This is created by *__Bingyun Li__*. It is only for reference. The instrument is Advanced ChemTech Apex 396.

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PART I: Chemfile Creations:

Sample synthesis sequence (40-well, 200mg of Fmoc protected AA substituted Wang resin with 0.63 mmol/g substitution)

__Calculations:__

(0.2g Wang resin)*(resin substitution)*(5 fold excess)=A
(A)*(1ml/0.5 mmol 0.5M HOBt)= B ml

For example: 0.2 g resin*0.60mmol/g*5*1ml/0.5mmol=**1200 µl**
The calculation result will be used in **Couple Protocol**.

__Swell Protocol: (swell.chm)__

1. Dispense System Fluid DMF [1] 4000 µl to RVBlock[1-2]
   (volumes dispensed and/or transferred in the Swell, Deprotection, and both Wash protocols depend on the initial amount of resin in the block)
2. Mix RVBlock for 3 minutes at 450 rpm
3. Empty RVBlock for 2 minutes
4.
5. Repeat from step 1, 1 times
6. Return

__Deprotection protocol 1: (dep-1.chm)__

1. Transfer 4000 µl from piperidine[1][PIP] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps (other parameters: as default, wash ratio=2)
2. Mix RVBlock for 3 minutes at 450 rpm
3. Empty RVBlock for 2 minutes
4.
5. Transfer 4000 µl from piperidine[1][PIP] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps (other parameters as default)
6. Mix RVBlock for 20 minutes at 450 rpm
7. Empty RVBlock for 2 minutes
8.
9. Return

__Deprotection protocol 2: (dep-2.chm)__

1. Transfer 4000 µl from piperidine[1][PIP] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps (other parameters: as default, wash ratio=2)
2. Mix RVBlock for 3 minutes at 450 rpm
3. Empty RVBlock for 2 minutes
4.
5. Transfer 4000 µl from piperidine[1][PIP] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps (other parameters as default)
6. Mix RVBlock for 30 minutes at 450 rpm
7. Empty RVBlock for 2 minutes
8. Return

First Wash Protocol: (wash-1.chm) To wash any trace of Piperidine from resin.

1. Dispense System Fluid DMF [1] 4000 µl to RVBlock[1-2]
2. Mix RVBlock for 3 minutes at 450 rpm
3. Empty RVBlock for 2 minutes
4.
5. Repeat from step 1, 8 times
6. Return

Couple Protocol: (couple.chm) HOBt/DIC double couple (Do not use repeats or returns in this file)

1. Dispense System Fluid DMF [1] 300 µl to RVBlock[1-2]
   (the porous frit will soak up this dead volume)
2. <Dispense Matrix> (Dispense List)
   —this is a place holder in software for dispensing amino acids; amino acids are dissolved in 0.5 M HOBt
3. Transfer **1200** µl from DIC [1][DIC] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps, other parameters as default. Wash ratio=2
4. Mix RVBlock for 1 hour at 450 rpm
5. Empty RVBlock for 2 minutes
6.
7. Dispense System Fluid DMF [1] 300 µl to RVBlock[1-2]
8. <Dispense Matrix>
9. Transfer **1200** µl from DIC [1][DIC] to RVBlock[1-2] using DMF [1]
   ** Advanced—recommend always using a 250 µl overdraw for Transfer steps. Wash ratio=2
10. Mix RVBlock for 1 hour at 450 rpm
11. Empty RVBlock for 2 minutes

Note: **1200** is the amount from above **Calculation**, each synthesis will be different.

Second Wash Protocol: (wash-2.chm) To wash any remaining amino acid that was not coupled.

1. Dispense System Fluid DMF [1] 4000 µl to RVBlock[1-2]
2. Mix RVBlock for 3 minutes at 450 rpm
3. Empty RVBlock for 2 minutes
4.
5. Repeat from step 1, 3 times
6. Return

Final-Deprotection Protocol: (fin-depr.chm)

1. Goto Chemfile C:\ACT\DEP-2.CHM, line 1
2. Goto Chemfile C:\ACT\WASH-1.CHM, line 1
3.
4. Return

DCM-FL.CHM:

1. Flush Arm 1 with DCM [1]
2. Repeat from step 1, 6 times
3.
4. Dispense System Fluid DCM [1] 4000µl to RVBlock[1-2]
5. Mix for 3.00 minutes at 450rpm
6. Empty RVBlock for 2 minutes
7.
8. Repeat from step 4, 8 times
9. Empty RVBlock for 20 minutes

10.

CLEAVAGE.CHM:

1. Empty CleavageBlock for 2.0 minutes
2. Transfer 4000µl from CocktailSolution[1][] to CleavageBlock[1-2] using DCM [1]
   Advanced—500µl, wash ratio=2 (as default).
3. Mix for 150.00 minutes at 450rpm
   Note: the time can be changed. 90-150minutes preferred.
4. Empty CleavageBlock for 3.00 minutes
5.
6. Transfer 4000µl from CocktailSolution[1][] to CleavageBlock[1-2] using DCM [1]
   Advanced—500µl, wash ratio=2.
7. Wait for 5.00 minutes
8. Empty CleavageBlock for 3.00 minutes
9.

Note: there should no mix step in the file after second flush, otherwise will cause contamination of the products.

FLU-2.CHM:

1. Flush Arm1 with DMF[1] and DMF[2]
2.
3. Repeat from step 1, 2 times
4.

Question: Each chemfile, there is EDIT→[SCALE]→ENTER SCALE FACTOR NUMBER? The scale factor will scale all volumes entered in the chemfile. For example if you wrote the chemfile with a volume of 1ml for a dispense, you could enter a scale factor of 4 and it would change the volume to 4ml.

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PART II: Calibrations

Racks needed to be calibrated: RVBlock, Monomer, Piperidine, DIC, CleavageBlock, Cocktail Solution.
Note: all chemicals should have the same properties (e.g. MW) as what we have, otherwise, need to recalibrate.

Calibrate Arm 1:

Coordinates	X	Y	Z
Reference Pos	720	492	688
Cleaner Station1	20	102	2340
Waste Station1	20	170	2340

RVBlock	Diameter=20mm		**X**	**Y**
No. of Col.	**5**	Arm 1		
No. of Rows	**8**	Straight		
Vessel cross section area	**314.16 mm^2**	First Corner Pos	**2344**	**2036**
		Second Corner Pos	**1831**	**634**
		Z-max Pos.	**2820**	
		Z-Start Pos	**2615**	
		Z-Dispense Pos	**2615**	
		Z-Travel Pos	**1**	
		Aspiration Speed	**18**	
		Dispense Speed	**15**	
Define Vessels: activate used vessels and deactivate unused vessels.		**Advanced**: Can Empty. Recommend to use valve Front Chamber (3), deactivate unused valve chambers.		

Z-Max Pos: calibrate the probe 2-3 mm below the septa.
Z-Start Pos: the probe must **below the innermost septum** but above the level of the liquid.
Z-Dispense Pos: is the distance the probe will descend when delivering liquid. The probe must be 2-3 mm below the innermost septum.
Z-Travel Pos: is the position of the probe as the arm travels point to point on the tabletop. This should always be left to its default setting.

Monomer	Diameter=27mm		**X**	**Y**
No. of Col.	**4**	Arm 1		
No. of Rows	**9**	Straight		
Vessel cross section area	**572.56 mm^2**	First Corner Pos	**934**	**1985**
		Second Corner Pos	**493**	**128**
		Z-max Pos.	**2760**	
		Z-Start Pos	**1065**	
		Z-Dispense Pos	**1065**	
		Z-Travel Pos	**1**	
		Aspiration Speed	**18**	
		Dispense Speed	**17**	
****Define Vessels** (Important!!) Activate used vessels and deactivate unused vessels. Let the vessel 36 active.		**Advanced**: Not required.		

*****IMPORTANT: Each time, you need to check and define your AAs. If not in the list, you need to calculate the MW. Different protecting groups will change the result.***
Z-Max Pos: calibrate the probe 2-3 mm from the bottom. **Note:** Z-Max high enough that the probe will always draw from the cylindrical region of the bottle.
Z-Start Pos: the probe must **below the innermost septum but above the level of the liquid** (Note: the bottle can not be too full).

CleavageBlock			**X**	**Y**
No. of Col.	**5**	Arm 1		
No. of Rows	**8**	Straight		
Vessel cross section area	**314.16 mm^2**	First Corner Pos	**2344**	**2036**
		Second Corner Pos	**1831**	**634**
		Z-max Pos.	**1826**	

		Z-Start Pos	**1768**
		Z-Dispense Pos	**1768**
		Z-Travel Pos	**1**
		Aspiration Speed	**20**
		Dispense Speed	**17**
Define Vessels: activate used vessels and deactivate unused vessels.		**Advanced**: Can Empty. Recommend to use valve Front Chamber (3), deactivate unused valve chambers.	

Piperidine	Diameter=90 mm		**X**	**Y**
No. of Col.	**1**	Arm 1		
No. of Rows	**1**	Straight		
Vessel cross section area	**6361.73 mm^2**	First Corner Position	**1312**	**1998**
		Second Corner Pos	**1312**	**1998**
		Z-max Pos.	**2928**	
		Z-Start Pos	**1629**	
		Z-Dispense Pos	**1751**	
		Z-Travel Pos	**1**	
		Aspiration Speed	**18**	
		Dispense Speed	**17**	
***Define Vessels** (Important!!)		**Advanced**: Not required.		

*Define:

Chemistry Database	**Reagent 1**
Vessel No.	1
Full Name	Piperidine
Abbreviation	PIP
MW	85.20 g/mol
Density	1.45 g/ml

DIC	Diameter=90mm		**X**	**Y**
No. of Col.	**1**	Arm 1		
No. of Rows	**1**	Straight		
Vessel cross section area	**6361.73 mm^2**	First Corner Position	**70**	**1014**
		Second Corner Pos	**70**	**1014**
		Z-max Pos.	**3023**	
		Z-Start Pos	**1554**	
		Z-Dispense Pos	**1719**	
		Z-Travel Pos	**1**	
		Aspiration Speed	**18**	
		Dispense Speed	**17**	
****Define Vessels** (Important!!)		**Advanced**: Not required.		

**Define:

Chemistry Database	**Reagent 1**
Vessel No.	1
Full Name	1,3-Diiso
Abbreviation	DIC
MW	126.20 g/mol
Density	0.81 g/ml

Cocktail Solution	Diameter=27mm		**X**	**Y**
No. of Col.	**1**	Arm 1		
No. of Rows	**1**	Straight		
Vessel cross section area	**572.56 mm^2**	First Corner Pos	**493**	**128**
		Second Corner Pos	**493**	**128**
		Z-max Pos.	**2760**	
		Z-Start Pos	**1065**	

		Z-Dispense Pos	**1065**
		Z-Travel Pos	**1**
		Aspiration Speed	**20**
		Dispense Speed	**17**
Define Vessels (Important!!)?		**Advanced**: Not required.	

Cocktail solution calibrated as bottle 36 in the amino acid rack.
Question: will the calibration (such as AA with different protecting group) influence the Calculation? Yes!!

If **HOBt** need to be calibrated, then it should be calibrated based on what we have: HOBt (1-hydroxybenzotriazole) hydrate (MW: 135.13; Density: 18.02 g/mole). HOBt•H2O: MW—153.10
DCM (Dichloromethane): MW—84.93, Density—1.326

System fluids define: ACP p3, Manual p41

System Fluids Needed		Aspiration Speed	Dispense Speed
DMF	Dilutor 1	18	15
DMF	Dilutor 2	18	15
DCM	Dilutor 1	20	18

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Procedures for reload the software:

1. open windows explorer and locate the ACT directory; delete ACT; Insert disk 1 of the software and open follow the instructions (model APEX SC).
2. re-set the comports (COM1)
3. re-calibrate Arm, all racks, system fluids, and re-create all chemfiles.

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PART III: Synthesis Preparation:

Synthesis Sequence: refer to ACP training manual p36-43.

Calculation: Here just show an example, yours may be different.

Example: Synthesis Sequence: Acyl Carrier Protein (ACP):
H-Val(V)-Gln(Q)-Ala(A)-Ala(A)-Ile(I)-Asp(D)- Ile(I)-Gly(G)-Tyr(Y)- OH

Add 15% as dead volume in the monomer bottles; add **30ml** as dead volume for **reagent bottles**. For small scale, 20% may be used instead of 15%. I recommend to add **20%** for all **AA solutions**. For AAs with small quantity, you may need to double the amounts.

Rack	Vessel	Reagent	MW	Dens	M	Vol, ml	Dvol, ml	Total Vol, ml	Grams
		DMF(1)				774.8		AA Total Vol:	
		MEOH(2)				176.0		***46.4***	
AA	8	Gly	297.3			5.04	0.76	5.8	0.86
	10	Ile	353.4			10.08	1.52	11.6	2.04
	4	Asp	411.5			5.04	0.76	5.8	1.2
(AlaH2O)	1	AlaH2O	329.3			10.08	1.52	11.6	1.9
	7	Gln	610.7			5.04	0.76	5.8	1.78
	20	Val	339.4			5.04	0.76	5.8	0.98
PIP	1	***PIP***	85.2			68	30	98	4.17
DIC	1	***DIC***	126.2			24.16	30	54.16	3.42

Cycle 1 includes: *Swell.chm*, Depro.chm, Wash.chm, Couple.chm, Wash2.chm;
(swell.cht, dep-1, wash-1, couple, wash-2)
Last cycle include: Depro.chm, Wash.chm, Couple.chm, Wash2.chm, *FinDepro.chm*;
(dep-2.cht, wash-1, couple, wash-2, fin-depr)
All other cycles: Depro.chm, Wash.chm, Couple.chm, Wash2.chm.
(1-19: dep-1.cht, wash-1, couple, wash-2)
(20-31: dep-2.cht, wash-1, couple, wash-2)

Synthesis Preparation:

1. Print the calibration result, and check whether we have enough required AAs and reagents.
Note: if the protecting group is different from the calibration or the molecular formula is different, some modifications need to be considered.

2. Calculate the total volume of 0.5 M HOBt needed by adding Vol and Dvol columns together from all AAs listed on the calculation sheet.
Here: 46.4 ml

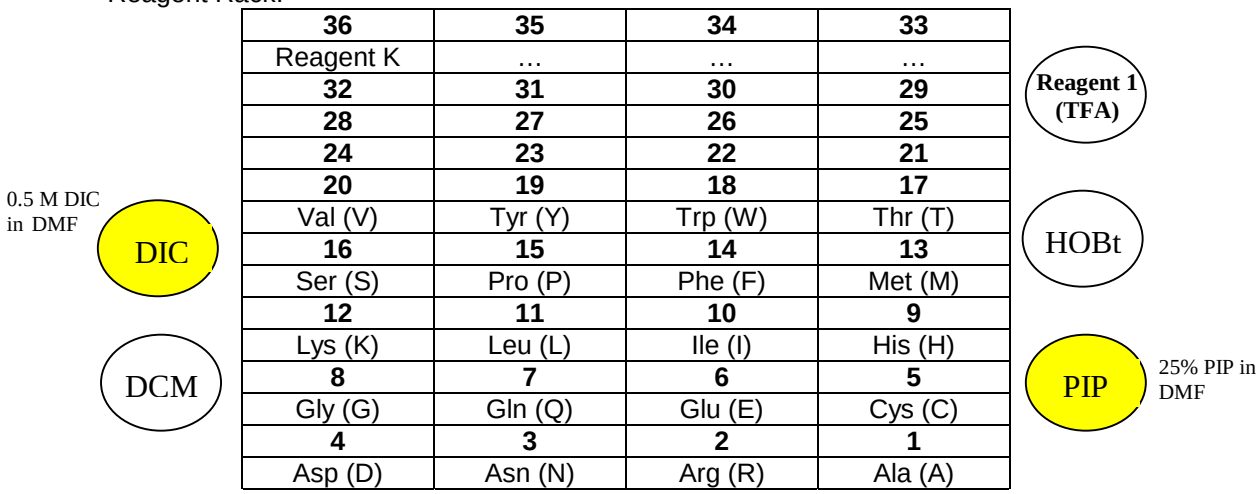
3. Prepare the **0.5 M HOBt solution in DMF (volume from above 2)**. For example, 38.27gHOBt+DMF⇒500ml (0.5M HOBt)}

HOBt.H2O, g	Total volume, ml
7.65	100
15.31	200
22.96	300
30.62	400
38.27	500
45.92	600

Note: weigh 38.27 g anhydrous HOBt (MW 135.1) into a cylinder (500ml). Add DMF until 500ml level is reached. Stir for 15 minutes with a magnetic stirring bar. Note: this solution is stable for >6 weeks.

4. Prepare a **25vol.% Piperidine in DMF** solution by adding the Vol and Dvol listed in calculations as the total volume of the solution, and place Piperidine in the calibrated location on the table top (**right front**). For example, 200ml Piperidine+600ml DMF⇒800ml (>Vol+DVol) 25vol% Piperidine in DMF (for deprotection).
Here: Total volume—98 ml, so prepare 160 ml (PIP: 40 + DMF: 120ml = Total 160ml)

Reagent Rack:



36	**35**	**34**	**33**
Reagent K	…	…	…
32	**31**	**30**	**29**
28	**27**	**26**	**25**
24	**23**	**22**	**21**
20	**19**	**18**	**17**
Val (V)	Tyr (Y)	Trp (W)	Thr (T)
16	**15**	**14**	**13**
Ser (S)	Pro (P)	Phe (F)	Met (M)
12	**11**	**10**	**9**
Lys (K)	Leu (L)	Ile (I)	His (H)
8	**7**	**6**	**5**
Gly (G)	Gln (Q)	Glu (E)	Cys (C)
4	**3**	**2**	**1**
Asp (D)	Asn (N)	Arg (R)	Ala (A)

5. Prepare **0.5 M DIC in DMF** solution by adding the Vol and Dvol listed in the calculations as the total volume of the solution, and place DIC in the calibrated location on the table top (**left behind**). For example, 23.23ml DIC+DMF⇒300ml (>Vol+DVol) 0.5 M DIC in DMF.
For example, Dilute 7.74 ml DIC with DMF to total 100 ml.
Here: Total Volume—54.16 ml, so prepare 100 ml.

DMF	DIC	
100 ml	7.74 ml	6.31 g
200 ml	15.48 ml	12.62 g
300 ml	23.23 ml	18.93 g

Check: place the **monomer bottles** in the corresponding location in the monomer rack and place the top plate. Place **DIC (left behind)** and **Piperidine (right front)** in the calibrated locations on the tabletop (Extra DIC and PIP solutions store in hood, extra AA solutions store in -20°C fridge).

6. Check the *waste Carboy bottles* (will they overflow?), Fill **DMF for Dilutor 1** and **Dilutor 2** (may need to re-fill during synthesis). Important: should have enough DMF at hand (see calculation table for DMF amount).

7. Weigh all AAs into labeled monomer bottles.

Rack	Vessel	Reagent	MW	Dens	M	Vol, ml	Dvol, ml	Total Vol, ml	Grams
		DMF(1)				774.8		AA Total Vol:	
		MEOH(2)				176.0		***46.4***	
AA	8	Gly	297.3			5.04	0.76	5.8	0.86
	10	Ile	353.4			10.08	1.52	11.6	2.04
	4	Asp	411.5			5.04	0.76	5.8	1.2
(AlaH2O)	1	AlaH2O	329.3			10.08	1.52	11.6	1.9
	7	Gln	610.7			5.04	0.76	5.8	1.78
	20	Val	339.4			5.04	0.76	5.8	0.98
PIP	1	***PIP***	85.2			68	30	98	4.17
DIC	1	***DIC***	126.2			24.16	30	54.16	3.42

8. Dissolve the AAs in 0.5M HOBt/DMF using corresponding volumes (Vol+DVol) of HOBt. Place the monomer bottles in the corresponding locations in the monomer rack (the calculation sheet lists the position number of each monomer). Important: extra AA solutions should be stored in –20°C fridge, and if the synthesis will not start soon, all AA solutions should be kept in the -20°C fridge.
9. Put a frit in each of the reaction vessels [1-4] of the reaction blocks that will be used, and weigh 200 mg of Fmoc-Gly (or other) Wang resin into these reaction vessels. Important, **all the reaction vessels not used should be blocked using silicon plugs, and first remove the filter frit then place the silicon side up (shinny side down)**. Especially, those unused vessels in the front two lines should be blocked.
   Reactors: 40 reactor positions

40	**39**	**38**	**37**	**36**
…	**…**	**…**	**…**	**…**
5	**4**	**3**	**2**	**1**
…				

10. place the ***reaction block*** and ***top plate*** on the instrument and secure with proper bolts.

11. check the ***nitrogen source*** to ensure proper pressure and quantity (9-12 psi, preferred **11psi**).

12. check ***vacuum source*** to ensure it is turned on and working correctly.

13. Flush the system using ***Flu-2.chm***.

14. No dripping probe!!(check) (otherwise see reference to fix it)

<u>RUN:</u>

15. **Run** the Chemfile.

16. During the synthesis, check on the ***reagent volumes*** (also check the filter positions in the system fluid bottles) and the ***pressure* of the nitrogen** tank. When complete click the EXIT button. The resin can be stored or proceed to the cleavage step. Resin should be washed with MeOH or THF if it is to be stored. Important: check **the synthesis conditions every 3-4 hours if possible**.

Note: In case of an unexpected interruption of Synthesis, first write down the **Synthesis Position** (or go to the log, refer to Manual p100).

After synthesis, recommend to go ahead with PART IV as soon as possible. The cleavage/deprotection should be done as quickly as possible to minimize the exposure of peptide to the cleavage reagent. Therefore, *I guess*, after finish, cleavage and washing should be done continuously.

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PART IV: Preparation for Cleavage

17. First replace the DMF bottle on Dilutor 1 with a bottle of DCM. Run DCM-FL.CHM. When complete, click EXIT.

Note: this program will not automatically start after the Synthesis, need to initiate.

18. Remove the top plate and reaction block from the instrument. Place the **cleavage block** (stored under CD drawer) on the instrument **with cleavage bottles** (please *label* the bottle) present in the correction locations. **Re-place the reaction block and top plate on the instrument**. Secure with cleavage bolts.
19. **Freshly** prepare a 25 ml of a solution containing 95% TFA, 2.5%D.I.Water, and 2.5% Triisipropyl silane (TFA—23.75ml, Water—0.625ml, Triisipropyl—0.625ml) (This can be replaced by Reagent K). **Place this solution in the bottle previously calibrated for the cocktail solution, monomer bottle 36** (should empty the bottle and flush using the solution first).

Or Prepare Reagent K (Ref: p79-81, Methods in Enzymology): phenol: 5vol%, water: 5vol%, thioanisole: 5vol%, 1,2-ethanedithiol (EDT), TFA: 82.5vol.%,: 2.5vol%. For example: 0.75 g Phenol (dangerous chemical), 0.5ml water, 0.5ml Thioanisole, 0.25ml EDT, 8.25 ml TFA. Total 10 ml.
Reagent preparation:

1) add the scavengers to a cylinder with a pipettor.
2) dilute with the TFA.
3) Mix vigorously for 30 seconds via shaking or nitrogen bubbling (recommended for sequences containing Cys or Met).

Note: for those cocktail containing phenol, use phenol crystals, not liquid phenol. The crystals may either be weighed out (w/v) or be melted and the subsequent liquid delivered to the cylinder (v/v).

About 5 ml of cleavage cocktail for 0.5 g of resin. Do not let cleavage time exceed 4 hours. One to two hours is optimal. Cleavage cocktails must be prepared fresh using high quality TFA and fresh scavengers.

20. Run CLEAVAGE.CHM.

Note: Ensure the cocktail solution is in the AA rack, monomer bottle position 36.

After start CLEAVAGE.CHM, put diethyl ether into –80°C fridge for centrifugation purpose.

21. After the Cleavage program finished, **remove the top plate and reaction vessel block**. Transfer the cleaved product from the cleavage vials and place in centrifuge tubes (*label or each peptide for one time, do not mess up*).

22. Add **9 parts** cold diethyl ether (HPLC grade) (pre-chilled in -80°C fridge). At this point the product will fall out of solution forming a fluffy white suspension. **Centrifuge for 8 minutes at 3300 rpm**. A well formed pellet should result. Decant the supernatant and resuspend the product in cold ether again. Repeat this process **6 times**, you should no longer detect an odor of TFA. (purity will be in the high eighties to low nineties, purity may be further improved by lyophilizing, which tends to remove lower MW impurities such as residual scavengers).

23. Allow the ether to evaporate slowly overnight (or See Methods in Enzymology: just partially dry for 1hr) In the hood from the open centrifuge tube. The resulting dry product is suitable for HPLC analysis or MassSpec.

24. After one day, wash and clean the RV blocks thoroughly after each synthesis.

Before the peptide is analyzed, it is recommended that neither the ether resolution nor the resin be discarded, and that they be stored under nitrogen or argon at 0°C. Typical synthesis yield: 80%

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Preventive maintenance: at the conclusion of each synthesis

1. wash and clean the RV blocks thoroughly after each synthesis. Before performing the next synthesis, test the RV block wells for clogged frits. Fill each vessel with 4 ml of MeOH and empty for 0.5 minutes at 6-10psi. Visually inspect for partially emptied wells. Partially emptied wells are an indication of clogged frits, replace with new frits.
2. flush the system 3 times to fully prime the system fluid lines. If the instrument will not be in use for an extended period of time, flush all system lines with MeOH or EtOH at least 3 times, then flush with air 3 times by pulling the system fluid lines out of the bottles.
3. Empty all waste containers if possible, check whether it will overflow for the next time synthesis.
4. remove the excess solutions from their respective reagent bottles.
5. ensure that the system fluid lines are fully immersed in the bottom of the solvent containers and the system fluid filters on the end of the system fluid lines are visibly clean.
6. clean the probe by gently wiping them with a soft, lint-free cloth containing methyl, ethyl or isopropyl alcohol. Ensure that the probe is neither bent or loose. Tighten the set-screw if necessary.
7. inspect the working surface; clean it using one of the above alcohols and a soft, lift-free cloth.
8. clean or replace syringes if needed.
9. inspect the conditions of al O-rings (nitrogen, reaction vessels, cleavage vessels).
10. inspect the condition of the reaction vessels septa; they should be replaced if they appear to be torn, discolored; or contain multiple punctures.
11. inspect the condition of the RV N2 pressure inlet filters. Replace as needed.
12. Avoid to use TFA in synthesis.
13. check the syringe for leaks
14. check the green Teflon coating on the straight probe, and check for clogged probe
15. check that there are no visible air bubbles in the system fluid lines. Tighten the tubing connections or replace the tubing as required.
16. Monthly: clean the stainless steel system fluid filters by using an ultrasonic bath. Clean and lubricate all linear motion rails. Use a KimWipe and methanol to clean, lubricate with silicone spray.

Routine reactor cleaning procedure:

1. move the reactor to a hood and rest it on a pad of paper towels.
2. remove all resin and frits from the reaction block and discard.
3. clean residual resin particles from the wells by squirting solvent into each and vacuum it out
4. allow the reactor to dry in the hood.
5. blow nitrogen or compressed air over the surface of the reactor to remove any remained resin particles.
6. store the reactor with the top plate and rubber gasket in place. This is very important. The surface finish of the reactor is critical. Storing the reactor in this manner protects it from accidental damage.

Note:

1. the drainage tubes must not form troughs or be allowed to dip into the accumulated waste liquid.
2. clean the probe tip in an ultrasonic bath with EtOH or MeOH after every 10-15 synthesis. Perform 1-2 flush operations after each synthesis to thoroughly clean the syringe barrels
3. when calibrating any rack, remember to include the lid or top plate. The Z-values are affected.
4. if air is seen within the syringe, check that the system fluid lines are below the level of the solvent and the syringe barrel is tight.

Plunger overload: recommended

1. check the rack calibration in the Z-direction where the plunger overload error occurred. It is possible to have the Z-direction not calibrated deep enough in a vessel. When this occurs the probe sees the septum as an obstruction and causes a plunger overload. Proceed to step 2 if error continues.
2. remove the system fluid lines from the solvent bottles and run airflush to dry flush the system 3 times. Then switch off the ACT instrument.
3. take off the probe and place it in a beaker of MeOH and sonicated for 15 minutes. Then re-install the probe.
4. turn on the instrument, recalibrate the reference point, cleaner and waste stations for the arm. It is also advisable to recheck the z-value of the racks associated with the arm.
5. place the system fluid filters back into the solvent bottles. Run the chemfile to flush the arm 3 times. At the end of the flush there should be no air bubbles present in the syringe or tubing. If so, repeat the flush chemfile.

Plunger overload (Procedure: Kranthi7):

1. Record the synthesis position (also check the log)
2. Utility, manual: ZR (Diluter 1 and 2)
3. Remove tubings, run AirFlush
4. Insert back the tubings and run flush
5. Run synthesis: run at the position problem occurred.

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