Impa (adenosine 5'-phosphorimidazolide) Synthesis

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Abstract

Synthesis helps to facilitate the chemical production process. As a mature performance of synthesis technology, carbohydrate synthesis service and enzyme synthesis service are easily accessed. Here is the protocol of impa (adenosine 5'-phosphorimidazolide) synthesis.

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Protocol

Impa (adenosine 5'-phosphorimidazolide) Synthesis

Step 1.

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			

1. Dissolve 174 mg (0.5 mmol) of 5'-AMP in 15 ml DMF. Keep a 50-µl aliquot for TLC analysis.

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.

Step 2.

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.

Step 3.

Purify ImpA

Step 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.

Let the precipitate settle to the bottom of the beaker for 1 hr and decant ~150 ml of the supernatant without perturbing the precipitate.

Step 5.

A large glass pipet connected to a pipetting aid may also be used to aspirate off the supernatant.

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.

Step 6.

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.

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.

Step 7.

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 40C.

Step 8.

Store the dried powder up to 3 weeks at -20C protected from humidity.

Step 9.

Perform quality control of synthesized ImpA

Step 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.

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.

Step 11.

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.

that's the protocol of this chemical synthesis.