

(6S)-5,6,7,8-Tetrahydrofolic acid

Product number 16.207

CAS number 71963-69-4

In a 1 l round bottom flask with a 3 cm egg shaped magnetic stir bar are added NaOH 30% (pH about 11.5).

16.9 g 37.5 g of folic acid and 150 ml of water. The folic acid is dissolved by the addition of NaBH₄ dissolved in 50 ml of water and 7.5 g of NaOH 30% are added. A condenser is installed and closed with a plastic foil (H₂ escapes!).

The flask is placed in a water bath 40°C. After 20 minutes the temperature of the water bath is increased to 70°C.

(The temperature of the solution is controlled with a thermometer. The reaction is exothermic).

After 5 hours the flask is cooled and the pH is lowered to 5.1 by the addition of about 215 ml HCl 37% arsenic free 1:3 through a dropping funnel within 4 hours. After 3 hours when about 90% of the HCl is added, the mixture is inoculated with crystalline 6S-FH₄.

The pH increases when FH₄ is precipitating.

If the pH is too low, add 1 N NaOH until the pH is 5.1.

The flask is set aside overnight.

The precipitated FH₄ is filtered through a filtering funnel. The filter cake is rinsed with 50 ml of water (2°C), placed in an evaporating dish and dried in a vacuum desiccator over NaOH to give 13.1 g of FH₄.

Recrystallisation

The raw, grinded FH₄ is filled in a 1 l two-neck round bottom flask with a 3 cm egg shaped magnetic stir bar and dissolved in 360 ml of water containing 3.1 g of NaBH₄. Argon is bubbled through the solution and a solution of 3.6 g NH₄OAc in 24 ml of water is slowly added.

The pH is lowered to 5.1 by the addition of about 26 ml of HCl 37% arsenic free 1:3 through a dropping funnel within about 4 hours.

The precipitated FH₄ is filtered and the filter cake is rinsed with 50 ml of water (2°C).

The filter cake is placed in an evaporating dish and dried in a vacuum desiccator over NaOH to give 10.9 g of 6S-5,6,7,8-tetrahydrofolic acid.

In order to reduce the proportion of 6R-FH₄ further, the recrystallization is repeated 2 or 3 times.

Determination of the **chiral ratio**:

chiral derivatizing reagent: 2,3,4,6-tetra-O-acetyl-beta-D-glucopyranosyl isothiocyanate

To 5 mg of FH₄ are added 10 mg of the isothiocyanate, 500 mg of acetonitrile, 300 mg of water and 3 drops of NH₃ 1:100. After 10 minutes a sample of this solution is diluted 1:4 and injected.

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HPLC:

Column: SAX

Eluent: 5 mM PB pH 7.5

Detection: 254 nm

HPLC of the free FH4:

Eluent: 25 mM PB pH 7.5 plus 2.5 mg of DDT/ml eluent

A sample of FH4 is dissolved in this solution and injected immediately. Every second counts! Inject the same solution again and you will see which peaks come from oxidation products.

Since tetrahydrofolic acid is extremely sensitive to oxygen, it must be filled in **ampules** for storage.

A glassblower manufactured for us ampules made from glass tubes with a wall thickness of 1.2 mm.

After filling, the neck of the ampule must be carefully cleaned with pipe cleaners. Kleenex paper towels were wrapped around the pipe cleaners.

A glass stopcock is mounted with a short piece of vacuum hose. The ampule is evacuated and closed with the help of a hot flame.

Purity: 98.0% (HPLC)

Description: beige powder

Data Sheet: There is a data sheet available for this compound.

Data sheets can be found in the price list by clicking on the product number of your choice