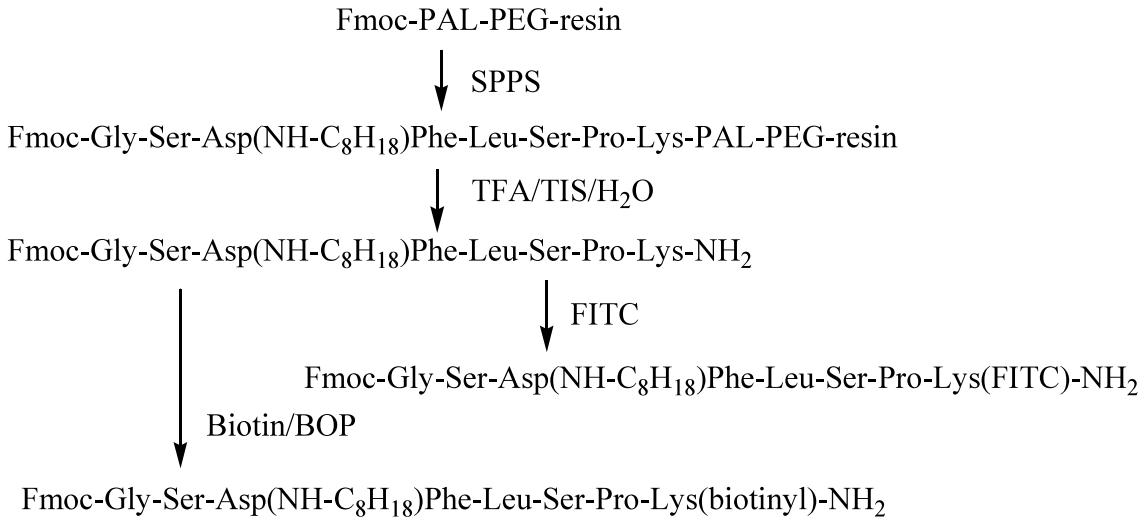
SUPPLEMENTARY DATA 1

Peptide description. The ghrelin peptides used throughout our work are:

JMV 4944: Fmoc-Gly-Ser-Asp(NH-C_8H_{18})Phe-Leu-Ser-Pro-Lys-NH_2
Calculated for $C_{61}H_{87}N_{11}O_{13}$, 1181.6; MS (ES), m/z: found 1182.8. $[M+H]^+$.
JMV 4945: Gly-Ser-Asp(NH-C_8H_{18})Phe-Leu-Ser-Pro-Lys-NH_2
Calculated for $C_{46}H_{77}N_{11}O_{11}$, 959.6; MS (ES), m/z: found 960.8. $[M+H]^+$.
JMV 4946: Gly-Ser-Asp(NH-C_8H_{18})Phe-Leu-Ser-Pro-Lys(FITC)-NH_2
Calculated for $C_{67}H_{88}N_{12}O_{16}S$, 1348.6; MS (ES), m/z: found 1349.7. $[M+H]^+$.
JMV 4947: Gly-Ser-Asp(NH-C_8H_{18})Phe-Leu-Ser-Pro-Lys(biotinyl)-NH_2
Calculated for $C_{56}H_{91}N_{13}O_{13}S$, 1185.7; MS (ES), m/z: found 1186.7. $[M+H]^+$.

All the peptides include the first five residues of the ghrelin that are those required for binding to GHSR-1a (1). In all cases, the ester bond in the side chain of residue in position 3 has been replaced by an amide to increase stability. This change does not induce any modification in the activity of these ghrelin analogues regarding native ghrelin (1).

Peptide synthesis. Peptides were synthesized manually on solid support starting with one gram of Fmoc-PAL-PEG-resin (Applied Biosystems, Courtaboeuf, France) substituted at 0.43 mmol/g.



Materials. DIEA, dichloromethane, methanol, acetonitrile, diethyl ether, trifluoroacetic acid, piperidine were purchased from Riedel-de Haën, Carlo Erba or Acros organics and used without purification. BOP reagent was purchased from Iris Biotech GmbH (Marktredwitz, Germany). Solvents used for HPLC and LC/MS were of HPLC grade. All final compounds were purified by reversed-phase HPLC and the purity assessed by analytical reversed-phase HPLC was found superior to 98 %
Anchoring on Fmoc-PAL-PEG-resin. Fmoc-amide resin was conditioned for 30 minutes in DMF and submitted to the standard deprotection cycle, using DMF/ piperidine 80/20 solution for 30 minutes. After washing steps, the first amino-acid (Fmoc-Lys(Boc)-OH (4 eq) was loaded onto the resin through a standard coupling cycle, using BOP (4 eq) as a coupling agent and DIEA (4 eq) as a base.
Deprotection step. Fmoc deprotection was carried out using DMF/piperidine 80/20 v/v solution two times for 30 minutes.
Coupling step. Coupling reaction was carried out manually in a glass reactor equipped with frits. BOP (4 eq) as a coupling agent, DIEA (4 eq) as a base and Fmoc-amino-acid (4 eq) (according to resin loading) were solubilized in DMF and added to the resin. The reaction was stirred for 0.5 hour at room temperature. The reaction was monitored by the standard Kaiser test. The following Fmoc-protected amino acids were successively introduced: Fmoc-Pro, Fmoc-Ser(tBu), Fmoc-Leu, Fmoc-Phe, Fmoc-Asp(NHC_8H_{18}), Fmoc-Ser(tBu) and Fmoc Gly.

Washing steps. These steps were performed after each coupling and deprotection steps: one time DMF, one time methanol, 2 times DMF.

Cleavage of PAL-PEG amide-PS resin. Fmoc-protected oligomer was cleaved from resin for 1.5 hours in TFA/Triisopropylsilane/H_2O : 95/2.2/2.5 (v/v/v). After removal of the resin by filtration, the trifluoroacetic acid was concentrated *in vacuo*. Compounds were precipitated by addition of diethyl ether and filtered. They were dissolved in acetonitrile/water 50/50 solution containing 0.1% TFA and freeze dried.

Preparation of the Fmoc-aspartyl derivative. Amino-octane (2.3 mmol) was acylated with Fmoc-Asp(OH)-OtBu (2 mmol) with BOP and DIEA for 1 hour at room temperature. After classical work-up, compound was treated without purification by a mixture of TFA/DCM, 50/50, v/v for 1 hour. The Fmoc-Asp(NHC8H18)-OH derivative was prepipitated by addition of diethylether and filtered.

FITC derivative. FITC reaction was carried out on the Fmoc-protected peptide in solution in DMF. 1.2 eq of FITC and 3 eq of NEt_3 were added. The reaction was stirred for 2 hours at room temperature. The reaction was monitored by LC/MS and found to be complete within 1.5 hour. Compound was precipitated by addition of a 1M potassium hydrogenosulfate, filtered and then dissolved in DMF containing 10% of diethylamine. After 1 hour, the deprotection step was complete and the mixture directly injected into the preparative HPLC apparatus.

Biotine derivative. Biotine (1eq) was coupled on the Fmoc-protected peptide in solution in DMF in the presence of BOP and DIEA. After 45 min, the LC/MS chromatogram showed a complete conversion into the biotinyl derivative. The desired compound was precipitated with diethylether, filtered and then dissolved in DMF containing 10% of diethylamine. After 0.5 hour, deprotection step was complete and the mixture directly injected into the preparative HPLC apparatus.

Purification of the compounds. All compounds were purified by preparative HPLC (Waters 4000 apparatus) carried out on a C18 reversed-phase column (C18 Deltapak column, 100 mm x 40 mm, 15 µm, 100Å) at a flow rate of 50 mL/min of a mixture of H_2O + 0.1% TFA and CH_3CN + 0.1% TFA in gradient mode with UV detection at 214 nm.

Analysis of the compounds. HPLC: Samples were prepared in an acetonitrile/water (50/50 v/v) mixture, containing 0.1% TFA. The HPLC system consisted of a Beckman 32 Karat System. All the analyses were carried out using a RP C18 VWR chromolith column, 50 mm x 3.9 mm. A flow rate of 5 mL/min and a gradient of (0–100)% B over 3 min were used. Eluent A: water/0.1% TFA; eluent B: acetonitrile/0.1% TFA. Detection was performed both at 214 nm and 254 nm.

LC/MS Analysis. Samples were prepared in acetonitrile/water (50/50 v/v) mixture, containing 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2690 HPLC, coupled to a Waters-Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using a RP C18 monolithic Onyx Phenomenex 25 x 4.6 mm column. A flow rate of 3 mL/min and a gradient of (0–100)% B over 3 min were used. Eluent A: water/0.1% formic acid; eluent B: acetonitrile/0.1% formic acid. Positive ion electrospray mass spectra were acquired at a solvent flow rate of 100–500 µL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode in 0.1 s intervals; 10 scans were summed up to get the final spectrum.

Reference

1. Kojima, M., and Kangawa, K. (2005). *Physiol Rev* **85**, 495-522.