

4-Amino-10-methylpteroic acid (APA, DAMPA)

Product number 16.403

As starting material for the synthesis of 4-amino-10-methylpteroic acid we used methotrexate. The side chain of methotrexate is cleaved by bacteria ATCC 29861. Since we had no bioreactor, we used a plastic basin with a diameter of about 40 cm. In this basin we gave 5 l of water and 400 ml of a culture medium. The culture medium had the following composition:

3.0 g KH_2PO_4
5.5 g K_2HPO_4
6.0 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
400 mg CaCl_2
300 mg $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$
90 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$
90 mg $\text{MoNa}_2\text{O}_4 \cdot \text{H}_2\text{O}$
30 ml of a fertilizer for hydroponics
4 l tap water

To the basin is added 30 g of methotrexate and a small amount of the bacteria. In the following batches we added 5 ml of the reaction solution of the preceding batch.

The level of the liquid is marked with a felt-tip pen and the evaporated water is occasionally replaced with ion-free water.

Methotrexate is light sensitive, so the basin must be stored in a dark place. We kept it in an utility shed, since the reaction solution stinks.

The temperature should be over 15°C.

The solution is stirred with a plastic rod and adjusted to pH 7.5 by adding 2 N NaOH.

The pseudomonas bacteria need oxygen, thus the liquid in the basin is stirred every third day and the pH is kept in the range between 7.3 and 8.0 by the addition of diluted phosphoric acid.

The reaction is finished when the pH does not raise anymore (after about 6 weeks).

Attention, remaining methotrexate is later difficult to remove!

The progress of the reaction can be observed by TLC.

A small sample must be filtered and diluted with water.

TLC foil: cellulose 400 UV254

The reference standards are dissolved in water by adding 1 drop of NH_3 .

Developing solvent: 5% NH_4HCO_3

The spots can be visualized by using ultraviolet light.

4-amino-10-methylpteroic acid is sparingly soluble and precipitates.

The crude 4-amino-10-methylpteroic acid is filtered through a 32 cm filtering funnel.

The first 500 ml of the filtrate may be turbid and are filtered once more. The filtration proceeds very slowly. A tap is installed at the suction bottle, so that the filtration can run overnight. The filter cake is rinsed with 3 x 500 ml of water. The filter cakes must well be sucked out and compacted with a glass stopper.

Recrystallisation with NaOH

The wet, crude 4-amino-10-methylpteroic acid is placed in a 20 l round bottom flask containing a magnetic stir bar and 12 l of water. 2 N NaOH is added until most of the 4-amino-10-methylpteroic acid is dissolved (pH about 13.0).

The mixture is filtered through a 32 cm filtering funnel. The filter may clog, please see the "General instructions for working with pteridines"

The filtrate is poured in a 20 l round bottom flask containing a magnetic stir bar. To the filtrate is added slowly with stirring through a dropping funnel AcOH until a pH of 7.5 is reached, following AcOH 1:10 is added until a pH of 6.0 is reached. The round bottom flask is left over night.

The precipitated 4-amino-10-methylpteroic acid is filtered through a 32 cm filtering funnel. The filter cake is rinsed with 1.0 l of water and then dried in a vacuum desiccator over NaOH.

Recrystallisation via sodium salt

4-amino-10-methylpteroic acid can be dissolved in water by adding NaOH. If more NaOH is added, the

4-amino-10-methylpteroic acid precipitates as sodium salt. It may be that the sodium salt forms before all

4-amino-10-methylpteroic acid is dissolved.

The 4-amino-10-methylpteroic acid is grounded and sieved.

15 g of 4-amino-10-methylpteroic acid are suspended in 70 ml of water and the suspension is mixed with a strong magnetic stir bar. Through a dropping funnel are slowly added 45 ml of 2 N NaOH. An orange precipitate forms.

The reaction vessel is allowed to stand overnight without stirring.

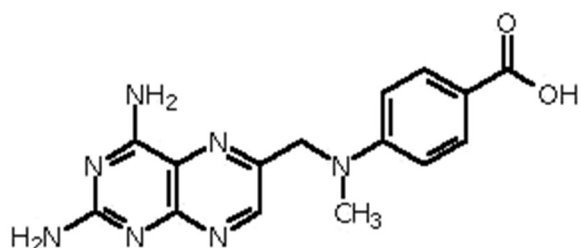
The precipitated sodium salt of 4-amino-10-methylpteroic acid is filtered and the filter cake is rinsed with 50 ml of EtOH in 3 portions and dried in a vacuum desiccator over NaOH.

To 12 g of the sodium salt of 4-amino-10-methylpteroic acid are added 250 ml of water and after that AcOH 1:10 is added with stirring very slowly through a dropping funnel until a pH of 6.0 is reached.

The precipitated 4-amino-10-methylpteroic acid is filtered and the filter cake is rinsed slowly with 150 ml of water and dried in a vacuum desiccator over NaOH.

Purity: 98.7%

Description: yellow powder



$C_{15}H_{15}N_7O_2$
325.32

C 55.38% H 4.65% N 30.14% O 9.84%

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4-Amino-10-methylpteroic acid

Schircks Laboratories

HPLC conditions:

Column:	Waters Spherisorb S5 ODS1 4.6x150 mm
Eluant:	10 mM Na ₂ HPO ₄ pH 7.0 - Methanol, (4:1)
Flow:	1.0 ml/min
Wavelength:	254 nm
Concentration:	1.0 mg/ml H ₂ O plus minimal ammonium hydroxide to dissolve