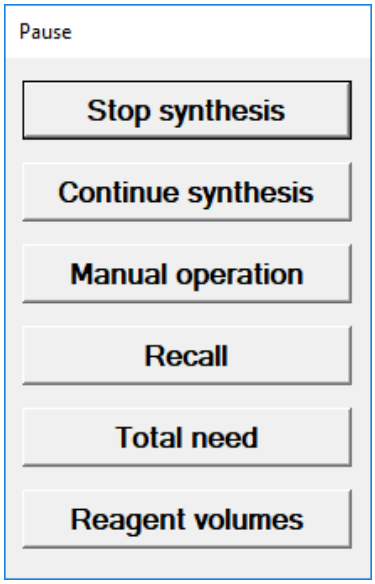
Synthesis will start and you will be able to follow the progress of the synthesis in graphic representation of the rotor with the syringes where amino acids addition is marked by red circle, reagent by green circle, base by blue circle, piperidine by brown circle. Additional information will be displayed in “Report Window” in text and graphic form giving you an estimate when the synthesis will be finished.



Simple information is also displayed on the front panel of the synthesizer.

If you will click the button **“Go On”,** which appears during the coupling incubation in the middle of the circle of rings, you will be able to skip the rest of the incubation time and continue the synthesis immediately. This can be used in situations when you are monitoring the progress of the reaction (for example with bromophenol blue*) and want to speed up the synthesis.



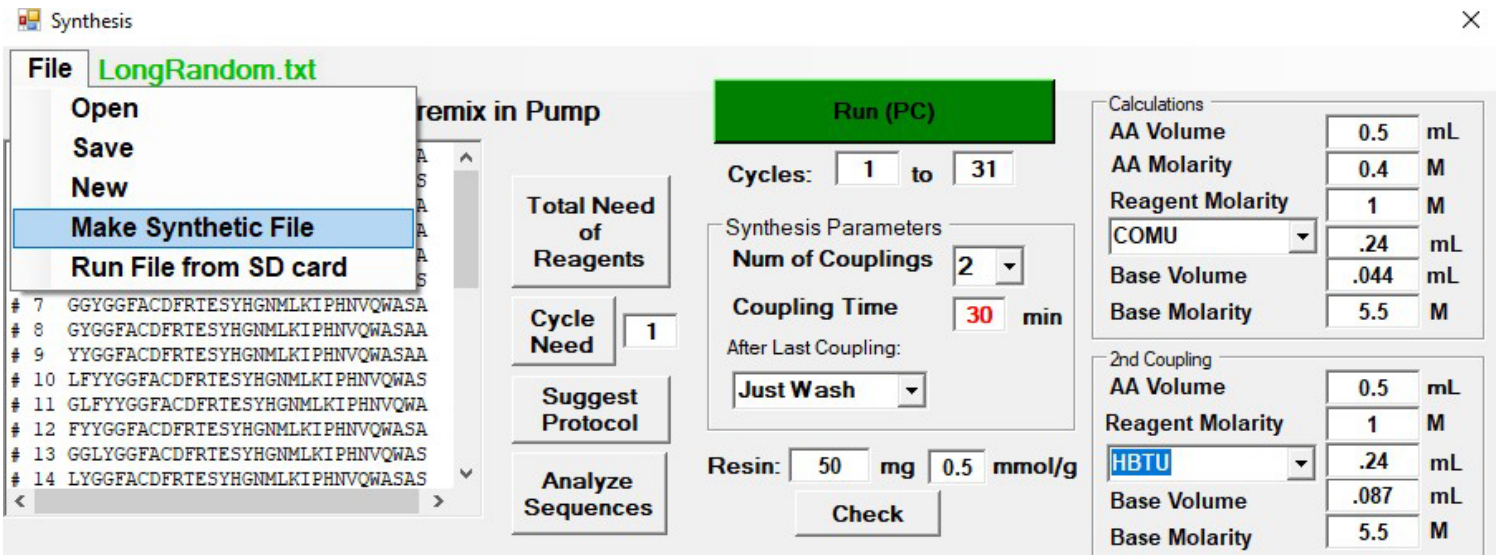


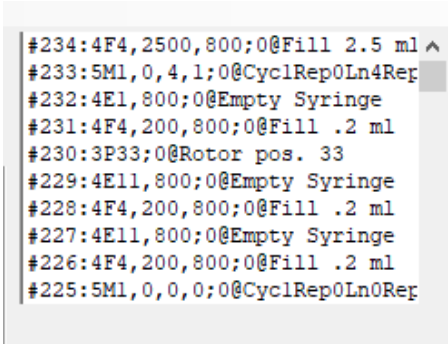
If you want to pause the synthesis, click the **“Pause”** button. From the “Pause” window you can go to the limited set of **“Manual operation”** of the machine, or you can **“Stop synthesis”.** If you just hit **“Continue synthesis”** button, the synthesis will continue. If you want to check where you were when the “Pause” button was pushed, click the **“Recall”** button. You can also check the total need of reagents calculated for this synthesis **(“Total Need”)** or check the **“Reagent volumes”** which you should have in the reagent bottles at this moment of the synthesis.

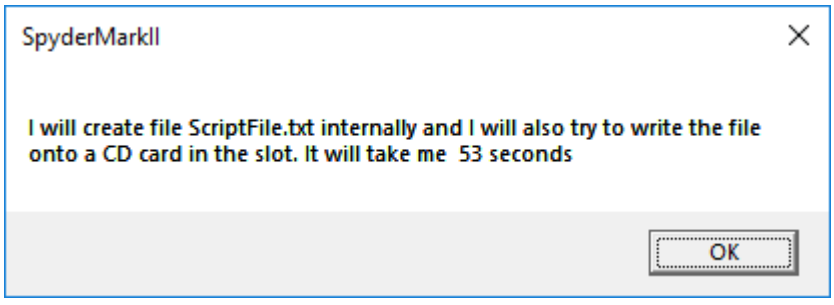
* Krchnak,V., Vagner,J., Safar,P., & Lebl,M. (1988) Noninvasive continuous monitoring of solid phase peptide synthesis by acid-base indicator. *Collect. Czech. Chem. Commun*., 53, 2542-2548

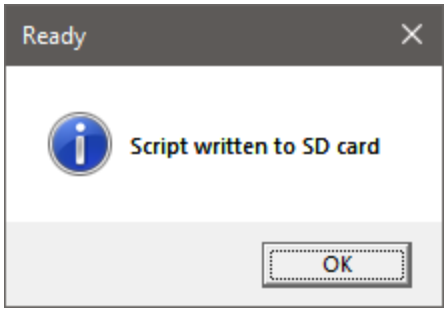
Synthesis without connected computer

You can also generate the script file which can be later inserted into the SD card slot of the machine and synthesis can be run without the need of computer. Just click “Make Synthetic File” button. The script will be generated and written onto SD card. The file “SCRIPT.txt” will be written into the directory “Script” and onto the SD card, if it was inserted in the slot in the machine. You can generate the script on another computer with installed software but remember that the parameters defined on that computer must match parameters defined on the synthesizer.

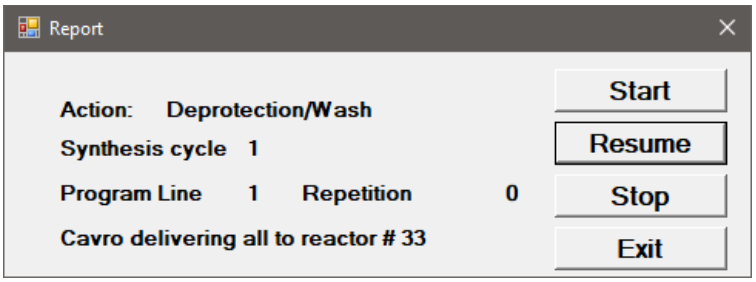


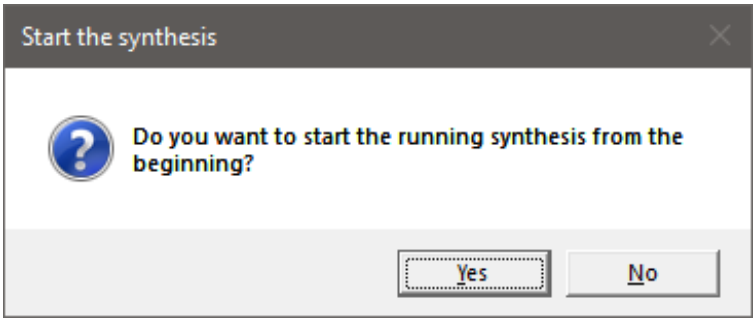


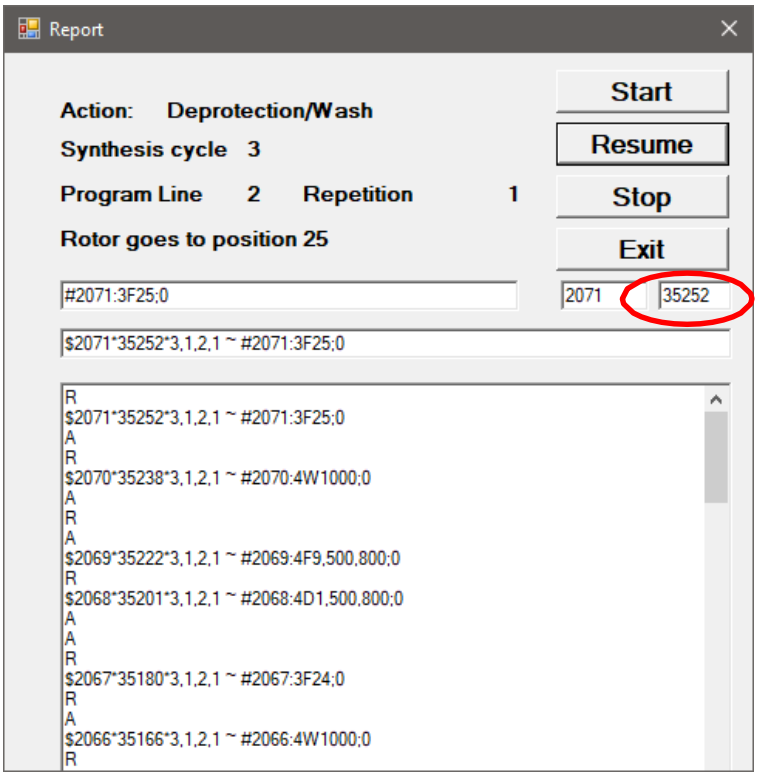




After you insert the SD card to the slot, you can start the synthesis either by pressing the buttons on the display, or select “SD card run” from the sequence: “Sequences file:” >> “New” >> “SD card run”. (If the machine is connected to the synthesizer.) This will open the following “Report” window:







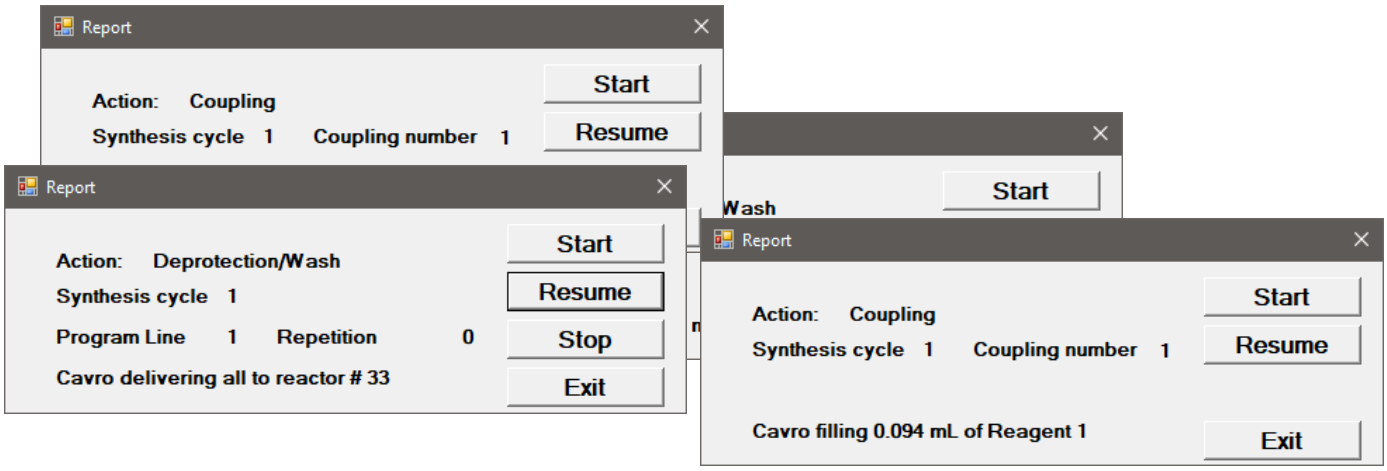
“Start” will initialize the synthesis defined in the file “Script.txt” which must be placed in the SD card slot. It will request confirmation that you want to start the synthesis from the beginning.

“Stop” will stop the execution of this script synthesis.

“Exit” will disconnect you from the synthesizer without interruption of the synthesis. Synthesis will continue using instructions from the SD card.

You can stretch the “Report” window and see the commands being executed in the real time.
“Resume” will continue the synthesis which was interrupted. You can “skip back” in the synthesis and define the new starting point. Just go in the list to the desired point and note the number between two * signs. (The first number is the line number from your script file.) Type *xxxxxx* number into the field marked with the circle and press “Resume”. If you just press “Resume” without changing the number in marked field, synthesis will continue where it was interrupted.
Example:
$2422*41418*4,1,0,0 ~ #2422:4F6,285,800;0
Number $2422 is a line number from your script file and number 41418 is the internal memory location from which you want to restart your synthesis.

“Report” window will follow your synthesis if the computer will stay connected to the synthesizer.

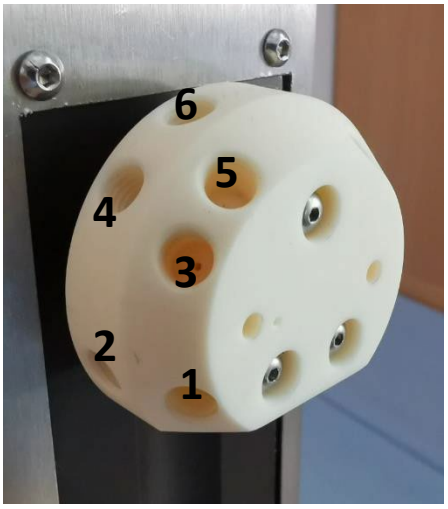


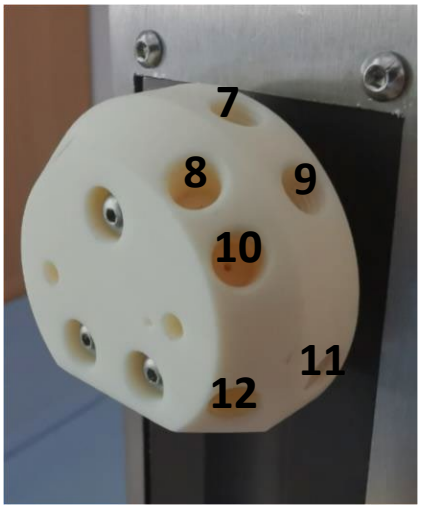
However, you can now switch off the computer and follow the synthesis progress on the display of the machine. Actually, in this case you don’t need the computer at all. You can just bring the SD card and start the synthesis using the machine display.

Appendix

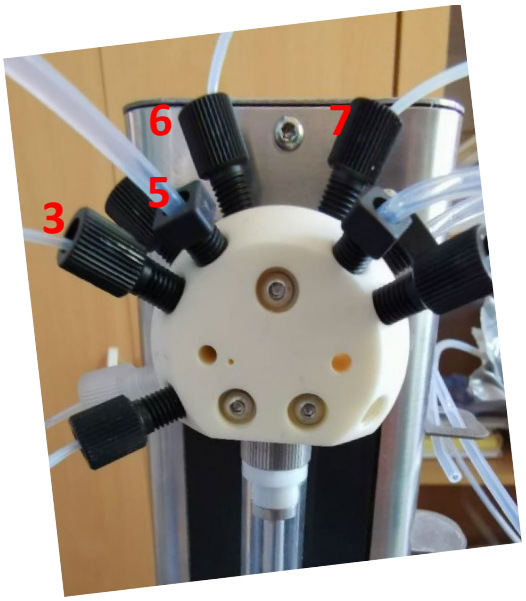
1. Cavro pump ports numbering
2. Intermediary transfer container (ITC) arrangement
3. Syringe rotor arrangements
4. Balancing rotors
5. Glass cover and delivery nozzle
6. Adjusting the shaking
7. Regional electric power setting
8. Use the appropriate and fresh syringes
9. Service functions

Cavro pump ports numbering



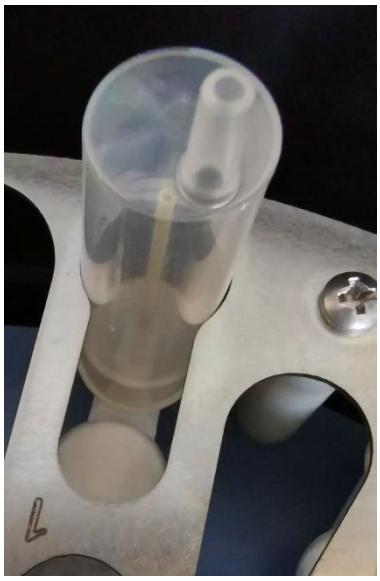


1 – connection to delivery nozzle (1/16 line)
2 – waste
3 – connection to 28-port selector valve (AA selection) (1/16 line)
4 – air
5 – DMF (1/8 line)
6 – reagent #1 (1/8 line)
7 – reagent #2 (1/8 line)
8 – base
9 – piperidin (1/8 line)
10 – HOBt solution (1/8 line)
11– final wash solvent (DCM, MeOH,…) (1/8 line)
12 – washing 28-port connection (1/8 line) or
another solution/solvent
(Line thickness is selected with regards to the minimization of wastage and potential of bubble formation)



Intermediary transfer container (ITC) arrangement

(only for 18 syringe rotor)



Good – exit of peek tubing is about 20 deg misaligned with ITC syringe output

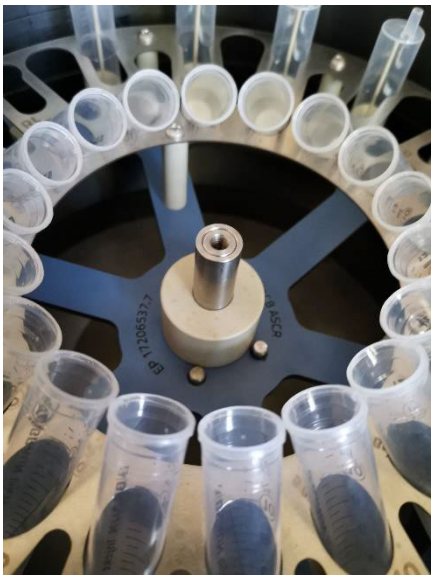
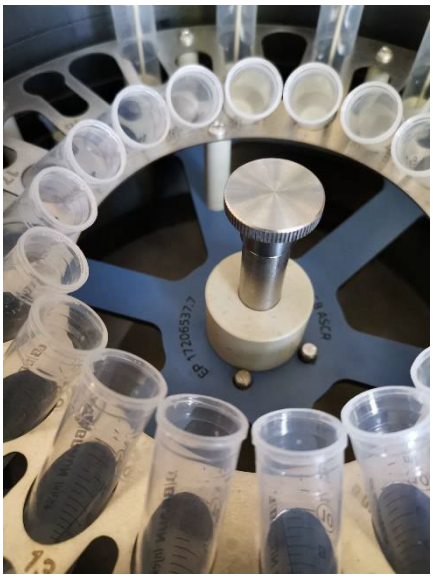
Bad – exit of peek tubing is aligned with syringe output or it is on the opposite side of the ITC syringe – liquid exiting the reactor during centrifugation will spray out of the top of ITC luer connector

Potentially Bad – exit peek tubing is more than 45 deg misaligned with syringe output. It may be OK for lower volumes used in the synthesis, but it may spray from the top of ITC when larger volumes will be used.

Syringe rotor arrangements

Arrangements for “Premix in pump” or “Direct add”

1. Make sure that three metal pins at the bottom are protruding through the plastic rotor bottom
2. Place the safety insert
3. Secure it by safety screw

Arrangements for “Use preactivation”

1. Make sure that three metal pins at the bottom are protruding through the plastic rotor bottom
2. Place the preactivation insert – pay attention to place the preactivation well with slightly deeper through to position 18 (33 syringe rotor wells are all equal size)
3. Secure it by safety screw



Balancing rotors

It is not necessary to have syringes attached to all positions in the rotor (if you are synthesizing fewer peptides than is the number of positions in the rotor). However, it is a good idea to balance the rotor by distributing the active syringes (those in which the synthesis will be performed) around the circle. When the rotor is not balanced, the shaking may become erratic and some splashing out of the syringes may occur.

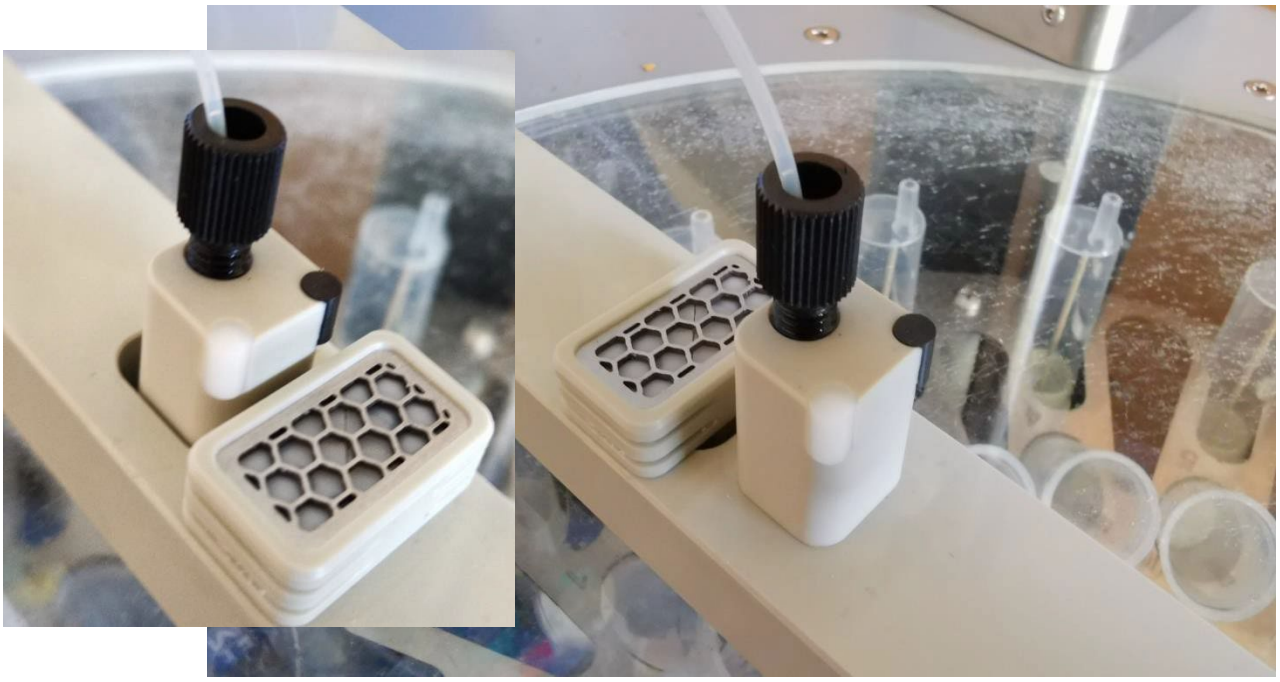
Good

Bad

Glass cover and delivery nozzle

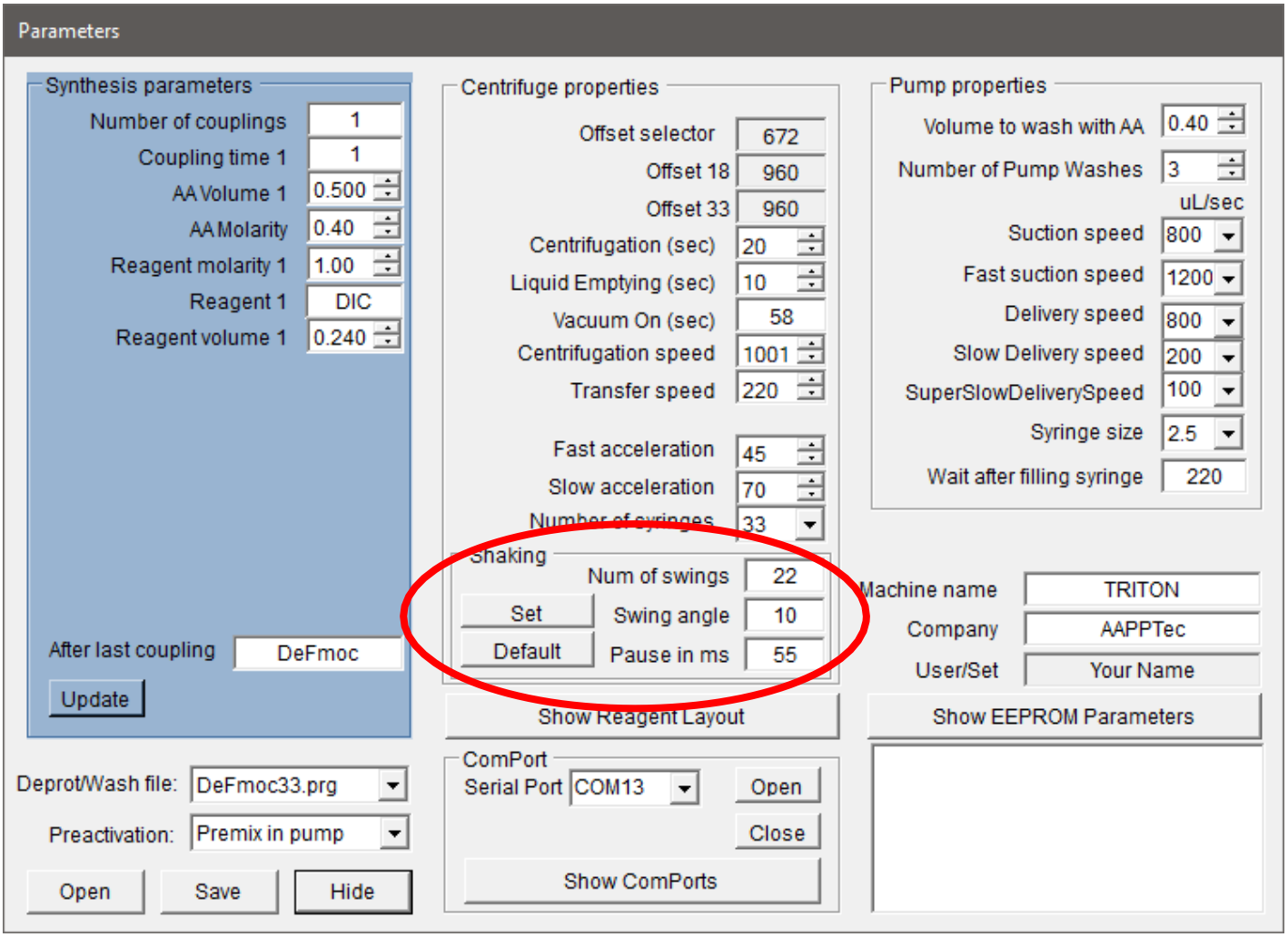
Do not take out the glass cover with the delivery nozzle in place. Take the nozzle out first and hang it in the holder on the side of Cavro pump. Hitting the synthesizer surface with the nozzle can deform its tip and the liquid delivery stream may be pointing in the wrong direction or be blocked.

The nozzle can be placed in two locations (closer to the center and farther from the center) and in two orientations (pointing to the center and pointing away from the center). The second opening should be filled with the safety plug which prevents creation of vacuum inside the reaction chamber.
Closer to the center = 18 reactors
Farther from the center = 33 reactors
Pointing to the center = “Use preactivation” (with preactivation insert)
Pointing away from the center = “Premix in pump” or “Direct add”



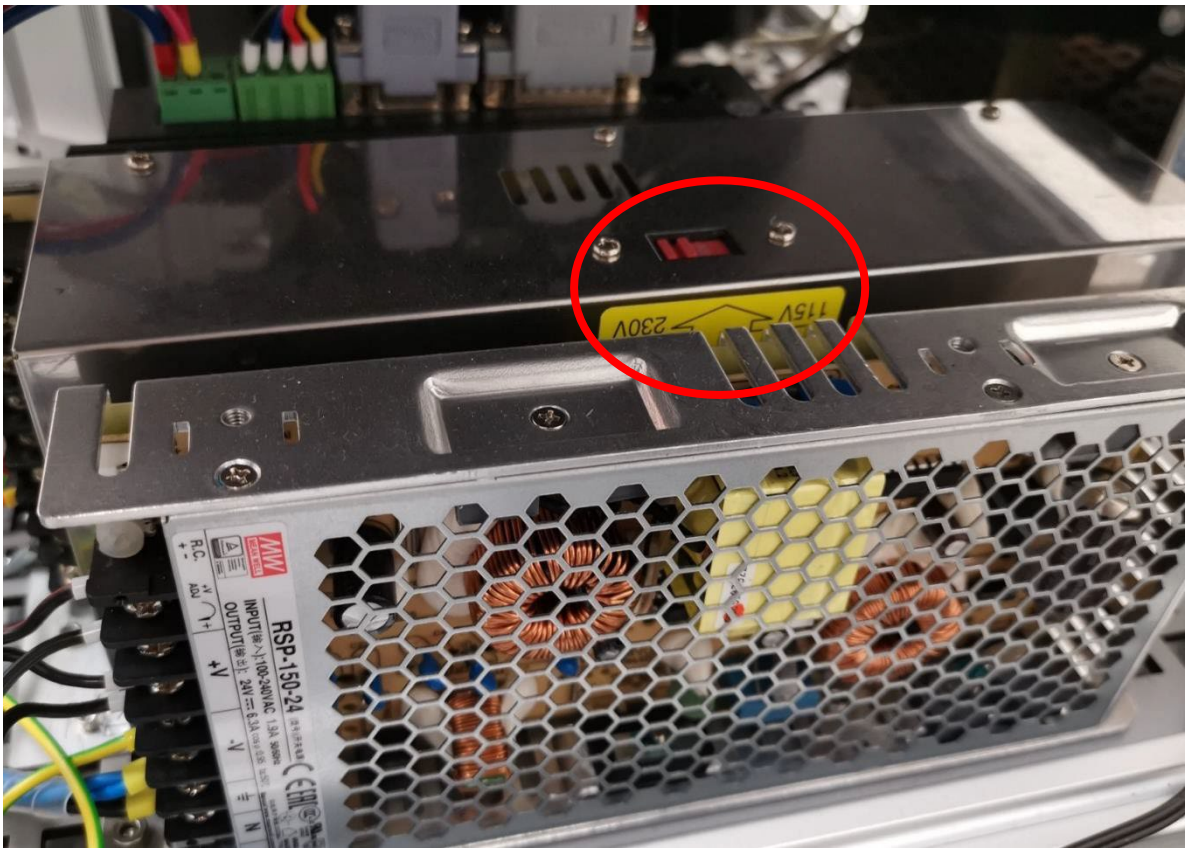
Adjusting the shaking

It is possible to fine tune the shaking motion of the rotor depending on the volume and characteristics of the solvents used in the synthesis.
Shaking is defined by three parameters – number of swings, swing angle and pause between individual swings. The default numbers 22 swings, 10 degrees angle and 55 milliseconds between individual motions were rehearsed on multiple machines and are producing good shaking motion. However, especially if you would be using larger volumes in the synthesis, they may result in splashing and should be modified. Just change the numbers and press **"Set"** button and test the shaking. If you like the shaking motion, save the new parameters set (your new parameters will then become default in your User/Set).
However, we strongly caution against adjusting the default parameters if not absolutely necessary.



Regional electric power setting

Make sure that the power is set to your country standard!



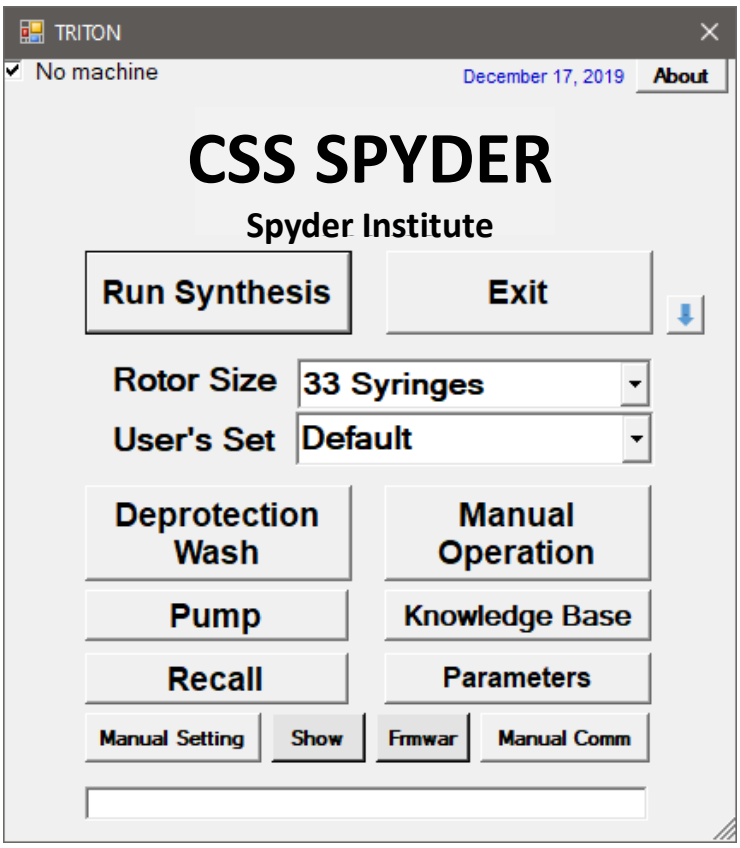
If you need to change the voltage setting, open the left side panel and position the red switch to appropriate value.

Use the appropriate and fresh syringes

If you have used the syringes for solid phase peptide synthesis, you probably know that you can use recycled syringes numerous times. However, this synthesizer does not use the plunger to move the resin to the bottom of the syringe and depends only on the centrifugal force to collect all solid support on the bottom. Therefore, any scratches on the syringe walls can catch the resin above the level of the liquid resulting in the incomplete exposure to the reagent. For that reason we strongly recommend to use new syringes for each synthesis – the cost of the new syringe is negligible in comparison of the cost of unsuccessful synthesis.

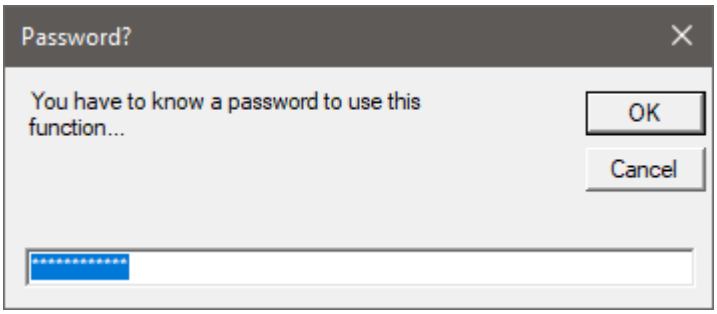
The best way to get appropriate syringes is to order them from the machine manufacturer – these syringes have a proper size fitting to the rotor and are equipped with the good frit at the bottom.

Service functions

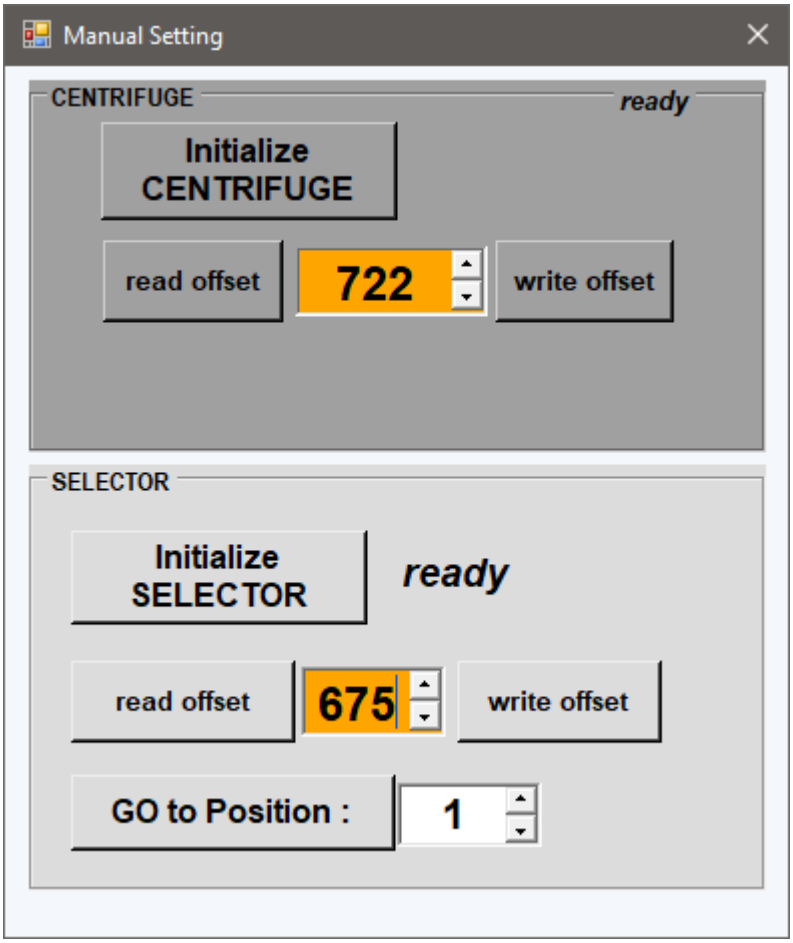


Pulling down the main window SPYDER reveals additional functions available only to the trained operators:
“Manual Setting”
“Show”
“Firmware”
“Manual Comm”

Clicking these buttons will open window asking you for password…



Service functions



“Manual setting” allows you to set offset of the selector valve and centrifuge. These values should be changed with utmost caution only after major mechanical modifications of the system.

“Manual comm” lets you send the lowest level commands to the system. To be able to use this function you have to know the syntax of the communication with the hardware which is available by clicking **“Firmware”** on the main window (if the computer is connected to the Internet). This button will also reveal the version of the firmware used by the system.

Clicking **“Show”** button will open window in which all communication of computer with synthesizer will be displayed.