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# Solid Phase Peptide Synthesis of the Fragment BPC 157 of Human Gastric Juice Protein BPC and its Analogues

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## Abstract

Human gastric juice contains protein BPC, which exhibits a wide range of biological activities. The 15-amino acid fragment (BPC 157) and its analogues were synthesized using SPPS on a preloaded Boc–Val-HYCRAM™ polymeric resin (the combination of Boc- and Fmoc-chemistry was used) and a preloaded Fmoc-Val-SASRIN™ polymeric resin (the Fmoc-chemistry was used). Yields, chromatographic purities and costs for synthesis of equal mmolar amounts of compounds on both polymeric resins were compared.

Key words: SPPS, BPC, peptides, Boc, Fmoc

## Introduction

Pentadecapeptide BPC 157 ($M_r$ 1419), with the sequence Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, a 15-amino acid fragment of body protection compound (BPC) peptide in gastric juice,[1-3] with apparently no sequence homology to other known gut peptides, is thought to be essential for BPC's activity.[4] Although the detailed mechanism is poorly understood, BPC 157 appears to be beneficial to almost all organ systems in many species when very low dosages (ng/kg or mg/kg) are used.[5]

Solid Phase Peptide Synthesis (SPPS), developed by Merrifield,[6] has proved to be the method of choice for efficiently producing peptides and small proteins. Chemical approaches also allow the incorporation of non-native elements, such as N-substituted and D-amino acids.

There are two main protocols that have been used for the chemical solid-phase chemical synthesis of peptides.

The first protocol uses tertiary-butyloxycarbonyl (Boc) group for $N^{\alpha}$-amino protection. Side-chain protecting groups are specifically designed to be stable to repeated cycles of Boc removal. In most cases the cleavage of the Boc group is performed with TFA in DCM. For coupling reactions numerous ways are available for the activation of the carboxylic function. DCC or DIC activation have been used from the first days of the solid phase technique and are still popular today.

In SPPS the monitoring of the completion of the Boc cleavage and of the coupling reaction is essential. Reliable methods detecting minute amounts of unreacted amino groups are essential for the monitoring of the coupling reaction. On the other hand the ability of small quantities of Boc peptide allows the control of the completion of the Boc cleavage. A range of colour tests for the qualitative monitoring of the coupling reaction has been developed. The Kaiser test,[7] which based on the reaction of ninhydrin with amines and which is very sensitive test for primary amines, is very often in use.

Finally, the peptide linker-support is cleaved to obtain the peptide. Ideally, the cleavage reagent should also remove the amino acid side-chain-protecting groups. In the most commonly employed anchoring system to peptide acids and amides are the 4-hydroxymethylphenylacetic acid (PAM) linker[8] and 4-methylbenzhydrylamine (MBHA)[9] resin. The peptide-resin anchorage and side–chain protecting groups are cleaved by the use of anhydrous HF. Linkers which are cleaved under non-acidolytic conditions are also known. Among these is **hy**droxyl-**cr**otonyl-**am**inomethyl (HYCRAM)[10] where for the cleavage tetrakis (triphenylphosphine) palladium ($Pd(PPh_3)_4$) is used.

The second protocol uses the 9-fluorenylmethyloxycarbonyl (Fmoc) group for $N^{\alpha}$-amino protection. During the synthesis, Fmoc is split off by a short treatment with piperidine in DMF. For coupling reactions DCC or DIC can be used and for the qualitative monitoring of the coupling reaction Kaiser test can be used. Side-chain protection that are compatible with $N^{\alpha}$-protection are removed at the same time as the appropriate anchoring linkages typically by the use of TFA. The Fmoc protocol is especially recommended for the synthesis of acid sensitive peptides and derivatives.

The choice of an adequate combination of protecting groups/solid support is the first step en route to achieve a successful synthesis.

For the synthesis of BPC fragment and its analogues both approaches were used. First, the fragment BPC 157 and analogues with exchanged L-Glu with D-Glu or N-Me-Glu were synthesized using SPPS on a preloaded Boc-Val-HYCRAM™.[11] Due to troubles by the use of Boc-chemistry alone (difficult sequence: hydrophobic chain association during synthesis and acidic catalyzed side reactions) the combination of Boc- and Fmoc-chemistry was used.

The second approach was the synthesis of the fragment BPC 157 and analogues with exchanged L-Asp with D-Asp and analogues with exchanged L-Asp with D-Asp and exchanged L-Glu with D-Glu or N-Me-Glu using SPPS on a preloaded Fmoc-Val-SASRIN™ (SASRIN™: 2-Methoxy-4-alkoxybenzyl-alcohol polymeric resin; **S**uper **A**cid-**S**insitive **Res**i**n**)[12] by the use of Fmoc-chemistry.

## Results and discussion

Peptide BPC 157 and analogues with the sequence GXPPPGKPAYYAGLV (X=L-Glu, Y=L-Asp: BPC 157 peptide **1a** (for the synthesis the combination of Boc- and Fmoc-chemistry on a preloaded Boc-Val-HYCRAM™ polymeric resin was used) and **1b** (for the synthesis Fmoc-chemistry on a preloaded Fmoc-Val-SASRIN™ polymeric resin was used); X=D-Glu, Y=L-Asp: peptide **2**; X=D-Glu, Y=D-Asp: peptide **3**; X=L-Glu, Y=D-Asp: peptide **4**; X=N-Me-Glu, Y=L-Asp: peptide **5**; X=N-Me-Glu, Y=D-Asp: peptide **6** were performed manually by the SPPS, cleaved from the resins and side chain protecting groups were removed. Finally, the crude products were purified by means of semi-preparative chromatography. The chromatographic purities and yields of BPC 157 and analogues are given in Table 1.

**Table 1.** Chromatographic purities and final yields of BPC 157 and its analogues.

| Peptide | purity [a] | purity [b] | Yield [c] (g) | Yield (%) |
|---|---|---|---|---|
| **1a** | 83.8 | 99.5 | 1.15 | 78.9 |
| **1b** | 87.3 | 99.5 | 1.29 | 85.2 |
| **2** | 79.3 | 99.5 | 1.11 | 73.8 |
| **3** | 70.9 | 99.0 | 1.25 | 83.1 |
| **4** | 72.2 | 99.0 | 1.20 | 79.4 |
| **5** | 82.5 | 99.3 | 1.12 | 73.4 |
| **6** | 72.8 | 99.2 | 1.19 | 77.8 |

[a] Chromatographic purity of crude products (area %). [b] Chromatographic purity of products (area %). [c] The final yields were calculated on first amino acid.

Costs for the synthesis and purifications of peptide BPC 157 (**1a** and **1b**) were calculated. The detailed calculations are given in Table 2.

**Table 2.** Comparison of preparation costs for 0.1 mmol of peptides **1a** and **1b**.

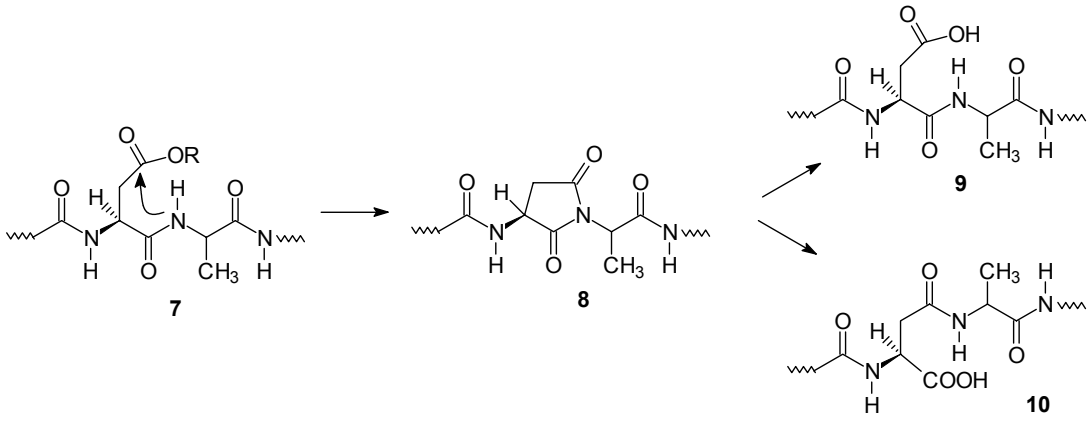
| | Costs for **1a** (EUR) | Costs for **1b** (EUR) |
|---|---|---|
| Preloaded Resin (0.1mmol of 1. AA) | 55 | 110 |
| Protected AA's (0.4 mmol of each AA) | 265 | 280 |
| Solvents, reagents | 390 | 420 |
| Costs Summary | 710 | 810 |
| Recalculated Costs /0.1 mmol of peptide [a] | 900 | 950 |

[a] For costs recalculations 78.9% yield calculated on first amino acid on Hycram polymeric resin and 85.2% yield calculated on first amino acid on Sasrin polymeric resin were used.

The development of Fmoc arose out of concern that repetitive TFA acidolysis in BOC-group deprotection could lead to alteration of sensitive peptide bonds as well as acidic catalyzed side reactions. In Fmoc synthesis, the growing peptide is subjected to mild base treatment using piperidine during Fmoc-group deprotection and TFA is required only for the final cleavage and deprotection of peptidyl resin. On the other hand, BOC chemistry is cheaper and using the palladium(0) catalyzed cleavage of an aminoacyl-4-hydroxycrotonyl-amidomethyl (HYCRAM) anchor group in polymer phase at neutral pH renders the gentle release of peptides possible even with fully intact protecting group.[13]

Choosing the best amino acid derivative is one of the most important and sometimes difficult aspect of peptide synthesis. Often compromises must be made in yield, purity, or both when choosing the most appropriate derivatives for a particular synthesis. Inappropriate choice of amino acid derivatives can lead to insurmountable problems later in the synthesis or during purification. Not all amino acids require side-chain protection. Those which do can undergo irreversible side reactions, either during the synthesis or during cleavage, if the protecting group is not compatible or if an inappropriate cleavage method is used. The choice of side-chain protection is not only dependent on the chemistry used, but also on the coupling and cleavage methods employed, solubility of the derivative in question, and the sequence of the peptide being synthesized.

The cyclization of aspartic acid residues to form aspartimides is a common side reaction in peptide synthesis, it is troublesome when Asp-Gly, Asp-Ser and Asp-Ala sequences are present.[14] The process is shown in Scheme 1, in which an Asp-Ala-containing peptide **7** undergoes aspartimide formation. On hydrolysis, aspartimide **8** gives a mixture of both α- and β-peptides, **9** and **10**, respectively. In Fmoc chemistry, this is normaly overcome by the use of Asp(OtBu) and Glu(OtBu) derivatives. With other amino acids the steric hindrance to




**Scheme 1.** The cyclization of aspartic acid and the formation of α- and β-peptides.

cyclization usually impedes the reaction, although the Asp-Asn sequence is problematicin Fmoc/tBu SPPS. An analogous cyclization can, but does not often, occur with Glu, leading to the formation of the corresponding glutarimides.[15-17]

Due to hydrophobic chain association during synthesis and acidic side reactions by the use of Boc-chemistry alone, the combination of Boc-chemistry for the first four amino acids and Fmoc-chemistry for the rest amino acids and Fmoc-chemistry alone were used.

Comparing the final chromatographic purities obtained using Fmoc-chemistry alone and the combination of Boc- and Fmoc-chemistry, no significant differences were observed. Due to lower costs for the synthesis of BPC 157 the combination of Boc- and Fmoc-chemistry is the method of choice.

## Conclusions

Peptide syntheses of pentadecapeptide BPC 157, the 15-amino acid fragment of human gastric protein BPC, and its analogues were performed manually by the solid phase method. The combination of Boc- and Fmoc-chemistry on a preloaded Boc-Val-HYCRAM™ polymeric resin and Fmoc-chemistry on a preloaded Fmoc-Val-SASRIN™ polymeric resin were used.

The analytical results show that the same quality of products can be achieved. A comparison of yields shows preference for Fmoc-based synthesis, but the costs for the BPC 157 synthesis from an equal mmolar amounts of preloaded resins shows that costs are higher using only Fmoc-chemistry in comparison with combination of Fmoc- and Boc-chemistry. The synthesis of BPC 157 on HYCRAM polymeric resin is the method of choice.

## Experimental

**Materials and methods.** All Boc-L-amino acids, Fmoc-L-amino acids and a preloaded Boc-Val-HYCRAM™ polymeric resin were obtained from Orpegen (Heidelberg, Germany), Fmoc-D-amino acids, Fmoc-N-Me-amino acids and a preloaded Fmoc-Val-SASRIN™ polymeric resin were obtained from Bachem (Bubendorf, Switzerland). All other solvents and reagents were obtained from Merck, Fluka or Sigma-Aldrich. Solvents were stored over molecular sieve and used without further purification.

Analytical RP-HPLC was performed on TSP chromatography system with membrane degaser, ConstaMetric 4000 ternary gradient pump and LDC analytical SpectroMonitor 5000 UV detector using Hypersil PEP 100 C-18 column (150 mm × 4.6 mm). After isocratic elution with 2% acetonitrile in 0.5% acetic acid in water for 9 min a linear gradient to 90% acetonitrile in 0.5% acetic acid in water in 15 min was used.

Semi-preparative RP-HPLC for all synthesized peptides was performed on RAININ chromatography system with two pumps Solvent Delivery System Model SD1 and Absorbance Detector Model UV1 using Kromasil 100 RP-18 column (250 mm × 22 mm). A linear gradient from 2% to 12% of isopropanol in 0.1% ammonium hydrogen carbonate in water was used in 18 min. Main fractions were collected and lyophilized.

IR spectra were measured as KBr pellets on Bio-Rad FTS-60. $^1$H-NMR spectra were recorded in INOVA-300 spectrometer. Optical rotations were measured with Perkin Elmer PE341 polarimeter at 20 °C. Mass spectra were recorded on a Finnigan-MAT TSQ 7000 mass spectrometer. N-terminal sequence was determined on Procise protein sequencing system 492A.

**Peptide synthesis on HYCRAM polymeric resin.** Peptide syntheses of **1a** and analogues **2** and **5** were performed manually by the solid phase method,[18,19] the combination of Boc- and Fmoc-chemistry was used (Chart).

Protected amino acids used: Boc-L-Ala, Boc-Gly, Boc-L-Leu, Fmoc-L-Ala, Fmoc-Gly, Fmoc-L-Pro,

Gly-Xxx-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val

Boc / Fmoc-chemistry / Boc-chemistry

**Chart**

Fmoc-L-Lys(BOC), Fmoc-L-Asp(OtBu), Fmoc-L-Glu(OtBu) or Fmoc-D-Glu(OtBu) or Fmoc-N-Me-L-Glu(OtBu).

The solid support was a preloaded Boc-Val-HY-CRAM™ polymeric resin (5605, 100-200 mesh, 0.65 mmol/g, 1.5 g).

The synthetic protocol for Boc-chemistry was: (1) DCM, 1 × 1 min, (2) 50% TFA in DCM, 10 + 15 + 15 min; (3) DCM, 6 × 1 min; (4) DMF,[20] 5 × 1 min; (5) 20% DMSO in DMF, 1 × 1 min; levels of free amino groups were assessed by Kaiser ninhydrin test;[21] (6) 4-fold molar excess of Boc-AA, HOBt, DCC, preactivation in minimum of DMF for 40 min at room temperature. The precipitated dicyclohexylurea was filtered off and solution was added to the resin. (7) After 45 min DMSO/DIEA was added. After the 90 min residual levels of free amino groups were assessed by Kaiser ninhydrin test. No double couplings were necessary. (8) DCM, 4 × 1 min; (9) $Ac_2O$-DIEA in DCM, 1 × 15 min; (10) DCM, 3 × 1 min.

The synthetic protocol for Fmoc-chemistry was: (1) DMF, 6 × 1 min; (2) 20% Piperidine in DMF, 3 × 10 min; (3) DMF, 5 × 1 min; (4) 20% DMSO in DMF, 1 × 1 min; levels of free amino groups were assessed by Kaiser ninhydrin test; (5) 4-fold molar excess of Fmoc-AA, HOBt, DCC, preactivation in minimum of DMF for 40 min at room temperature. The precipitated dicyclohexylurea was filtered off and solution was added to the resin. (6) After 45 min DMSO/DIEA was added. After the 90 min residual levels of free amino groups were assessed by Kaiser ninhydrin test. No double couplings were necessary. (7) DCM, 4 × 1 min; (8) $Ac_2O$-DIEA in DCM, 1 × 15 min; (9) DCM, 3 × 1 min.

Cleavage and final deprotection. The peptides were cleaved from the resin by treatment with tetrakis-(triphenylphosphine) palladium ($Pd(PPh_3)_4$ / $PPh_3$ in 40% morpholine in DMF at room temperature for 3 h.[22,23] The peptides were precipitated with cold diethyl ether and washed three times with diethyl ether and dried. The crude product was dissolved in TFA at –5 °C. After 90 min the peptides were precipitated with cold diethyl ether and washed three times with diethyl ether and dried.

**Peptide synthesis on SASRIN polymeric resin.** Peptide syntheses of **1b** and analogues **3**, **4** and **6** were performed manually by the solid phase method, the Fmoc-chemistry was used.

Protected amino acids used: Fmoc-L-Ala, Fmoc-Gly, Fmoc-L-Leu, Fmoc-L-Pro, Fmoc-L-Asp(OtBu) or Fmoc-D-Asp(OtBu), Fmoc-L-Lys(BOC), Fmoc-L-Glu(OtBu) or Fmoc-D-Glu(OtBu) or Fmoc-N-Me-L-Glu(OtBu).

The solid support was a preloaded Fmoc-Val-SASRIN™ polymeric resin (0536991, 200-400 mesh, 0.73 mmol/g, 1.4 g).

The synthetic protocol for Fmoc-chemistry was: (1) DMF, 6 × 1 min; (2) 20% Piperidine in DMF, 3 × 10 min; (3) DMF, 5 × 1 min; (4) 20% DMSO in DMF, 1 × 1 min; levels of free amino groups were assessed by Kaiser ninhydrin test; (5) 4-fold molar excess of Fmoc-AA, HOBt, DCC, preactivation in minimum of DMF for 40 min at room temperature. The precipitated dicyclohexylurea was filtered off and solution was added to the resin. (6) After 45 min DMSO/DIEA was added. After the 90 min residual levels of free amino groups were assessed by Kaiser ninhydrin test. Usually no double couplings were necessary. (7) DCM, 4 × 1 min; (8) $Ac_2O$-DIEA in DCM, 1 × 15 min; (9) DCM, 3 × 1 min.

Cleavage and final deprotection. The peptides were cleaved from the resin and simultaneously all the side chain protecting groups were removed by treatment with 2% water in TFA at room temperature for 1 h. The peptides were precipitated with cold diethyl ether and washed three times with diethyl ether and dried.

**Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val (1a).** White solid, 78.9% yield, HPLC purity 99.5 area %. $[\alpha]^{20}_D$ –155.1(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 7.1 Hz), 1.6 (8H, m), 2 (19H, m), 2.36 (7H, m), 2.7 (5H, m), 3 (2H, t, *J* 7.1 Hz), 3.9 (15H, m), 4.15 (2H, m), 4.25 (2H, m), 4.4 (2H, m), 4.6 (2H, m), 7.64 (1H, d, *J* 8.6 Hz), 8 (1H, d, *J* 7.2 Hz), 8.15 (1H, d, *J* 6.8 Hz), 8.25 (2H, t, *J* 5.2 Hz), 8.33 (1H, d, *J* 7.2 Hz), 8.4 (2H, m), 8.45 (1H, d, *J* 5.1 Hz), 8.67 (1H, d, *J* 6.7 Hz). MS: m/z 1420 $(M+H)^+$, 710 $(M+2H)^{2+}$. N-terminal sequence: compliant.

**Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val (1b).** White solid, 85.2% yield, HPLC purity 99.5 area %. $[\alpha]^{20}_D$ –155.5(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 7.2 Hz), 1.6 (8H, m), 2 (19H, m), 2.36 (7H, m), 2.7 (5H, m), 3 (2H, t, *J* 7.1 Hz), 3.9 (15H, m), 4.15 (2H, m), 4.25 (2H, m), 4.4 (2H, m), 4.6

(2H, m), 7.64 (1H, d, *J* 8.8 Hz), 8 (1H, d, *J* 7.3 Hz), 8.15 (1H, d, *J* 6.9 Hz), 8.25 (2H, t, *J* 5.2 Hz), 8.33 (1H, d, *J* 6.6 Hz), 8.4 (2H, m), 8.45 (1H, d, *J* 5.3 Hz), 8.67 (1H, d, *J* 6.7 Hz). MS: m/z 1420 $(M+H)^+$, 710 $(M+2H)^{2+}$. N-terminal sequence: compliant.

**Gly-D-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val (2).**White solid, 73.8% yield, HPLC purity 99.5 area %. $[\alpha]^{20}_D$ –124.9(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 7.3 Hz), 1.6 (8H, m), 2 (19H, m), 2.36 (7H, m), 2.7 (5H, m), 3 (2H, t, *J* 7.2 Hz), 3.9 (15H, m), 4.15 (2H, m), 4.25 (2H, m), 4.4 (2H, m), 4.6 (2H, m), 7.64 (1H, d, *J* 8.8 Hz), 8 (1H, d, *J* 7.3 Hz), 8.15 (1H, d, *J* 7.0 Hz), 8.22 (2H, t, *J* 5.6 Hz), 8.31 (1H, d, *J* 6.6 Hz), 8.4 (2H, m), 8.46 (1H, d, *J* 5.3 Hz), 8.53 (1H, d, *J* 7.8 Hz). MS: m/z 1420 $(M+H)^+$, 710 $(M+2H)^{2+}$. N-terminal sequence: compliant.

**Gly-D-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-D-Asp-D-Asp-Ala-Gly-Leu-Val (3).**White solid, 83.1% yield, HPLC purity 99.0 area %. $[\alpha]^{20}_D$ –108.1(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m, 14H), 1.4 (10H, t, *J* 6.7 Hz), 1.6 (8H, m), 2 (19H, m), 2.36 (7H, m), 2.7 (5H, m), 3 (2H, t, *J* 7.1 Hz), 3.9 (15H, m), 4.15 (2H, m), 4.25 (2H, m), 4.4 (2H, m), 4.6 (2H, m), 7.64 (1H, d, *J* 8.7 Hz), 8 (1H, d, *J* 7.3 Hz), 8.15 (1H, d, *J* 7.0 Hz), 8.4 (5H, m), 8.49 (1H, d, *J* 5.5 Hz), 8.53 (1H, d, *J* 7.7 Hz). MS: m/z 1420 $(M+H)^+$, 710 $(M+2H)^{2+}$. N-terminal sequence: compliant.

**Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-D-Asp-D-Asp-Ala-Gly-Leu-Val (4).**

White solid, 79.4% yield, HPLC purity 99.0 area %. $[\alpha]^{20}_D$-126.3(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 6.7 Hz), 1.6 (8H, m), 2 (19H, m), 2.36 (7H, m), 2.7 (5H, m), 3 (2H, t, *J* 6.9 Hz), 3.9 (15H, m), 4.15 (2H, m), 4.28 (2H, m), 4.4 (2H, m), 4.6 (2H, m), 7.65 (1H, d, *J* 8.7 Hz), 8 (1H, d, *J* 7.3 Hz), 8.14 (1H, d, *J* 7.1 Hz), 8.4 (5H, m), 8.49 (1H, d, *J* 5.3 Hz), 8.65 (1H, d, *J* 6.7 Hz). MS: m/z 1420 $(M+H)^+$, 710 $(M+2H)^{2+}$. N-terminal sequence: compliant.

**Gly-N-Me-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val (5).**White solid, 73.4% yield, HPLC purity 99.3 area %. $[\alpha]^{20}_D$–154.5(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O$/$H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 7.4 Hz), 1.6 (8H, m), 2 (18H, m), 2.3 (7H, m), 2.7 (4H, m), 2.9 (3H, s), 3 (3H, t, *J* 7.1 Hz), 3.6 (6H, m), 3.8 (4H, m), 3.9 (4H, m), 4. 5 (3H, m), 4.25 (2H, m), 4.4 (3H, m), 4.6 (1H, m), 5.25 (1H, m), 7.63 (1H, d, *J* 8.8 Hz), 8 (1H, d, *J* 7.3 Hz), 8.13 (1H, d, *J* 7.0 Hz), 8.22 (2H, t, *J* 5.9 Hz), 8.31 (1H, d, *J* 6.7 Hz), 8.4 (3H, m). MS: m/z 1434 $(M+H)^+$, 717 $(M+2H)^{2+}$.

**Gly-N-Me-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-D-Asp-D-Asp-Ala-Gly-Leu-Val (6).** White solid, 77.8% yield, HPLC purity 99.2 area %. $[\alpha]^{20}_D$ –127.7(c 1, $H_2O$). IR (KBr): 3450, 3300, 2950, 2880, 1650, 1550, 1460, 1400, 1340, 1250, 1210, 1180, 1070cm$^{-1}$. $^1$H NMR (20% $D_2O/H_2O$) δ 0.9 (14H, m), 1.4 (10H, t, *J* 6.9 Hz), 1.6 (8H, m), 2 (18H, m), 2.3 (7H, m), 2.7 (4H, m), 2.9 (3H, s), 3 (3H, t, *J* 6.7 Hz), 3.6 (6H, m), 3.8 (4H, m), 3.9 (4H, m), 4. 5 (3H, m), 4.25 (2H, m), 4.4 (3H, m), 4.6 (1H, m), 5.25 (1H, m), 7.65 (1H, d, *J* 8.7 Hz), 8 (1H, d, *J* 7.3 Hz), 8.13 (1H, d, *J* 7.1 Hz), 8.26 (1H, d, *J* 6.1 Hz), 8.32 (4H, m), 8.49 (1H, d, *J* 5.4 Hz). MS: m/z 1434 $(M+H)^+$, 717 $(M+2H)^{2+}$.

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## Povzetek

Želodčni sok človeka vsebuje protein BPC s širokim območjem bioloških delovanj. Fragment s petnajstimi aminokislinami (BPC 157) in analogi so bili sintetizirali z uporabo sinteze na netopnih polimernih nosilcih SPPS (Solid Phase Peptide Synthesis) in to z Boc-Val-HYCRAM™ (na polimerni nosilec že vezana ustrezno zaščitena aminokislina zadnja v sekvenci želenega peptida) in kombinacijo uporabljenih Boc- in Fmoc- zaščitenih aminokislinin ter z Fmoc-Val-SASRIN™ (na polimerni nosilec že vezana ustrezno zaščitena aminokislina zadnja v sekvenci želenega peptida) z uporabljenimi Fmoc- zaščitenimi aminokislinami. Primerjali smo izkoristke, kromatografske čistosti in stroške za sintezo ekvivalentnih količin peptidov.