Chapter 6
Solid Phase Synthesis of Biologically Active Peptides Using PS-TRPGGDA Supports
6.1 Introduction

Synthetic peptides and proteins are widely used in the field of life sciences. They play crucial roles in a number of diverse biological processes. The recombinant DNA-based expression of proteins in genetically engineered cell has its own limitation like over expression of protein and the molecular studies are inherently limited to the 20 genetically encoded amino acids. The use of polymer supported synthesis of peptide compared to molecular cloning, promises the unlimited variation in the primary structure. It is the best economical way to generate peptides and proteins in useful quantities. The synthesis of large peptides using PS-DVB support with high purity and homogeneity is still a challenging problem. Several studies revealed that the successful peptide synthesis is hampered mainly due to the poor solvation of the growing peptidyl resin chain, which often leads to incomplete reactions. These problems could be solved to a certain extent by a judicious selection of the monomers and their quantities used for developing the polymer support. The nature of the cross-linker and degree of cross-linking are the two factors that control the mechanical stability, compatibility, and swellability of the polymer in a wide range of polar/non polar solvents. The PS-TRPGGDA resins of various cross-linking densities were utilised for the successful synthesis of biologically active peptides. The suitable hydrophobic/hydrophilic balance of the resin allows the mass transport of the reagents and solvents through the resin matrix to various reaction sites in the polymer. The secondary hydroxyl functional groups present in the cross-linker avoids further reaction steps which are used for the initial resin functionalisation steps for most of the polymers. The resin is further incorporated with various linkers like 4-(hydroxymethyl)phenoxyacetic acid and 4-(4-hydroxymethyl-3-methoxyphenoxy) butyric acid anchoring groups inorder to cleave the target peptide from the resin effectively. The degree of cross-linking is selected according to the required quantities of the synthetic peptide since degree of functional group depends upon the amount of the cross-linker in the resin.
Incorporation of linkers also allows the cleavage of the target peptide in acid form or other C-terminal modified forms.

The capability of the new polymer support for effective assembly of amino acids present in a peptide/protein sequences either by Boc or Fmoc-chemistries is illustrated here. Side reactions like the formation of diketopiperazine were not observed during the synthesis. In the initial stages Boc-chemistry is used for the synthesis of peptides. Further studies on the resin confirmed that the peptidyl resin has to be suspended more time in TFA compared to Fmoc-synthesis for the cleavage of the support from the polymeric support. This problem was largely avoided by synthesising the peptide using Fmoc-amino acids on linker attached polymeric supports. All these studies clearly supported the efficiency of the new resin over the various PS-DVB resins; it is as good as that of the Sheppard resin and even better when compare the chemical and mechanical stability.

6.2 Results and Discussion

6.2.a. Synthesis of peptides using Boc-chemistry

1. Leu-Gly-Ala-Leu-Gly-Ala

Boc-Ala was attached to 2% PS-TRPGGDA resin by MSNT method. The quantitative incorporation of amino acid to the resin was estimated by picric acid titration method. After removing the temporary Boc-protection with 30% TFA in DCM and neutralization with 5% DIEA/DCM, the successive amino acids were incorporated by HOBt active ester method using HOBt and DCC. The pre-formed HOBt active ester is transferred to the reaction vessel after removing the DCU precipitated. Each coupling step was monitored by Kaiser test. The target peptide was cleaved from the support using TFA in presence of water. The crude peptide was obtained in 84% yield. HPLC profile of the peptide gives a single peak corresponding to the target peptide (Fig. 1).
Figure 1: HPLC time-course analysis of peptide (Leu-Gly-Ala-Leu-Gly-Ala) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water. Gradient used: 0% B in 5 min and 100% B in 60 min.

2. Ala-Ala-Ala-Ala

The C-terminal Boc-Ala was attached to the 2% PS-TRPGGDA resin by MSNT method. The temporary Boc-deprotection, neutralization and coupling of the respective amino acids using HOBT active ester were performed till the target peptide was completely formed. The peptide was cleaved from the support using TFA in presence of water, in 83% yield. HPLC profile of the peptide showed only a single peak (Fig. 2).
3. Synthesis of 17 residue chicken IL-2 cDNA peptide

Leu-Gly-Val-Val-Thr-Lys-Lys-Glu-Thr-Glu-Asp-Asp-Thr-Glu-Ile-Lys-Glu

The C-terminal Boc-Glu was attached to the 2% PS-TRPGGDA support using active ester of Boc Glu with MSNT. Boc-deprotection, neutralisation, and the incorporation of the successive amino acids were carried out using HOBut active ester of the respected Boc-amino acids. The target peptide was cleaved from the support using TFA in presence of phenol, ethanedithiol, thioanisole and water. The crude peptide was obtained in 82% yield. HPLC profile showed only a single peak corresponding to the target peptide (Fig. 3). Amino acid analysis data agreed with the target peptide sequence. Thr showed a low value due to the partial degradation during hydrolysis.
Biologically Active Peptides Using PS-TRPGGDA...

Figure 3: HPLC time-course analysis of peptide (Leu-Gly-Val-Val-Thr-Lys-Lys-Glu-Thr-Glu-Asp-Asp-Thr-Glu-Ile-Lys-Glu) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water. Gradient used; 0% B in 5 min and 100% B in 67 min.

4. Ala-Cys-Ala-Pro-Pro-Ala-Asp-Arg-Ala-Thr-Arg-Ala

Mutated residue of HIV Rev. nuclear export signal Ala-Cys-Ala-Pro-Pro-Ala-Asp-Arg-Ala-Thr-Arg-Ala where all leucine were replaced by alanine was synthesised on 4% PS-TRPGGDA support. Boc-Ala was attached to the polymer support as active ester using MSNT. Boc-deprotection, neutralization and the amino acid incorporations were carried out as described earlier. The target peptide was cleaved from the support using TFA in presence of phenol, ethanedithiol, thioanisole and water. The crude peptide was obtained in 83% yield. HPLC profile of the peptide using C18 RPC column. (Fig. 4a) showed a single peak corresponding to the target peptide. The amino acid analysis and MALDI-TOF-MS also agreed with the identity of the target peptide (Fig. 4b).
Figure 4: (a) HPLC time-course analysis of peptide (Ala-Cys-Ala-Pro-Pro-Asp-Arg-Ala-Thr-Arg-Ala) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 70% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Ala-Cys-Ala-Pro-Pro-Asp-Arg-Ala-Thr-Arg-Ala

6.2.b Synthesis of peptides using Fmoc-chemistry

6.2.b.1 Synthesis of peptide substrates of Ca^{2+}/Calmodulin binding peptide

Calcium/Calmodulin dependent protein kinase type II (CaMK II) is a neuronal enzyme found in high concentrations in the postsynaptic density. It is thought to be involved in the molecular events underlying learning and memory. It is known to bind with high affinity to the C-terminal region of the NR2B subunit of the NMDA-type glutamate receptor found in neuronal synapses at more than one site. The binding is thought to be responsible for the localization of CaMKII in the post synaptic density just beneath the post synaptic membrane, in response to increases in free Ca^{2+} concentration. The postsynaptic density also has \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) type glutamate receptors. The binding of CaMKII to the NR2B subunit thus positions the enzyme proximal to the AMPA-type glutamate receptor, which also gets phosphorylated when the NR2B-bound CaMKII is activated by NMDA-receptor mediated Ca^{2+} influx. One of the
binding sites on NR2B for CaMKII is near its phosphorylation site, serine-1303. The high affinity binding of CaMKII also shows specificity for the NR2B subunit. The NR2A sub unit, which has very similar sequences near the phosphorylation site, does not show affinity binding to CaMKII. It has been suggested that the site responsible for high affinity binding of CaMKII lies in the region of residues 1260-1309 of NR2B.

In order to define the features on the NR2B sub unit that determines the binding affinity, a series of peptide substrates of increasing N-terminal chain length identical to the sequence in the vicinity of serine-1303 of NR2B subunit were synthesised. Peptides of analogous sequence on NR2A sub unit were also synthesised for comparative study of the efficiency of these peptides to act as substrates for the enzyme.

**NR2A:** SQNALQFQKKNKLIRINRQHPDNL

**NR2B:** TNSKAQKKRNKLR- -RQHSPYDFVD

6.2.b.1.a Synthesis of NR2B peptide substrates of Ca\(^{2+}\)/Calmodulin binding peptide


Synthesis of NR2B peptide substrates of increasing N-terminal chain length were carried out on 4-(4-hydroxymethyl-3-methoxy) phenoxypyramido 2% PS-TRPGGDA support. The NR2B substrates synthesised were of 11, 17, 20 and 23 residues. The C-terminal amino acid Fmoc-Val was attached to the polymer support as active ester by MSNT method in presence Melm. Fmoc-deprotection was carried out using 20% piperidine in DMF. The respective Fmoc-amino acids were incorporated to the support using the coupling reagent HBTU in presence of HOBT and DIPEA. The coupling was monitored by...
Kaiser test. After the completion of the 11th residue i.e., leucine at the N-terminus about ¼ of the resin bound peptide was withdrawn from the reaction mixture and kept aside for cleavage. The NR2B 11 residue peptide substrate was cleaved by suspending the peptidyl resin in TFA in presence of thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 96% yield. HPLC profile shows a single peak corresponding to the target peptide (Fig. 5a).

![Figure 5: (a) HPLC time-course analysis of peptide (Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val](image)

Amino acid analysis data agreed with the target peptide sequence. Low values for Ser and Thr due to its partial degradation during hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 5b).

The CD spectrum of the peptide in TFE showed negative band at 207 nm (amide π-π* transition) and 228 nm (amide n-π* transition) suggesting a right handed α-helical conformation (Fig. 5c).
The stepwise synthesis of NR2B substrate was continued with \(N^\alpha\)-protected leucine terminally attached peptidyl resin. The deprotection was carried out using 20% piperidine in DMF. The synthesis was continued until the 17\(^{\text{th}}\) amino acid residue from the C-terminal by incorporating the respective Fmoc-amino acid using HBTU, HOBr in presence of DIEA. About \(\frac{1}{4}\) of the resin bound peptide was withdrawn from the reaction mixture and kept aside for cleavage. The NR2B 17 residue peptide substrate was cleaved by suspending the peptidyl resin in TFA in presence of thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 96% yield. HPLC profile shows a single peak corresponding to the target peptide (Fig. 6a). Amino acid analysis data agreed with the target peptide sequence. Low values for Ser and Thr are due to partial degradation during hydrolysis and high value for Asp is due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 6b).
Biologically Active Peptides Using PS-TRFGGDA...

Figure 6: (a) HPLC time-course analysis of peptide (Lys-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used: 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Lys-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val

The CD spectrum of the peptide in TFE showed negative band at 207 nm (amide $\pi-\pi^*$ transition) and 228 nm (amide $n-\pi^*$ transition) suggesting a right handed $\alpha$-helical conformation for the peptide (Fig. 6c).

Figure 6(c): CD spectrum of Lys-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val in TFE

The stepwise synthesis was continued with remaining peptidyl resin by incorporating the respective amino acid using HBTU, HOBt as coupling reagents in presence of DIEA. After the successful completion of the 20th residue from the C-terminal about $\frac{1}{4}$ of the peptidyl resin was withdrawn from
the synthesiser and kept aside for cleavage. The NR2B 20 residue peptide substrate was cleaved by suspending the peptidyl resin in TFA in presence of thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 95% yield. HPLC profile (Fig. 7a) shows a single peak indicating the high purity of the NR2B 20 mer peptide. Amino acid analysis data agreed with the target peptide sequence. Low values for Ser and Thr due to its partial degradation during hydrolysis and high value for Asp due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 7b).

**Figure 7:** (a) HPLC time-course analysis of peptide (Lys-Ala-Gln-Lys-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Tyr-Asp-Thr-Phe-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Lys-Ala-Gln-Lys-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Tyr-Asp-Thr-Phe-Val

The CD spectrum of the peptide in TFE showed intense negative band at 208nm (amide $\pi-\pi^*$ transition) and 222nm (amide n-\(\pi^*\) transition) suggesting a right handed $\alpha$-helical conformation for the peptide (Fig. 7c)
Figure 7(c): CD spectrum of Lys-Ala-Gln-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val in TFE

The stepwise synthesis was carried out until the 23 residue target peptide was achieved by incorporating the respective amino acid using HBTU, HOBt as coupling reagents in presence of DIEA. Most of the couplings were successfully completed in the first coupling stage itself. Each coupling step was monitored by ninhydrin test. After the successful synthesis of 23 residue fragment of NR2B it was cleaved from the support by suspending the resin in TFA in presence of acid scavenger thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 94% yield. HPLC profile shows a single major peak indicating the high purity of the NR2B 23 mer peptide (Fig. 8a). Amino acid analysis data agreed with the target peptide sequence. Low values for Ser and Thr due to its partial degradation during hydrolysis and high value for Asp due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 8b).
Figure 8: (a) HPLC time-course analysis of peptide (Thr-Asn-Ser-Lys-Ala-Gln-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Thr-Asn-Ser-Lys-Ala-Gln-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val

The CD spectrum of the peptide in TFE showed intense negative band at 208 nm (amide π-π* transition) and 222 nm (amide π-π* transition) suggesting a right handed α-helical conformation for the peptide (Fig. 8 c).

Figure 8(c): CD spectrum of Thr-Asn-Ser-Lys-Ala-Gln-Lys-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val in TFE
6.2.b.1.b Synthesis of NR 2A 24 residue peptide substrates of Ca\(^{2+}\)/Calmodulin binding peptide


Synthesis of NR 2A peptide substrates of increasing chain length were carried out on 4-(4-hydroxymethyl-3-methoxy)phenoxybutyramido 2% PS-TRPGGDA support. The NR 2A substrates of 12 and 24 residues were synthesised. The C-terminal amino acid Asp was attached to the polymer support as Fmoc-Asp active ester by MSNT method in the presence MeIm. Fmoc-deprotection was carried out using 20% piperidine in DMF. The respective Fmoc-amino acids were incorporated to the support using the coupling reagent HBTU in presence of HOBt and DIEA. Each coupling reaction was monitored by ninhydrin test. After the completion of the 12\(^{th}\) residue (i.e., isoleucine at the N-terminus), about \(\frac{1}{4}\) of the resin bound peptide was withdrawn from the reaction mixture and kept aside for cleavage. The NR2B 12 residue peptide substrate was cleaved by suspending the peptidyl resin in TFA in presence of thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 96% yield. The peptide gave a single peak on HPLC analysis (Fig. 9a) indicating the homogeneity of the target peptide. Amino acid analysis data agreed with the target peptide sequence. Low values for Ser due to its partial degradation during hydrolysis and high value for Asp due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 9b).
Figure 9: (a) HPLC time-course analysis of peptide (Ile-Asn-Arg-Gln-His-Ser-Tyr-Asp-Asn-Ile-Leu-Asp) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Ile-Asn-Arg-Gln-His-Ser-Tyr-Asp-Asn-Ile-Leu-Asp

The CD spectrum of the peptide in TFE showed negative band at 205 nm (amide $\pi-\pi^*$ transition) and 225 nm (amide $n-\pi^*$ transition) suggesting a right handed $\alpha$-helical conformation for the peptide (Fig. 9c).

Figure 9(c): CD spectrum of Ile-Asn-Arg-Gln-His-Ser-Tyr-Asp-Asn-Ile-Leu-Asp in TFE

The stepwise synthesis was further carried out until the 24 residue target peptide was achieved by incorporating the respective amino acid using HBTU, HOBt as coupling reagents in presence of DIEA. In the above
synthesis, most of the coupling reactions were complete in the first coupling itself. Each coupling steps were monitored by Kaiser test. After the successful synthesis of 24 residue fragment of NR 2A it was cleaved from the support by suspending the resin in TFA in presence of acid scavenger thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 94% yield. HPLC profile (Fig. 10a) shows a single major peak indicating the high purity of the NR2B 24 mer peptide. Amino acid analysis data agreed with the target peptide sequence. Low values for Ser due to its partial degradation during hydrolysis and high value for Asp due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 10b).

![Figure 10: (a) HPLC time-course analysis of peptide (Asn-Asn-Ala-Leu-Gln-Phe-Gln-Lys-Asn-Lys-Leu-Arg-Ile-Asn-Arg-Gln-His-Ser-Tyr-Asp-Asn-Ile-Leu-Asp) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used: 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Asn-Asn-Ala-Leu-Gln-Phe-Gln-Lys-Asn-Lys-Leu-Arg-Ile-Asn-Arg-Gln-His-Ser-Tyr-Asp-Asn-Ile-Leu-Asp](image_url)

Enzyme Binding Studies indicated that the peptide sequences analogues with the amino acid sequence in the vicinity of serine 1303 shows an increased affinity for the binding of NR2B to CaMKII. However, the peptide substrates from the closely related NR2A subunit showed lower
Biologically Active Peptides Using PS-TRPGGDA...145

affinity for CaMKII. The result showed that the amino acid residues particularly between 1289-1294 regions is unique to NR2B peptide substrate and this region largely influences its high binding affinity to the enzyme.  

6.2.b.1.c Synthesis of NR 2B mutated peptide substrate of \( \text{Ca}^{2+}/\text{calmodulin} \) binding peptide using semi-automatic peptide synthesiser

Thr-Asn-Ser-Lys-Ala-Gln-Ala-Ala-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val

Synthesis of NR 2B 23 residue mutated peptide substrate was carried out on 4-(4-hydroxymethyl-3-methoxy)phenoxybutyramido 2% PS-TRPGGDA support. The C-terminal amino acid Fmoc-Val was attached to the polymer support as the active ester by MSNT method in presence MeIm. Fmoc-deprotection was carried out using 20% piperidine in DMF. The respective Fmoc amino acids were incorporated to the support using the coupling reagent HBTU in presence of HOBT and DIEA. Each coupling reaction was monitored by ninhydrin test. After the synthesis, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 94% yield. HPLC profile of the peptide shows only a single peak corresponding the target peptide (Fig. 11a). Amino acid analysis data agreed with the target peptide sequence. Low values for Ser and Thr due to its partial degradation during hydrolysis and high value for Asp due to the hydrolysis of Asn to Asp. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 11b).
Figure 11: (a) HPLC time-course analysis of peptide (Thr-Asn-Ser-Lys-Ala-Gln-Ala-Ala-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water. Gradient used: 0% B in 5 min and 100% B in 60 min (b) MALDI-TOF-MS of Thr-Asn-Ser-Lys-Ala-Gln-Ala-Ala-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val.

The CD spectrum of the peptide in TFE showed negative band at 208 nm (amide \( \pi - \pi^* \) transition) and 225 nm (amide \( \pi - \pi^* \) transition) suggesting a right handed \( \alpha \)-helical conformation for the peptide (Fig. 11c).

Figure 11(c): CD spectrum of Thr-Asn-Ser-Lys-Ala-Gln-Ala-Ala-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val in TFE.
6.2.b.2 Synthetic peptide fragments of Hepatitis C viral protein

Hepatitis C virus (HCV) is the aetiological agent responsible for most cases of non-A, non-B hepatitis. Hepatitis C is a disease of clinical importance because of its high infection rate in blood donors and its persistence as chronic infection that may lead to cirrhosis and hepatocellular carcinoma. HCV is a positive-sense single-stranded RNA virus with a genome size of about 9.5 kb. The viral nucleic acid codes for single polyprotein varying in size from 3010-3011 amino acids, which are subsequently spliced into at least ten functional protein units which are coded as: core (C); two envelope (E1 & E2); and at least six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A & NS5B). The genomic sequences of HCV vary to a great extent in different isolates with differences at the nucleotide level of up to 40%. At present HCV variants are divided into 11 genotypes including the 6 types and several sub-types. The variability of the HCV genome has posed serious problems in serological detection of HCV. The best available method (for the detection of HCV) is the ultra sensitive polymerase chain reaction (PCR) assay using serum samples of patients with hepatitis, chronic hepatitis and cirrhosis. Due to the genetic variability of HCV strains commercially used primers are found to be unsuitable for the detection of the Indian strain of the virus by PCR. This method is costly, requires highly trained manpower and specially designed primers for the detection of the virus. Because of these limitations, Das et al. developed an easy sensitive method to detect the presence of anti-viral antibodies in patient sera using synthetic peptide ELISA.

6.2.b.2.a Synthesis of NS2 peptide fragment of Hepatitis C viral protein

His-Val-Trp-Ile-Pro-Pro-Leu-Asn-Ala-Arg-Arg-Gly-Gly-Arg-Asp-Ala-Ile-Ile-Leu-Leu-Met-Cys-Ala-Val

23-residue fragment of NS2 peptide was synthesised on 4-(hydroxymethyl) phenoxyacetamido 2% PS-TRPGGDA support. Fmoc-Val was attached to the
support by the MSNT method in presence of Melm. After removing the Fmoc-protection with 20% piperidine in DMF, respective amino acids were incorporated by using coupling reagent HBTU in presence of HOBt and DIEA. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 95% yield. HPLC profile of the peptide shows only a single peak corresponding the target peptide (Fig. 12a).

Amino acid analysis data agreed with the target peptide sequence. Asn was hydrolysed to Asp and Trp destroyed during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS (Fig. 12b).

**Figure 12:** (a) HPLC time-course analysis of peptide (His-Val-Trp-Ile-Pro-Pro-Leu-Asn-Ala-Arg-Arg-Gly-Gly-Arg-Asp-Ala-Ile-Leu-Leu-Met-Cys-Ala-Val) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water. Gradient used; 0% B in 5 min and 100% B in 45 min (b) MALDI-TOF-MS of His-Val-Trp-Ile-Pro-Pro-Leu-Asn-Ala-Arg-Arg-Gly-Gly-Arg-Asp-Ala-Ile-Leu-Leu-Met-Cys-Ala-Val
6.2.b.2.b Synthesis of NS3 peptide fragment of Hepatitis C viral protein

Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Ser-Ala-Leu

Fmoc-Leu was attached to the 4-(hydroxymethyl)phenoxyacetamido 2% PS-TRPGGDA support as MSNT active ester in presence of MeIm. After removing the Fmoc-protection by 20% piperidine in DMF, respective amino acids were incorporated by using coupling reagent HBTU in presence of HOBt and DIEA. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 13a). Amino acid analysis data agreed with the target peptide sequence. Ser showed a low value due to its partial degradation during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 13b).

Figure 13: (a) HPLC time-course analysis of peptide (Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Ser-Ala-Leu) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 45 min (b) MALDI-TOF-MS of Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Ser-Ala-Leu
6.2.b.2.c Synthesis of NS4 peptide fragment of Hepatitis C viral protein

Arg-Glu-Val-Leu-Tyr-Gln-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu

The 22-residue NS4 peptide fragment of HCV was synthesised on 4-(hydroxymethyl)phenoxyacetamido 2\% PS-TRPGGDA support. Fmoc-Glu was attached to the support by MSNT active ester in presence of MeIm. After removing the Fmoc-protection by 20\% piperidine in DMF, respective amino acids were incorporated by using coupling reagent HBTU in presence of HOBt and DIEA. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 95\% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 14a).

Figure 14: (a) HPLC time-course analysis of peptide (Arg-Glu-Val-Leu-Tyr-Gln-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80\% acetonitrile in water, Gradient used; 0\% B in 5 min and 100\% B in 50 min (b) MALDI-TOF-MS of Arg-Glu-Val-Leu-Tyr-Gln-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu

Amino acid analysis data agreed with the target peptide sequence. Ser showed a low value due to its partial degradation during the hydrolysis. Gln residues
were hydrolysed to Glu. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 14b).

6.2.b.2.d Synthesis of NS5 peptide fragment of Hepatitis C viral protein

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\text{Asp-Glu-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Val-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg}
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Fmoc-Arg was attached to the 4-(hydroxymethyl)phenoxyacetamido 2% PS-TRPGGDA support as MSNT active ester in presence of MeIm. After removing the Fmoc-protection by 20% piperidine in DMF, respective amino acids were incorporated by using coupling reagent HBTU in presence of HOBT and DIEA. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 15a).

\[\text{Figure 15: (a) HPLC time-course analysis of peptide (Asp-Glu-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Val-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Asp-Glu-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Val-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg}\]
Amino acid analysis data agreed with the target peptide sequence. Ser showed a low value due to its partial degradation during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 15b).

6.2.b.3 Synthesis of HIV-1 Rev. NES peptide

Ala-Cys-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Arg-Ala

The 12-residue HIV-1 Rev. NES peptide was synthesised on 4-((hydroxymethyl)phenoxacectamido 4% PS-TRPGGDA support. The peptide was synthesised to determine its efficiency as a nuclear export signal. This NES is highly transferable and it is characterised by a short stretch of hydrophobic amino acid primarily rich in leucine residues. Formoc-Ala was attached to support by MSNT active ester method in presence of MeIm. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 16a). Amino acid analysis data agreed with the target peptide sequence. Thr showed a low value due to its partial degradation during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 16b).

The CD spectrum of the peptide in TFE showed intense negative band at 207 nm (amide $\pi-\pi^*$ transition) and 228 nm (amide n-$\pi^*$ transition) suggesting a right handed $\alpha$-helical conformation for the peptide (Fig. 16c).
Biologically Active Peptides Using PS-TRPGGDA...153

Figure 16: (a) HPLC time-course analysis of peptide (Ala-Cys-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Arg-Ala) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Ala-Cys-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Arg-Ala

Figure 16(c): CD spectrum of Ala-Cys-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Arg-Ala

6.2.b.4 Synthesis of M10 mutant NES peptide

Ala-Cys-Leu-Pro-Pro-Leu-Asp-Arg-Leu-Thr-Arg-Ala

The 12-residue M10 mutant NES peptide (in which the glutamic acid of HIV-1 Rev. NES peptide replaced by aspartic acid) was synthesised on 4-hydroxymethyl phenoxyacetamido 4% PS-TRPGGDA support. Fmoc-Ala
was attached to support by MSNT active ester method in presence of MeIm. Each coupling step was monitored by ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 17a). Amino acid analysis data agreed with the target peptide sequence. Thr showed a low value due to its partial degradation during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 17b).

![Figure 17: (a) HPLC time-course analysis of peptide (Ala-Cys-Leu-Pro-Pro-Leu-Asp-Arg-Leu-Thr-Arg-Ala) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used: 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Ala-Cys-Leu-Pro-Pro-Leu-Asp-Arg-Leu-Thr-Arg-Ala](image)

The CD spectrum of the peptide in TFE showed intense negative band at 204 nm (amide $\pi-\pi^*$ transition) and 225 nm (amide $\pi-\pi^*$ transition) suggesting a right handed $\alpha$-helical conformation for the peptide (Fig. 17c).
6.2.b.5 Synthesis of Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr

The decapeptide Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr was synthesised on 4-(4-hydroxymethyl-3-methoxyphenoxybutylamido PS-TRPGGDA support. The peptide is effective in stimulating melanin cells and currently used in trial basis for the patients suffering from albinism. Fmoc-Tyr was attached to support by MSNT active ester method in presence of MeIm. Each coupling step was monitored by Kaiser’s semi quantitative ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 18a). Amino acid analysis data agreed with the target peptide sequence. Ser showed a low value due to its partial degradation during the hydrolysis. Trp destroyed during the hydrolysis. The identity of the target peptide was further confirmed by MALDI-TOF-MS. (Fig. 18b).
**Figure 18:** (a) HPLC time-course analysis of peptide (Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min (b) MALDI-TOF-MS of Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr

The CD spectrum of the peptide in TFE showed intense negative band near 210 nm (amide n-π* transition) suggesting a β-conformation for the peptide (Fig. 18c).

**Figure 18(c):** CD spectrum of Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr

A synthetic step for the propionylation of the peptide was then carried out with the remaining N⁰-protected peptidyl resin. Fmoc-deprotection was carried out using 20% piperidine in DMF. The deprotected resin was then
propionylated by 3-(p-hydroxyphenyl)-propionic acid using HBTU/HOBt as coupling reagents in presence of DIEA. Coupling reaction was monitored by Kaiser test. The propionylated peptide was cleaved from the support by suspending the resin in TFA in presence of acid scavenger thioanisole, ethanedithiol, phenol and water at room temperature for 4 h. The crude peptide was obtained in 94% yield. HPLC profile shows a single major peak indicating the high purity of the propionylated peptide (Fig. 19).

![Figure 19: HPLC time-course analysis of peptide (Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr-propionylated) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min](image)

### 6.2.b.6 Synthesis of Asn-Pro-Val-Tyr

The peptide Asn-Pro-Val-Tyr was synthesised on a 4-(4-hydroxymethyl-3-methoxy)phenoxybutylamido 8% PS-TRPGGDA support. Fmoc-Tyr was attached to support by MSNT active ester method in presence of MeIm. Each coupling step was monitored by Kaiser's semi quantitative ninhydrin test. After the attachment of amino acids to the resin, peptide was cleaved from the support by treating with TFA in presence of thioanisole, phenol, ethanedithiol and water. The crude peptide was obtained in 96% yield. HPLC profile of the peptide shows only one major peak corresponding the target peptide (Fig. 20). Amino acid analysis of the peptide also agreed with that of the target peptide.
The extreme purity and the high yield of peptide shows that the new support can be used for polypeptide synthesis even with high crosslinking densities.

![Figure 20: HPLC time-course analysis of peptide (Asn-Pro-Val-Tyr) using the buffer (A) 0.5 mL TFA in 100 mL water; (B) 0.5 mL TFA in 80% acetonitrile in water, Gradient used; 0% B in 5 min and 100% B in 67 min]

6.3 Experimental

6.3.a Materials

4-(hydroxymethyl)phenoxyactic acid (HMPA), 4-(4-hydroxymethyl 1-3-methoxyphenoxy)butyric acid (HMPB), 2-(1 H benzotriazol-1-yl) 1, 1, 3, 3-tetramethyl uronium hexafluoro phosphate (HBTU), 1-hydroxy benzotriazole (HOBt), Boc and Fmoc-amino acids, were purchased from Nova Biochem Ltd. UK. Diisopropylethyl amine (DIEA), piperidine, trifluoroactic acid (TFA), ethanedithiol, phenol, thioanisole, Sephadex G-10, G-25, and G-50 were purchased from Sigma-Aldrich Corp., USA. All solvents used were of HPLC grade purchased from E. Merck (India), BDH (India) and SISCO Chemicals (Mumbai). HPLC was done on a Pharmacia Akta purifier using C-18 reverse phase semi preparative HPLC column. The amino acid analysis was carried out on an LKB 4151 α-plus amino acid analyzer. Mass spectra of peptides
were recorded in a Kratos PC Kompact MALDI-TOF-mass spectrometer. CD was taken in Jasco spectropolarimeter J 715.

6.3.b Synthesis of peptides using Boc-chemistry

1. Synthesis of Leu-Gly-Ala-Leu-Gly-Ala

2% PS-TRPGGDA dried resin (400 mg, 0.04 mmol) was swelled in dry DCM (40 mL). After 30 min, excess DCM was removed; Boc-Ala (15 mg, 0.08 mmol), MSNT (24 mg, 0.08 mmol) and N-methyl imidazole (4.8 μL, 0.06 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min. in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 × 10 mL), EtOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum. The amount of Ala attached to the resin was estimated by picric acid titration method (0.095 mmol/g).

Boc-Ala-PS-TRPGGDA resin (180 mg, 0.017 mmol) was used for the Leu-Gly-Ala-Leu-Gly-Ala peptide synthesis. Boc-protection of the C-terminal amino acid bound resin was removed with 30% TFA in DCM. The resin was washed with DCM (5 × 10 mL), the resulting salt was neutralized with 5% DIEA in DCM. After 10 min. the resin was washed with DCM (5 × 10 mL) and NMP (5 × 10 mL). The remaining amino acids Boc-Gly (10.5 mg, 0.06 mmol), Boc-Leu (14.4 mg, 0.06 mmol), Boc-Ala (11.3 mg, 0.06 mmol), were coupled by HOBT active ester method. The HOBT active ester was prepared by dissolving DCC (12 mg, 0.06 mmol), HOBT (8 mg, 0.06 mmol) in minimum amount of NMP and stirred with respective amino acid for 40 min. DCU formed was filtered off and the active ester was added into the resin. 40 min. was given for each coupling reaction. Each coupling step was monitored by semi-quantitative ninhydrin test. After the synthesis, the peptidyl resin was washed with NMP (5 × 10 mL), DCM (5 × 10 mL), MeOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum.

The peptide was cleaved from the support by suspending the peptidyl resin in TFA (2.8 mL), thioanisole (150 μL), ethanedithiol (150 μL), and water.
(150μL) for 12 h at room temperature. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 6.8 mg of crude peptide. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5%TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

2. Synthesis of Ala-Ala-Ala-Ala

Boc-Ala-PS-TRPGGDA resin (200 mg, 0.019 mmol) was used for the Ala-Ala-Ala-Ala peptide syntheses. Boc-protection of the C-terminal amino acid bound resin was removed with 30% TFA in DCM. The resin was washed with DCM (5×10 mL), the resulting salt was neutralized with 5% DIEA in DCM. After 10 min. the resin was washed with DCM (5×10 mL) and NMP (5×10 mL). Boc-Ala (12.4 mg, 0.066 mmol) was coupled to the deprotected amino acid bound resin by HOBt active ester method. Active ester was prepared by dissolving DCC (13.6 mg, 0.066 mmol), HOBt (9 mg, 0.066 mmol) in minimum amount of NMP and stirred with the Boc-Ala (12.4 mg, 0.066 mmol) for 40 min. DCU formed was filtered off and the active ester was added into the resin. Forty min. was given for each coupling reaction. The coupling was monitored by semi quantitative ninhydrin test. After the synthesis the peptidyl resin was washed with NMP (5×10 mL), DCM (5×10 mL), MeOH (5×10 mL), ether (5×10 mL) and dried under vacuum.

The peptide was cleaved from the support by suspending the peptidyl resin in TFA (2.8 mL), thioanisole (150μL), ethanedithiol (150μL), and water (150μL) for 12 h at room temperature. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 4.5 mg of crude peptide. HPLC analysis of the peptide was carried out by injecting a small amount of peptide
Biologically Active Peptides Using PS-TRPGGDA...

3. **Synthesis of 17 residue Chicken IL-2 cDNA peptide**

Leu-Gly-Val-Val-Thr-Lys-Lys-Glu-Thr-Glu-Asp-Asp-Thr-Glu-Ile-Lys-Glu

The 2% PS-TRPGGDA dried resin (300 mg, 0.03 mmol) was swelled in dry DCM (30 mL). After 30 min, excess DCM was removed, Boc-Glu (18 mg, 0.06 mmol), MSNT (17.7 mg, 0.06 mmol) and N-methyl imidazole (3.5 μL, 0.04 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min. in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 × 10 mL), EtOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum. The amount of Glu attached to the resin was estimated by picric acid titration method (0.09 mmol/g).

Boc-Glu-PS-TRPGGDA resin (280 mg, 0.025 mmol) was used for the Leu-Gly-Val-Val-Thr-Lys-Lys-Glu-Thr-Glu-Asp-Asp-Thr-Glu-Ile-Lys-Glu peptide synthesis. Boc-protection of the C-terminal amino acid bound resin was removed with 30% TFA in DCM. The resin was washed with DCM (5 × 10 mL), the resulting salt was neutralized with 5% DIEA in DCM. After 10 min. the resin was washed with DCM (5 × 10 mL) and NMP (5 × 10 mL). The remaining amino acids Boc-Leu (20 mg, 0.088 mmol), Boc-Ile (20 mg, 0.088 mmol), Boc-Asp (28 mg, 0.088 mmol), Boc-Val (19 mg, 0.088 mmol), Boc-Thr (27 mg, 0.088 mmol), Boc-Glu (27 mg, 0.088 mmol), Boc Lys (36.5 mg, 0.088 mmol) and Boc-Gly (15.4 mg, 0.088 mmol) were coupled by HOBT active ester method. The HOBT active ester was prepared by dissolving DCC (18 mg, 0.088 mmol), HOBT (12 mg, 0.088 mmol) in minimum amount of NMP and stirred with respective amino acid for 40 min. DCU formed was filtered off and the active ester was added into the resin. Forty min. was given for each coupling reaction. Each coupling step was monitored by semi-quantitative ninhydrin test. After the synthesis the peptidyl resin was washed with...
NMP (5 x 10 mL), DCM (5 x 10 mL), MeOH (5 x 10 mL), ether (5 x 10 mL) and dried under vacuum.

The peptide was cleaved from the support by suspending the peptidyl resin in TFA (2.8 mL), thioanisole (150 µL), ethanedithiol (150 µL), phenol (200 µL) and water (150 µL) for 12 h at room temperature. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 39 mg of crude peptide. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Leu, 1.00 (1); Ile, 1.02 (1); Asp, 2.02 (2); Thr, 2.68 (3); Val, 1.88 (2); Gly, 0.91 (1); Glu, 3.98 (4); Lys, 2.98 (3).

4. Synthesis of Ala-Cys-Ala-Pro-Pro-Ala-Asp-Arg-Ala-Thr-Arg-Ala

4 % PS-TRPGGDA resin (300 mg, 0.057 mmol) was swelled in dry DCM (30 ml). After 30 min, excess DCM was removed, C-terminal Boc-Ala (21.5 mg, 0.114 mmol), MSNT (33.7 mg, 0.114 mmol) and N-methyl imidazole (9 µL, 0.085 mmol) mixture in dry DCM (5 ml) was added. The reaction mixture was kept at room temperature for 30 min. in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 x 10 ml), EtOH (5 x 10 ml), ether (5 x 10 ml) and dried under vacuum. The amount of Ala attached to the resin was estimated by picric acid titration method (0.18 mmol/g).

Boc-Ala-PS-TRPGGDA (267 mg, 0.051 mmol) was used for the Ala-Cys-Ala-Pro-Pro-Ala-Asp-Arg-Ala-Thr-Arg-Ala peptide synthesis. Boc-protection of the C-terminal amino acid bound resin was removed with 30% TFA in DCM. The resin was washed with DCM (5 x 10 ml), the
resulting salt was neutralized with 5% DIEA in DCM. After 10 min. the resin was washed with DCM (5 × 10 ml) and NMP (5 × 10 ml). The remaining amino acids Boc-Arg (86.6 mg, 0.178 mmol), Boc-Thr (49 mg, 0.178 mmol), and Boc-Asp (33 mg, 0.178 mmol), Boc-Pro (24.5 mg, 0.178 mmol), Boc-Ala (21.5 mg, 0.178 mmol) and Boc-Cys (53 mg, 0.178 mmol) were then successively coupled to Boc-deprotected resin. The coupling was carried out using DCC (37 mg, 0.178 mmol), HOBT (24 mg, 0.178 mmol) and the respective amino acid in each coupling step using NMP as a solvent. The DCU formed was filtered off and the active ester was added into the resin. Forty min. was given for each coupling reaction. The coupling steps were monitored by semi quantitative ninhydrin test. After the synthesis the peptidyl resin was washed with NMP (5 × 10 mL), DCM (5 × 10 mL), MeOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum.

The peptide was cleaved from the support by suspending the peptidyl resin in TFA (2.8 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) for 12 h at room temperature. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 49.5 mg of peptide. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 70% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Ala, 5.03 (5); Cys, 0.97 (1); Pro, 1.89 (2); Asp, 0.96 (1); Arg, 1.95 (2); Thr, 0.75 (1). MALDI-TOF-MS: m/z 1200.31 (M+H)
\(^+\), \(\text{C}_{48}\text{H}_{82}\text{O}_{16}\text{N}_{18}\text{S}_1\) requires M\(^+\) 1199.27.
6.3.c Synthesis of peptides using Fmoc-chemistry

1. Synthesis of NR 2B peptide substrates of Ca\(^{2+}\)/Calmodulin binding Peptide

Thr-Asn-Ser-Lys-Ala-Gln-Lys-Lys-Asn-Arg-Arg-Asn-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val

2 % PS-TRPGGDA-HMPB resin (700 mg, 0.07 mmol OH) was used for the synthesis of NR 2B 23-residue peptide substrate of Ca\(^{2+}\)/calmodulin binding peptide. The dried resin was swelled in dry DCM (100 mL). After 1 h, excess DCM was removed. C-terminal amino acid Fmoc-Val (47.5 mg, 0.14 mmol), MSNT (41 mg, 0.14 mmol) and N-methylimidazole (8.3 µl, 0.1 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min and dried under vacuum. The amino capacity of the resin: 0.095 mmol/g.

Fmoc-Val-HMPB-PS-TRPGGDA resin (680 mg, 0.065 mmol) was taken in a manual peptide synthesiser and swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF (20 mL x 30 min), wash the resin with DMF (5 x 15 mL). The remaining amino acids of the target peptide sequence, Phe (88 mg, 0.227 mmol), Thr (90 mg, 0.227 mmol), Asp (93 mg, 0.227 mmol), Tyr (104 mg, 0.227 mmol), Ser (87 mg, 0.227 mmol), His (140 mg, 0.227 mmol), Gln (139 mg, 0.227 mmol), Arg (138 mg, 0.227 mmol), Leu (80 mg, 0.227 mmol), Lys (106 mg, 0.227 mmol), Asn (136 mg, 0.227 mmol), Ala (71 mg, 0.227 mmol) were successively incorporated by using the respective amino acid (0.227 mmol), with HBTU (86 mg, 0.227 mmol), HOBt (31 mg, 0.227 mmol) and DIEA (39 µl, 0.227 mmol). All acylation reactions were performed twice for confirming the quantitative conversion. All coupling and deprotection steps were monitored by ninhydrin test. The resin was washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum.

The synthesis was stopped at the 11th residue from the C-terminal and 1/4 of the peptidyl resin was removed. Fmoc-protection of the target peptidyl resin
was removed and the resin was washed with washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum. The NR2B 11 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 22 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-10 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Amino acid analysis: Leu, 1.02 (1); Arg, 1.97 (2); Glu, 0.98 (1); Tyr, 0.82 (1); Ser, 0.68 (1); Asp, 0.95 (1); Thr, 0.71 (1); Phe, 1.02 (1); Val, 1.00 (1); His, 0.91 (1). MALDI-TOF-MS: m/z 1422.60 (M+H)^+, C_{63}H_{96}O_{18}N_{20} requires M^+ 1421.53

Stepwise synthesis of NR2B peptide substrate was continued with the remaining resin by incorporation of the successive amino acids using HBTU/HOBt procedure in presence of DIEA. The synthesis was stopped at the 17th residue from the C-terminal and ¼ of the peptidyl resin was removed. Fmoc-protection of the target peptidyl resin was removed and the resin was washed with washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum. The NR2B 17 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 36 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-10 column. The
peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Leu, 1.01 (1); Arg, 2.98 (3); Glu, 0.97 (1); Tyr, 0.81 (1); Ser, 0.67 (1); Asp, 3.01 (3); Thr, 0.73 (1); Phe, 1.01 (1), Val, 1.01 (1); His, 0.90 (1); Lys, 3.03 (3). MALDI-TOF-MS: m/z 2191.43 (M+H)^+, C_{98}H_{156}O_{28}N_{34} requires M^+ 2190.42

Stepwise synthesis of NR2B peptide substrate was continued with the remaining resin by incorporation of the successive amino acids using HBTU/HOBt procedure in presence of DIEA. The synthesis was stopped at the 20th residue from the C-terminal and % of the peptidyl resin was removed. Fmoc-protection of the target peptidyl resin was removed and the resin was washed with washed with DMF (5 × 15 mL), methanol (5 × 15 mL), ether (5 × 15 mL) and dried in vacuum. The NR2B 20 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 43 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Leu, 1.02 (1); Arg, 2.96 (3); Glu, 1.98 (2); Tyr, 0.82 (1); Ser, 0.68 (1); Asp, 3.02 (3); Thr, 0.75 (1); Phe, 1.00 (1), Val, 1.02 (1); His, 0.90 (1); Lys, 4.01 (4); Ala, 1.02 (1). MALDI-TOF-MS: m/z 2518.90 (M+H)^+, C_{109}H_{181}O_{30}N_{39} requires M^+ 2517.79
The stepwise synthesis was carried out until the 23 residue target peptide was achieved by incorporating the respective amino acid using HBTU, HOBt as coupling reagents in presence of DIEA. Fmoc-protection of the target peptidyl resin was removed and the resin was washed with washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum. The NR2B 23 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 µL), ethanedithiol (150 µL), phenol (200 µL) and water (150 µL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 68.6 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Leu, 1.01 (1); Arg, 2.97 (3); Glu, 1.97 (2); Tyr, 0.82 (1); Ser, 1.59 (2); Asp, 3.98 (4); Thr, 1.63 (2); Phe, 1.05 (1), Val, 1.00 (1); His, 0.91 (1); Lys, 4.11 (4); Ala, 1.01 (1). MALDI-TOF-MS: m/z 2821.17 (M+H)+, C120H199O36N43 requires M+: 2820.06.

2. Synthesis of NR 2A peptide substrates of Ca²⁺/Calmodulin binding peptide


2% PS-TRPGGDA-HMPB resin (600 mg, 0.06 mmol) was used for the synthesis of NR 2B 24-residue peptide substrate of Ca²⁺/calmodulin binding peptide. The dried resin was swelled in dry DCM (50 ml). After 1 h, excess DCM was removed, C-terminal Fmoc-Asp (49 mg, 0.12 mmol), MSNT (35 mg, 0.12 mmol) and N-methyl imidazole (7 µl, 0.09 mmol) mixture in dry
DCM (5 ml) was added. The reaction mixture was kept at room temperature for 30 min in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 x 15 mL), EtOH (5 x 15 mL), ether (5 x 15 mL) and dried under vacuum. The amino capacity of the resin: 0.092 mmol/g.

Fmoc-Asp-HMPB-PS-TRPGGDA resin (565 mg, 0.052 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 x 15 mL). The remaining amino acids, Ile (64 mg, 0.182 mmol), Phe (70 mg, 0.182 mmol), Thr (72 mg, 0.182 mmol), Asp (75 mg, 0.182 mmol), Tyr (84 mg, 0.182 mmol), Ser (70 mg, 0.182 mmol), His (113 mg, 0.182 mmol), Gin (111 mg, 0.182 mmol), Arg (110 mg, 0.182 mmol), Leu (64 mg, 0.182 mmol), Lys (85 mg, 0.182 mmol), Asn (109 mg, 0.182 mmol), Ala (57 mg, 0.182 mmol) were successively incorporated by using the respective amino acid (0.182 mmol), with HBTU (69 mg, 0.182 mmol), HOBut (25 mg, 0.182 mmol) and DIEA (31 μL, 0.182 mmol). All acylation reactions were performed twice for confirming the quantitative conversion. All coupling and deprotection steps were monitored by ninhydrin test. The resin was washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum.

The synthesis was stopped at the 12th residue from the C-terminal and ¼ of the peptidyl resin was removed. Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum. The NR2A 12 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 22 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-10 column. The peptide fraction was collected and lyophilized. HPLC analysis of
HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Leu, 1.00 (1); Ile, 2.02 (2); Arg, 0.96 (1); Asp, 3.82 (4); Tyr, 0.83 (1); Ser, 0.78 (1); His, 0.91 (1); Glu, 1.08 (1). MALDI-TOF-MS: m/z 1488.63 (M+H)^+, C_{63}H_{98}O_{22}N_{20} requires M^+ 1487.55.

The stepwise synthesis was carried out until the 24 residue target peptide was achieved by incorporating the respective amino acid using HBTU, HOBr as coupling reagents in presence of DIEA. Fmoc-protection of the target peptidyl resin was removed and the resin was washed with washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum. The NR2A 24 residue peptide was cleaved from the resin by suspending the peptidyl resin in TFA (3 mL), thioanisole (150 µL), ethanedithiol (150 µL), phenol (200 µL) and water (150 µL) at room temperature for 4 h. The solution was filtered and filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the precipitate was washed with ether until the scavengers are removed and dried in vacuum to yield 120 mg of crude peptide. The peptide was dissolved in 1% acetic acid/water and passed through a sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Ser, 0.73(1); Glu, 3.02(3); Ala, 1.02(1); Leu, 3.01(3); Phe, 1(1); Lys, 1.95 (2); Arg, 1.98(2); Ile, 2(2); His, 0.94(1); Tyr, 0.84(1); Asp, 6.96(7). MALDI-TOF-MS: m/z 2944.24 (M+H)^+, C_{127}H_{204}O_{39}N_{42} requires M^+ 2943.19.
3. **Synthesis of NR 2B mutated peptide substrate of Ca\(^{2+}\)/Calmodulin binding peptide using semi-automatic peptide synthesiser**

Thr-Asn-Ser-Lys-Ala-Gln-Ala-Ala-Asn-Arg-Asn-Lys-Leu-Arg-Arg-Gln-His-Ser-Tyr-Asp-Thr-Phe-Val

2% PS-TRPGGDA-HMPB resin (700 mg, 0.07 mmol) was used for the synthesis of NR 2B 23-residue mutated peptide substrate of Ca\(^{2+}\)/Calmodulin binding peptide. The dried resin was swelled in dry DCM (50 mL). After 1 h, excess DCM was removed and C-terminal Fmoc-Val (47.5 mg, 0.14 mmol), MSNT (41 mg, 0.14 mmol) and N-methyl imidazole (8.3 μL, 0.1 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 x 15 mL), EtOH (5 x 15 mL), ether (5 x 15 mL) and dried under vacuum. The amino capacity of the resin: 0.095 mmol/g.

Fmoc-Val-HMPB-PS-TRPGGDA resin (680 mg, 0.065 mmol) was taken in a glass column of a semi-automatic Novasyn peptide synthesiser. Initially the resin was swelled by flowing in DMF for 30 min. through the column. Fmoc-group protection was removed by flowing 20% piperidine in DMF through the column for 10 min. at a rate of 3 mL/min. The deprotected resin was washed with DMF for 5 min. The remaining amino acids, Phe (88 mg, 0.227 mmol), Thr (90 mg, 0.227 mmol), Asp (93 mg, 0.227 mmol), Tyr (104 mg, 0.227 mmol), Ser (87 mg, 0.227 mmol), His (140 mg, 0.227 mmol), Gln (139 mg, 0.227 mmol), Arg (138 mg, 0.227 mmol), Leu (80 mg, 0.227 mmol), Lys (106 mg, 0.227 mmol), Asn (136 mg, 0.227 mmol), Ala (71 mg, 0.227 mmol) were successively incorporated by adding the respective amino acid (0.227 mmol), with HBTU (86 mg, 0.227 mmol), HOEt (31 mg, 0.227 mmol) and DIEA (39 μL, 0.227 mmol) in to the column and recirculated in the synthesiser for 40 min. Deprotection and acylation was continuously monitored by measuring the OD at 309 nm using a spectrophotometer attached to the synthesiser. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed by flowing DMF through the column for 5 min. at
the flow rate of 3ml/min. The resin was taken out of the column and then washed with methanol (5 × 15 mL), ether (5 × 15 mL) and dried in vacuum.

The synthesised peptide was removed from the polymer support by suspending the peptidyl resin in TFA, thioanisole, ethanedithiol, phenol and water for 4 h. at room temperature. The solution was filtered and the filtrate was concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the peptide was thoroughly washed with ice-cold ether to yield 164 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5%TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA. Amino acid analysis: Leu, 1.05 (1); Arg, 2.94 (3); Glu, 1.98 (2); Tyr, 0.82 (1); Ser, 1.63 (2); Asp, 4.01 (4); Thr, 1.65 (2); Phe, 1.02 (1), Val, 1.01 (1); His, 0.91 (1); Lys, 2.07 (2); Ala, 3.10 (3). MALDI-TOF-MS: m/z 2706.92 (M+H)+, C_{114}H_{185}O_{26}N_{41} requires M+ 2705.86.

4. **Synthesis of NS2 peptide fragment of Hepatitis C viral protein**

His-Val-Trp-Ile-Pro-Pro-Leu-Asn-Ala-Arg-Gly-Gly-Arg-Asp-Ala-Ile-Leu-Leu-Met-Cys-Ala-Val

2 % PS-TRPGGDA-HMPA resin (500 mg, 0.05 mmol) was used for the synthesis of the 23-residue NS2 peptide chosen from the nonstructural region (857-880) of hepatitis C viral protein. The dried resin was swelled in dry DCM (50 mL). After 1 h, excess DCM was removed; C-terminal Fmoc-Val (34 mg, 0.1 mmol), MSNT (30 mg, 0.1 mmol) and N-methyl imidazole (5.8 μL, 0.075 mmol) mixture in dry DCM (5 ml) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with DCM (5 × 15 mL), EtOH (5 × 15 mL), ether (5 × 15 mL) and dried under vacuum. The amino capacity of the resin: 0.094 mmol/g.
Fmoc-Val-HMPAPS-TRPGGDA resin (470 mg, 0.044 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 × 15 mL). The remaining Fmoc-amino acids, Cys (90 mg, 0.154 mmol), Met (57 mg, 0.154 mmol), Gly (46 mg, 0.154 mmol), Pro (52 mg, 0.154 mmol), Trp (66 mg, 0.154 mmol), His (95 mg, 0.154 mmol), Asp (67 mg, 0.154 mmol), Arg (94 mg, 0.154 mmol), Leu (54 mg, 0.154 mmol), Asn (92 mg, 0.154 mmol), Ile (54 mg, 0.154 mmol), Ala (48 mg, 0.154 mmol) were successively incorporated by using the respective Fmoc-amino acid (0.154 mmol), with HOBt (21 mg, 0.154 mmol), HBTU (58 mg, 0.154 mmol) and DIEA (26 μL, 0.154 mmol). All acylation reactions were performed twice for ensuring quantitative conversion. All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 × 15 mL), methanol (5 × 15 mL), ether (5 × 15 mL) and dried in vacuum.

The finished peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4h the suspension was filtered, and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the peptide was thoroughly washed with ice-cold ether to yield 107 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: His, 0.96(1); Val, 2(2); Ile, 3.02(3); Pro, 1.78(2); Leu, 3.02(3); Asp, 1.95(2); Ala, 3.03(3); Arg, 3.05(3); Gly, 1.97(2); Met,
1.03(1); Cys, 0.91(1). One Asn was hydrolyzed to Asp and Trp destroyed during hydrolysis. MALDI-TOF-MS: \( m/z \) 2673.23 \((M+H)^+\), \( \text{C}_{126}\text{H}_{199}\text{O}_{28}\text{N}_{37}\text{S}_{2} \) requires \( M^+ \) 2672.17.

5. **Synthesis of NS3 peptide fragment of Hepatitis C viral protein**

Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Ser-Ala-Leu

2% PS-TRPGGDA-HMPA resin (500 mg, 0.05 mmol) was used for the synthesis of 22 residue NS3 peptide chosen from the nonstructural region (1388-1410) of hepatitis C viral protein. The dried resin was swelled in dry DCM (50 mL). After 1 h, excess DCM was removed, C-terminal Fmoc-Leu (35 mg, 0.1 mmol), MSNT (30 mg, 0.1 mmol) and N-methyl imidazole (6 \( \mu \)L, 0.075 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with dry DCM (5 \( \times \) 15 mL), EtOH (5 \( \times \) 15 mL), ether (5 \( \times \) 15 mL) and dried under vacuum. The amino capacity of the resin: 0.09 mmol/g.

Fmoc-Leu-HMPA-PS-TRPGGDA resin (450 mg, 0.045 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 \( \times \) 10 mL). The remaining amino acids, Ala (49 mg, 0.158 mmol), Ser (60 mg, 0.158 mmol), Lys (74 mg, 0.158 mmol) Glu (67 mg, 0.158 mmol), Cys (93 mg, 0.158 mmol), Phe (61 mg, 0.158 mmol), His (98 mg, 0.158 mmol), Asp (65 mg, 0.158 mmol), Arg (96 mg, 0.158 mmol), Leu (56 mg, 0.158 mmol), Ile (56 mg, 0.158 mmol) were successively incorporated by using the respective amino acid (0.158 mmol), with HBTU (60 mg, 0.158 mmol), HOBt (21.3 mg, 0.158 mmol) and DIEA (27 \( \mu \)L, 0.158 mmol). All acylation reactions were performed twice for confirming the quantitative conversion. The coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl
resin was removed and the resin was washed with DMF (5 x 15 mL), methanol (5 x 15 mL), ether (5 x 15 mL) and dried in vacuum.

The peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4h the suspension was filtered, and the filtrate were concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the peptide was thoroughly washed with ice-cold ether to yield 107 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: His, 1.89(2); Ile, 1.03(1); Ser, 1.68(2); Leu, 3.92(4); Asp, 0.92(1); Ala, 3.08(3); Arg, 1.05(1); Glu, 0.95(1); Phe, 1.03(1); Cys, 1.95(2); Lys, 4.07(4). MALDI-TOF-MS: m/z 2513.00 (M+H)+, C_{111}H_{187}O_{29}N_{33}S_{2} requires M^+ 2511.95.
6. Synthesis of NS4 Peptide fragment of Hepatitis C viral protein

Arg-Glu-Val-Leu-Tyr-Gln-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-
Gln-His-Leu-Pro-Tyr-Ile-Glu

2 % PS-TRPGGDA-HMPA resin (500 mg, 0.05 mmol) was used for the synthesis of 22 residue NS4 peptide chosen from the nonstructural region (1697-1719) of hepatitis C viral protein. The dried resin was swelled in dry DCM (100 ml). After 1 h, excess DCM was removed, C-terminal Fmoc-Glu (42.5 mg, 0.1 mmol), MSNT (29.6 mg, 0.1 mmol) and N-methyl imidazole (6 μL, 0.075 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with dry DCM (5 × 15 mL), EtOH (5 × 15 mL), ether (5 × 15 mL) and dried under vacuum. The amino capacity of the resin: 0.092 mmol/g.

Fmoc-Glu-HMPA-PS-TRPGGDA resin was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, washed the resin with DMF (5 × 15 mL). The remaining amino acids, Glu (63 mg, 0.147 mmol), Ile (52 mg, 0.147 mmol), Tyr (68 mg, 0.147 mmol), Pro (49.5 mg, 0.147 mmol), Leu (52 mg, 0.147 mmol), His (91 mg, 0.147 mmol), Gln (90 mg, 0.147 mmol), Ser (56 mg, 0.147 mmol), Cys (86 mg, 0.147 mmol), Asp (60 mg, 0.147 mmol), Val (50 mg, 0.147 mmol), Phe (57 mg, 0.147 mmol), Arg (89 mg, 0.147 mmol), Ile (52 mg, 0.147 mmol) were successively incorporated by using the respective amino acid (0.147 mmol), with HBTU (56 mg, 0.147 mmol), HOBt (20 mg, 0.147 mmol) and DIEA (25 μL, 0.147 mmol) All acylation reactions were performed twice for confirming the quantitative conversion. All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 × 15 mL), methanol (5 × 15 mL), ether (5 × 15 mL) and dried in vacuum.
The peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4h the suspension was filtered, and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the peptide was thoroughly washed with ice-cold ether to yield 105 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5%TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Val, 1.00(1); His, 0.89(1); Ile, 1.01(1); Tyr, 1.82(2); Pro, 0.77(1); Ser, 0.58(1); Leu, 2.02(2); Asp, 0.92(1); Arg, 1.03(1); Glu, 7.88(8); Phe, 1.04(1); Cys, 1.01(1); Met, 0.88(1). MALDI-TOF-MS: m/z 2789.05 (M+H)+, C122H179O42N29S2 requires M+ 2787.97.

7. **Synthesis of NS5 peptide fragment of Hepatitis C viral protein**

Asp-Glu-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Val- Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg

2 % PS-TRPGGDA-HMPA resin (400 mg, 0.04 mmol) was used for the synthesis of 23 residues NS5 peptide chosen from the nonstructural region (2253-2276) of hepatitis C viral protein. The dried resin was swelled in dry DCM (100 ml). After 1 h, excess DCM was removed; C-terminal Fmoc-Arg (49 mg, 0.08 mmol), MSNT (24 mg, 0.08 mmol) and N-methyl imidazole (56 μL, 0.06 mmol) mixture in dry DCM (5 mL) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with dry DCM (5 × 10 mL), EtOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum. The amino capacity of the resin: 0.088 mmol/g.
Bicologically Active Peptides Using PS-TRPGGDA...

Fmoc-Arg-HMPA-FS-TRPGGDA resin (363 mg, 0.032 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 x 10 mL). The remaining Fmoc-amino acids, Arg (68 mg, 0.112 mmol), Leu (40 mg, 0.112 mmol), Ile (40 mg, 0.112 mmol), Glu (48 mg, 0.112 mmol), Ala (35 mg, 0.112 mmol), Pro (39 mg, 0.112 mmol), Val (38 mg, 0.112 mmol), Ser (43 mg, 0.112 mmol), Asp (46 mg, 0.112 mmol), were successively incorporated by using the respective Fmoc-amino acid (0.112 mmol), with HBTU (42 mg, 0.112 mmol), HOBt (15 mg, 0.112 mmol) and DIEA (19 µL, 0.112 mmol). All acylation reactions were performed twice for confirming the quantitative conversion. All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 x 10 mL), methanol (5 x 10 mL), ether (5 x 10 mL) and dried in vacuum.

The peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 µL), ethanediol (150 µL), phenol (200 µL) and water (150 µL) at room temperature. After 4 h the suspension was filtered, and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether and the peptide was thoroughly washed with ice-cold ether to yield 81 mg of peptide.

The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Pro, 1.88(2); Ile, 1.02(1); Ser, 0.66(1); Leu, 2.03(2); Asp, 2.92(3); Ala, 2.08(2); Arg, 1.91(2); Glu, 5.95(6); Phe, 1.03(1); Val, 3.07(3). MALDI-TOF-MS: m/z 2658.90 (M+H)+, C_{115}H_{181}O_{45}N_{29} requires M+ 2657.75.
8. **Synthesis of HIV-1 Rev. NES Peptide**

Ala-Cys-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Arg-Ala

4 % PS-TRPGGDA-HMPA resin (300 mg, 0.054 mmol) was used for the synthesis of HIV-1 Rev. NES peptide. The dried resin was swelled in dry DCM (30 mL). After 1 h, excess DCM was removed and C-terminal Fmoc-Ala (34 mg, 0.108 mmol), MSNT (32 mg, 0.108 mmol) and N-methyl imidazole (6.4 μL, 0.081 mmol) mixture in dry DCM (3 mL) was added. The reaction mixture was kept at room temperature for 30 min in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 × 10 mL), EtOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum. The amino capacity of the resin: 0.175 mmol/g.

Fmoc-Ala-HMPA-PS-TRPGGDA resin (257 mg, 0.045 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 × 10 mL). The remaining Fmoc-amino acids, Ala (49 mg, 0.157 mmol), Cys (92 mg, 0.157 mmol), Leu (55 mg, 0.157 mmol), Pro (53 mg, 0.157 mmol), Glu (67 mg, 0.157 mmol), Arg (96 mg, 0.157 mmol), Thr (62 mg, 0.157 mmol) were successively incorporated by using the respective Fmoc-amino acid (0.157 mmol), with HBTU (59 mg, 0.157 mmol), HOBt (21.2 mg, 0.157 mmol) and DIEA (27 μL, 0.157 mmol). All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 × 10 mL), methanol (5 × 10 mL) and ether (5 × 10 mL) and dried in vacuum.

The peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4 h the suspension was filtered and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether. The peptide was thoroughly washed with ice-cold ether to yield 57 mg of peptide. The
peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilised. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Ala, 2.04 (2); Cys, 0.95 (1); Leu, 2.91 (3); Pro, 1.76 (2); Glu, 0.94 (1); Arg, 1.89(2); Thr 0.63(1). MALDI-TOF-MS: m/z 1340.60 (M+H)+, C_{58}H_{102}O_{16}N_{18}S_{1} requires M+ 1339.57.

9. Synthesis of M10 mutant NES peptide

Ala-Cys-Leu-Pro-Pro-Leu-Asp-Arg-Leu-Thr-Arg-Ala

4 % PS-TRPPGDA-HMPA resin (300 mg, 0.054 mmol) was used for the synthesis of M10 mutant NES peptide. The dried resin was swelled in dry DCM (100 ml). After 1 h, excess DCM was removed; C-terminal Fmoc-Ala (34 mg, 0.108 mmol), MSNT (32 mg, 0.108 mmol) and N-methyl imidazole (6.4 µL, 0.081 mmol) mixture in dry DCM (3 ml) was added. The reaction mixture was kept at room temperature for 30 min in a septum-stoppered flask under nitrogen atmosphere. The resin was washed with dry DCM (5 x 10 mL), EtOH (5 x 10 mL), ether (5 x 10 mL) and dried under vacuum. The amino capacity of the resin : 0.176 mmol/g.

Fmoc-Ala-HMPA-PS-TRPPGDA resin (270 mg, 0.048 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 x 10 ml). The remaining Fmoc-amino acids, Cys (98 mg, 0.168 mmol), Leu (59 mg, 0.168 mmol), Pro (57 mg, 0.168 mmol), Asp (69 mg, 0.168 mmol), Arg (102 mg, 0.168 mmol), Thr (67 mg, 0.168 mmol) were successively incorporated by using the respective amino acid (0.168 mmol), with HBTU (64 mg, 0.168 mmol), HOBt (23 mg, 0.168 mmol) and DIEA (29 µL, 0.168
mmol). All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 × 10 mL), methanol (5 × 10 mL), ether (5 × 10 mL) and dried in vacuum.

The peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4h the suspension was and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether. The peptide was washed thoroughly washed with ice-cold ether to yield 60 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilised. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5%TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Ala, 2.03 (2); Cys, 0.96 (1); Leu, 3.03 (3); Pro, 1.79 (2); Asp, 1.03 (1); Arg, 1.92 (2); Thr 0.65(1). MALDI-TOF-MS: m/z 1326.56 (M+H)^+, C_{57}H_{100}O_{18}N_{18}S_{1} requires M^+1325.54.
10. **Synthesis of Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr**

2 % PS-TRPGDA-HMPB resin (500 mg, 0.05 mmol) was used for the synthesis of peptide Tyr-Ser-Arg-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr. The dried resin was swelled in dry DCM (50 mL). After 1 h, excess DCM was removed, C-terminal Fmoc-Tyr (46 mg, 0.1 mmol), MSNT (30 mg, 0.1 mmol) and N-methyl imidazole (5.8 μL, 0.075 mmol) mixture in dry DCM (5 ml) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with dry DCM (5 × 10 mL), EtOH (5 × 10 mL), ether (5 × 10 mL) and dried under vacuum. The amino capacity of the resin: 0.092 mmol.

Fmoc-Tyr-HMPB-PS-TRPGDA resin (480 mg, 0.044 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 × 10 mL). The remaining Fmoc-amino acids, Tyr (70 mg, 0.154 mmol), Ser (59 mg, 0.154 mmol), Lys (72 mg, 0.154 mmol), Trp (66 mg, 0.154 mmol), Arg (94 mg, 0.154 mmol) were successively incorporated by using the respective Fmoc-amino acid (0.154 mmol), with HBTU (58 mg, 0.154 mmol), HOBt (21 mg, 0.154 mmol) and DIEA (27 μL, 0.154 mmol). All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, about ½ of the resin bound peptide was withdrawn from the reaction mixture and kept aside for cleavage. Fmoc-protection peptidyl resin was removed and the resin was washed with DMF (5 × 10 mL), methanol (5 × 10 mL) and ether (5 × 10 mL) and dried in vacuum.

The decapeptide peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4 h the suspension was filtered and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether. The peptide was washed thoroughly with cold ether (5 × 10 mL) to remove all the scavengers. The yield of the crude peptide is 26.8 mg. The peptide was...
dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilised HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Tyr, 2.87(3); Ser, 2.56(3); Arg, 1.98(2); Lys, 1.02(1). MALDI-TOF-MS: m/z 1396.51 (M+H)+, C_{65}H_{90}O_{17}N_{18} requires M+ 1395.48.

Propionylation of the peptide was then carried out with the remaining Nα protected peptidyl resin. Fmoc-deprotection was carried out using 20% piperidine in DMF. The N-terminal deprotected peptidyl resin was then propionylated by 3-(p-hydroxyphenyl)-propionic acid (19 mg, 0.115 mmol) using HBTU (44 mg, 0.115 mmol), HOBr (16 mg, 0.115 mmol) and DIEA (20 μL, 0.115 mmol) as activation reagents. Propionylation reactions were performed twice for confirming the quantitative conversion. The coupling step was monitored by ninhydrin test. The resin was washed with DMF (5 × 10 mL), methanol (5 × 10 mL) and ether (5 × 10 mL) and dried in vacuum. The propionylated peptide was cleaved from the support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 μL), ethanedithiol (150 μL), phenol (200 μL) and water (150 μL) at room temperature. After 4h the suspension was filtered and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether. The peptide was washed thoroughly with ice-cold ether to yield 37 mg of peptide. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.
11. Synthesis of Asn-Pro-Val-Tyr

8% PS-TRPGGDA-HMPB resin (100 mg, 0.04 mmol) was used for the synthesis of peptide Asn-Pro-Val-Tyr. The dried resin was swelled in dry DCM (20 ml). After 1 h, excess DCM was removed, C-terminal Fmoc-Tyr (37 mg, 0.08 mmol), MSNT (23.7 mg, 0.08 mmol) and N-methyl imidazole (4.9 µL, 0.06 mmol) mixture in dry DCM (2 mL) was added. The reaction mixture was kept at room temperature for 30 min. The resin was washed with dry DCM (5 × 6 mL), EtOH (5 × 6 mL), ether (5 × 6 mL) and dried under vacuum. The amino capacity of the resin : 0.37 mmol/g.

Fmoc-Tyr-HMPB-PS-TRPGGDA resin (90 mg, 0.033 mmol) was taken in a manual peptide synthesiser and the resin was swelled in DMF for 1 h. Fmoc group protection was removed by using 20% piperidine in DMF, wash the resin with DMF (5 × 6 mL). The remaining Fmoc-amino acids, Asn (69 mg, 0.115 mmol), Val (39 mg, 0.115 mmol), Pro (39 mg, 0.115 mmol), were successively incorporated by using the respective Fmoc-amino acid (0.115 mmol), with HBTU (44 mg, 0.115 mmol), HOBt (16 mg, 0.115 mmol), and DIEA (20 µL, 0.115 mmol). All coupling steps were monitored by ninhydrin test. After the incorporation of all amino acids, Fmoc-protection of the target peptidyl resin was removed and the resin was washed with DMF (5 × 6 mL), methanol (5 × 6 mL), ether (5 × 6 mL) and dried in vacuum.

The finished peptide was removed from the polymer support by suspending the peptidyl resin in a mixture of TFA (3 mL), thioanisole (150 µL), ethanediethiol (150 µL), phenol (200 µL) and water (150 µL) at room temperature. After 4h the suspension was filtered and the filtrate concentrated under reduced pressure. The peptide was precipitated by adding ice-cold ether. The peptide was washed thoroughly with cold ether (5 × 10 ml) to remove all the scavengers and dried. The yield of the peptide was 15 mg. The peptide was dissolved in 1% acetic acid water mixture and passed through sephadex G-25 column. The peptide fraction was collected and lyophilized. HPLC analysis of the peptide was carried out by injecting a small amount of peptide
peptide dissolved in water to C-18 RPC and eluting using a gradient of solvent A: nanopure water containing 0.5% TFA and solvent B 80% acetonitrile in nanopure water containing 0.5% TFA.

Amino acid analysis: Tyr, 0.88(1); Asp, 0.98(1); Pro, 0.91(1); Val, 1.02(1).
References


