Pteroyltri-y-L-glutamic acid

Product number 16.253 CAS number 89-38-3

Step 1: Coupling reaction
If the starting material TFA-Pte is not pure enough, it will be difficult to dissolve it in DMF.
Solution 1:
9.6 g of Glu3-OtBu4 HCl are dissolved in 50 ml 0f DMF (anhydrous) and cooled to 0°C.
Solution 2:
4.8 g of TFA-Pte are dissolved in 65 ml of DMF and cooled to 0°C.
3.0 ml of 4-methylmorpholine are added and the solution is mixed for a short time.
1.6 ml of isobutyl chloroformate (stored in a freezer) are added and the solution is mixed immediately and kept at 0°C for 15 minutes.
Solution 1 is added to solution 2, mixed for one hour and set aside overnight.
The solution is poured on 560 ml of crushed ice/water, mixed and sonicated for 3 minutes. Then it is stored in a fridge for 1 hour.
The mixture is filtered through a 16 cm filtering funnel. When all ice is melted, the filter cake is rinsed with water 3°C and dried in a vacuum desiccator over NaOH.

Step 2: Removal of the OtBu protecting group
The reaction product of step 1 is filled in a 1 l round bottom flask and 100 ml of TFA 0°C are added.
The solution is set aside for 1 hour and following it is evaporated by means of a rotary evaporator. The water bath should not be warmer than 25°C.
150 ml of ether are added, the flask is shaken and evaporated. This is repeated once more.
If the work cannot be continued, the flask must be stored in a freezer.

Step 3: Removal of the protecting group TFA
In the 1 l round bottom flask are filled 200 ml of water and the residue is dissolved.
NH3 1:1 is added until a pH of 9.5 is reached.
The solution is set aside for 2 hours and following it is evaporated by means of a rotary evaporator to about 50 ml.

Step 4: **Column chromatography (Whatman CC 31)**
To the remaining 50 ml of step 3 is added NH3 until a pH of 8.5 is reached. (If the Pte-Glu3 is heavily contaminated, the pH must be increased).
Preparation of the column:
Column diameter: 9 cm, height of the stationary phase: 26 cm
A slurry of water with cellulose CC31 from Whatman is poured through a funnel into the column. The top of the cellulose is protected by filter papers.
The column is washed with 0.2 N NaOH and then equilibrated with
5% NH4OAc.
The solution with the crude Pte-Glu3 (from step 3) is loaded onto the column.
The column must be protected from light.
The column is run with 5% NH4OAc.

The yellow fractions are collected and tested by HPLC.
Column: Spherisorb S5 ODS1
Eluant: 10 mM Na2HPO4 pH 6.0 plus 5% methanol
Detection: 254 nm

All Pte-Glu3 containing solutions must always be protected from light.
The best fractions (about 400 ml) are collected and the water is evaporated. To the yellow oil is added 100 ml of EtOH anhydrous and evaporated. 150 ml of EtOH anhydrous are added. The content in the round bottom flask is mixed and stored at 3°C overnight.
The Pte-Glu3 is filtered through an 8 cm filtering funnel. The filter cake is rinsed with 100 ml of EtOH 3°C and then dried in a vacuum desiccator over NaOH to give 3.7 g of Pte-Glu3.

Step 5: Column chromatography (DEAE-Sephadex)
Preparation of the column:
Column diameter: 8 cm, height of the stationary phase: 15 cm
(Fresh DEAE material may retain Pte-Glu3 stronger, so we recommend to begin with a height of the stationary phase of only 6-10 cm).
A slurry of water with DEAE-Sephadex is poured through a funnel into the column.
The top of the DEAE-Sephadex is protected by filter papers.
The column is washed with 0.2 N NaOH and then equilibrated with 5% NH4OAc.
3.7 g of Pte-Glu3 of step 3 are dissolved in 60 ml of water and NH3 1:1 is added until a pH of 7.5 is reached.
The solution is loaded onto the column.
The column must be protected from light.
The column is run with 5% NH4OAc.
The yellow fractions are collected and tested by HPLC.
Column: Spherisorb S5 ODS1
Eluant: 10 mM Na2HPO4 pH 6.0 plus 5% methanol
Detection: 254 nm

All Pte-Glu3 containing solutions must always be protected from light.
The best fractions (about 2.0 l) are collected and the water is evaporated.
To the yellow oil is added 100 ml of EtOH anhydrous and evaporated. 600 ml of EtOH anhydrous are added. The content in the round bottom flask is mixed and stored at 3°C overnight.
The Pte-Glu3 is filtered through an 8 cm filtering funnel. The filter cake is rinsed with 50 ml of EtOH 3°C and then dried to give 2.7 g of Pte-Glu3.

Step 6: Conversion to the free acid
2.7 g of Pte-Glu3 ammonium salt of step 4 are dissolved in 150 ml of water and 0.5 N HCl is added until a pH of 1.85 is reached. The flask is stored at 3°C for 3 days. (*If stored shorter, the filter is clogged).*
The precipitated Pte-Glu3 is filtered through a 12 cm filtering funnel. The filter cake is rinsed with 50 ml of water 3°C and then dried to give 2.1 g of pteroyltri-y-L-glutamic acid.

Purity: 98.7%

Description: yellow powder

Solubility: soluble in alkaline solvents

HPLC

Sample: 1 mg/ml 0.01 N NaOH
Column: Spherisorb S5 ODS1, 150 mm x 4.6 mm
Eluant: 10 mM Na_2HPO_4 pH 6,0 plus 3% MeOH
Flow: 1 ml/min
Detection: 254 nm

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