

Protocol for Peptoid Synthesis

Purpose: Preparation of resin beads to build peptoids, cleave and lyophilize compounds

Materials:

Reaction flask with cap	Plastic tubing
10-15 3 ml syringes with needles	Latex gloves
10-15 15 ml polypropylene test tubes	Micropipette with solvent safe tips (1000 μ l)
Resin beads	
Rubber stopper with hole	
Glass pipettes	

Chemicals:

N,N Dimethylformamide	Bromoacetic acid (BMA)
Anhydrous Dimethylformamide	Piperidine
Acetonitrile	3-diisopropylcarbodiimide (DIC)
Trifluoroacetic acid (leave on hood)	
Dichloromethane (DCM)	
Various amines in peptoid sequence	

Peptoid Preparation:

- Note:** Advisable to do steps 1-2 the night before planning to do peptoid synthesis. Also, calculate the concentration of each amine following the formula on next page.

First, clean reaction flask with soap and water. Then rinse with acetone. To rinse, fill the flask with acetone and drain using vacuum line in hood. Drain with acetone 2-3 times. Set flasks in oven to dry for 15 minutes and then cool on bench for another 10 minutes.

- Label the test tubes:
 - 20% Piperidine
 - 2M Bromoacetic acid
 - 50% DIC/DMF
 - 2M Name of each amine

Solution Preparation:

- Make a 10 ml solution of 20% piperidine (v/v) using anhydrous DMF as the solvent. Use the appropriately labeled test tube.
Ex: 2ml piperidine plus 8ml anhydrous DMF
- Make a 10 ml solution of 2M Bromoacetic acid with the following Concentration:
[1.3 g BMA/5 ml anhydrous DMF] **or** [2.6 g BMA/10 ml DMF]

- (c) Make a 10 ml solution of 50% DIC/anhydrous DMF (v/v). Bring to volume with the DMF. (5 ml DIC plus 5 ml anhydrous DMF)
- (d) 2M amine solutions

Use the following equation to get a 2M concentration for each amine. The formula weight and the density of each amine are needed to calculate this concentration.

$V = \text{formula weight} / \text{density}$

Divide this volume by 1000 to get milliliters

Multiply the milliliters by 2M. This will give you the volume of each amine needed per ml.

Note: For peptoid synthesis, 1 ml is needed for each time an amine is repeated on the peptoid chain.

Calculate the volume needed for each amine and bring to volume with anhydrous DMF. ($V = \text{FW} / \text{d} / 1000 \times 2\text{M} \times 5\text{ml}$)

Procedure:

Step 1

Swelling of the resin beads.

- (a) Measure approximately 50-100 milligrams of resin beads and place into a clean dried reaction flask.
- (b) Measure 5 ml of hydrous DMF and add to the beads. Leave on the beads 30-40 minutes. It is possible to leave on beads overnight.

Step 2

Put reaction flask into top of waste flask - vacuum

- (a) After swelling beads, wash 3 times with hydrous DMF using the vacuum line under hood.
- (b) Add 2.5 ml of 20% piperidine solution
- (c) After adding piperidine, place the reaction flask on the shaker/incubator for 20 minutes, set at 200 rpm @ 25 °C .
- (d) Then wash 8-10 times with hydrous DMF. Each washing: use 5 ml of DMF.

Step 3

- (a) Add 1 ml of Bromoacetic acid using syringe.
Note: Remember to use corresponding labeled syringe)
 - (b) Add 1 ml of 50% DIC/DMF solution.
 - (c) Put cap on tube. Microwave for 15 seconds @ 10% power.
- Do these 3 steps 2 times swirling the flask side to side between sets of microwaving..

Note: Very important: A white precipitate should be visible between each microwaving

Wash beads 8-10 times with DMF.

Step 4

- (a) Add 1 ml of the first amine in your sequence and shake the vessel slightly to evenly distribute the amine on the beads.
- (b) Microwave for 15 seconds @ 10% power 2 times.

Note: You do not need to see a white precipitate in between microwaving

- (c) Wash the beads with hydrous DMF 8-10 times.

Repeat steps 3 and 4 until all amines have been added in the peptoid sequence.

Step 5

- (a) Wash with dichloromethane (DCM) 3 times. The Dichloromethane will dry and remove DMF from the beads. When the beads are dry, they will stick to the side of the reaction flask. Can leave on bench overnight.

Step 6

Cleaving of peptoid

Remember to use a polypropylene test tube and solvent safe P-1000 tips.

- (a) Make 5 ml of a 95% trifluoroacetic solution.
Ex: 4.75 ml TFA, bring to volume with 0.25 ml Millipore H₂O
- (b) Place a lid on the test tube and invert slowly to mix.
Important note: Trifluoroacetic acid is highly caustic. It is cautioned to wear eye protection as well as gloves and lab coat when handling TFA
- (c) Add 1.5 ml of TFA to the peptoids. The mixture should become orange-red in color. Place in incubator for 1 hour at 200 rpm 25°C. Amines are in the solution.

Step 7

- (a) When finished, remove the reaction vessel from the incubator. Take to hood, remove the stopper from the reaction vessel and place a test tube underneath the reaction vessel to collect the peptoid compound.
- (b) Attach a piece of plastic tubing to the argon outlet in the hood. On the other end, attach the glass pipette into a rubber stopper. Break off the slender end of the pipette and insert into the rubber stopper.
- (c) Place the rubber stopper over the mouth of the reaction vessel creating a vacuum.
- (d) Slowly turn on the argon. This will drain the peptoid compound off the beads into the test tube below.

- (e) Then add a 50% (v/v) acetonitrile (500 μ l) and H₂O (500 μ l) to wash the beads. The acetonitrile will drain into the test tube.
- (f) To evaporate the rest of the TFA from the peptoid, blow argon gas onto the compound for approximately 30 minutes until half of the compound inside the test tube has evaporated.

Step 8

Lyophilizing the compound

Obtain a lyophilizing jar from drawer. Place an adapter on an available arm of the machine. Poke holes into the cap covering the tube containing the compound. Fill a styrofoam cup ~half full with liquid nitrogen. Place the tube into the cup and sit the cup in the sink. Move all the **active** knobs on lyophilizer to between “Vac” and “Vent”. Put the compound tube into the jar and be sure the knob is in “Vac” position. Look at the controls for the machine and when the top bar is green, you can change all the other knobs back to “Vac”. The compound can stay in the lyophilizer for a **minimum** of 3-4 hours. Transfer any leftover liquid nitrogen back to the container.

Ready for the HPLC

