Glu4-OtBu5 hydrochloride

Product number 16.904b

Step 1: Coupling reaction
S o l u t i o n 1:
21.9 g of Glu3-OtBu4 hydrochloride are dissolved in 150 ml of AcOEt and 3.6 ml of morpholine are added, mixed and placed in a freezer for 2 hours.
S o l u t i o n 2:
12.2 g of Z-Glu-OtBu are dissolved in 200 ml of AcOEt and 4.4 ml of morpholine are added and mixed.
The solution is placed in a freezer for 2 hours.
5.2 ml of isobutyl chloroformate (stored in a freezer) dissolved in
12 ml of AcOEt are added and the solution is mixed immediately and is placed in a freezer for 5 minutes.

Solution 1 is added to solution 2 and mixed for 2 hours and then set aside overnight at about 22°C.
The mixture is purified by solvent extraction in a separating funnel. The AcOEt-layer is rinsed in turn with NaCl 10%, NaHCO3 5% (shake for 3 minutes), NaCl 10%, KHSO4 0,05M (shake for 3 minutes) and NaCl 10%.
The AcOEt solution is dried with Na2SO4, evaporated and the drying is finalized by connecting the flask with a vacuum pump to give 32.1 g of Z-Glu4-OtBu5.

Purity: 51.3% (HPLC)

HPLC:

Sample	1 mg/ml eluent
Column	Spherisorb ODS 2, 150 mm x 4,6 mm
Eluent	10 mM PB pH 6.0 / MeCN 1 :2
Flow	1.5 ml/min
Detection	200 nm

Step 4: Column chromatography
Preparation of the column:
Column diameter: 11.5 cm, height of the stationary phase: 71 cm
A slurry of gasoline : AcOEt (7:3) with silica gel is poured through a funnel into the column. The column is equilibrated with the same solvent mixture.
32.1 g of Z-Glu4-OtBu5 is dissolved in 200 ml of AcOEt and 400 ml of gasoline are added. This solution is loaded onto the column.
The column is run with gasoline : AcOEt (7:3) 10 l
then gasoline : AcOEt (1:1) 10 l
then gasoline : AcOEt (4:6)
The product came after 18 l eluent.
The fractions are tested by thin layer chromatography.
TLC plate: silica gel polyester sheet
Developing solvent: AcOEt / gasoline (1:1)

The spots can be visualized by using hot steam (see “General instructions ……”)
The best fractions (about 14 l) are collected and the solvent is evaporated. The drying is finalized by connecting the flask with a vacuum pump to give 18.9 g of Z-Glu4-OtBu5.

Purity: 86.8% (HPLC)

HPLC :

Sample	1 mg/ml eluent
Column	Spherisorb ODS 2, 150 mm x 4,6 mm
Eluent	10 mM PB pH 6.0 / MeCN (1:2)
Flow	1.5 ml/min
Detection	200 nm

Step 5: Removal of the protecting group Z
(*Please see the “General instructions for working…)*
In a 1 l three-necked round bottom flask with an egg-shaped stir bar are added 1.0 g of Pd on carbon in 10 ml of water.
18.9 g of Z-Glu4-OtBu5 are dissolved in 200 ml of MeOH and 18.9 ml 1 N HCl are added and the hydrogenation is started. After about 3 hours the hydrogenation is finished.
The course of the hydrogenation is monitored by thin layer chromatography.
TLC plate: silica gel polyester sheet
Developing solvent: AcOEt / gasoline (1:1)
The spots can be visualized by using hot steam (see *“General instructions … “)*

The Pd on carbon is filtered out, rinsed with MeOH (attention: the dry filter paper may start to burn) and the filtrate is evaporated to dryness by means of a rotary evaporator.
The flask with the residue is dried in a vacuum desiccator over NaOH to give 16.4 g of Glu4-OtBu5 hydrochloride.

Purity: 87.6% (HPLC)

HPLC:

Sample	1 mg/ml eluent
Column	Spherisorb ODS 2, 150 mm x 4,6 mm
Eluent	10 mM PB pH 6.0 / MeCN 1:2
Flow	1.5 ml/min
Detection	200 nm

Recrystallisation:
Glu4-OtBu5 hydrochloride cannot be recrystallized.