Chemical Synthesis of ImpA (adenosine 5'-phosphorimidazolide)

Based on 26.4.4 Current Protocols in Molecular Biology 2005.

This protocol is used to synthesize ImpA which is required to make the intermediate reaction product of RNA/DNA ligases. This is desirable when the target molecule has a 5'-phosphate because use of an activated linker oligo allows ligation without ATP and avoids self-circularization of the molecule of interest.

Work must be done in a fume hood.
Materials:

Reagent	Supplier	Product #	CAS #
Adenosine 5 -monophosphoric acid (5 -AMP)	MP Biomedical	210008001	18422-05-4
Dimethylformamide (DMF)	Sigma	D-4551	68-12-2
Triphenylphosphine	Sigma	T84409	603-35-0
2,2 -Dipyridyldisulfide	Sigma/Fluka	43791	2127-03-9
Imidazole	Sigma	I2399	288-32-4
Triethylamine	Sigma	T0886	121-44-8
Sodium perchlorate	Sigma	410241	7601-89-0
Acetone	Sigma	154598	67-64-1
Anhydrous ethyl ether	Sigma	346136	60-29-7
Thin layer chromatography (TLC) cellulose plates with a 254-nm fluorescence indicator (cellulose-F TLC)	Analtech	06011	
(NH4)2SO4	Sigma	A4418	7783-20-2
100% ethanol	Gold Shield		
MgCl2 Hexahydrate Enzyme Grade	Fisher	BP214-500	7791-18-6
DTT			
glycerol	Fisher	BP229-1	56-81-5
HEPES	VWR	VWR1481-04	7365-45-9
acetylated bovine serum albumin	Sigma	B8894	9048-46-8
KOH			

Methods:
Synthesize ImpA

1. Dissolve 174 mg (0.5 mmol) of 5'-AMP in 15 ml DMF. Keep a 50-µl aliquot for TLC analysis.
2. Dissolve 262 mg (1 mmol) of triphenylphosphine, 220 mg (1 mmol) of 2,2'-dipyridyldisulfide, and 170 mg (2.5 mmol) of imidazole in 15 ml DMF and 0.9 ml (2.5 mmol) triethylamine. Keep a 50-µl aliquot for TLC analysis.
3. Add the AMP solution dropwise to a vigorously stirred triphenylphosphine containing solution. Cover beaker and stir for 1.5 hr at room temperature under fume hood.

Purify ImpA
4. Precipitate the ImpA by adding the reaction mixture dropwise into a vigorously stirred solution of 1.1 g (9 mmol) sodium perchlorate, 115 ml acetone, and 55 ml anhydrous ethyl ether.

5. Let the precipitate settle to the bottom of the beaker for 1 hr and decant ~150 ml of the supernatant without perturbing the precipitate.
A large glass pipet connected to a pipetting aid may also be used to aspirate off the supernatant.
6. Once the volume has been reduced to ~20 ml, resuspend the precipitate with the residual supernatant and transfer the suspension to 30-ml Corex tubes. Transfer the residual precipitate by rinsing the beaker with small volumes (5 ml) of acetone. Collect the precipitate by centrifuging 10 min at 3000 × g (5000 rpm with a Sorvall SS34 rotor), room temperature.
7. Remove the supernatant and wash the pellet two times by resuspending it with 10 ml acetone and then centrifuge 5 min at 3000 × g, room temperature.
8. Resuspend the pellet in 10 ml anhydrous ethyl ether and centrifuge 20 min at 3000 × g, room temperature. Dry the pellet overnight in a vacuum oven at 40Ã¢â€”Â¦C.
9. Store the dried powder up to 3 weeks at −20Ã¢â€”Â¦C protected from humidity.
The yield of ImpA is ~80 mg. The molecular weight of ImpA is 396.28 g/mol.

Perform quality control of synthesized ImpA
10. Soak cellulose-F TLC plates in 10% saturated aqueous (NH4)2SO4 and dry the TLC plates in open air for 1 hr or blow dry.
11. Dissolve 1 mg ImpA in 50 µl water and spot the sample on pre-treated TLC plates with aliquots of the AMP solution and triphenylphosphine solutions using glass capillaries. Develop the TLC by using 80% ethanol and visualize the spots under 254-nm UV light.
The AMP should have the lowest retention factor (Rf = 0.26), ImpA should run faster (Rf = 0.4), and the components of the triphenylphosphine solution all run near the solvent front.
Comments:

Step 1. Note that 5'-AMP does not dissolve in DMF. There is a lot of material floating about.

Step 2. Use pasture pipette with bulb. Add slowly.

Step 4. A fluffy white ppt. forms.

Step 7. Keep washing until yellow color is gone and solution is clear and colorless.

Step 8. There is a vacuum oven in the tissue sectioning room but it is set at 60C. If the oven is in use, it is okay to let air dry in the fume hood for at least 2 hours.

Step 9. Store under nitrogen gas (HSW-6 Torsten Wittmann lab) at -20C.

Step 10. Do not pour solution directly on to plate. Soak plate by gently submerging plate into pan with solution.

Step 11. Prior to run, let atmosphere equilibrate for 1h with the solution in a sealed beaker. Run plate under a tight seal.