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A NOVEL AND SCALABLE SYNTHETIC PROCESS FOR THE PREPARATION OF PARALLEL AND ANTIPARALLEL VASOPRESSIN IMPURITIES

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ABSTRACT

Sulfur-containing peptide drugs such as Vasopressin, Oxytocin, Calcitonin, Lanreotide and Octreotide are susceptible to the formation of parallel and antiparallel disulfide-linked dimers during synthesis, which poses significant challenges for impurity identification, process control and regulatory evaluation. In the present study, vasopressin was employed as a model peptide to develop a novel, safe and scalable synthetic process for the selective preparation of these impurities. The strategy is based on an orthogonal Trt/Acm cysteine protection scheme combined with 2, 2′-dithiodipyridine-mediated thiol activation, enabling controlled and sequential disulfide bond formation under mild reaction conditions. The process avoids hazardous reagents and allows reproducible gram-scale synthesis of the target dimers with high purity and yield. This method provides a practical and industrially relevant approach for the preparation and characterization of disulfide-linked vasopressin impurities, supporting pharmaceutical analysis, impurity profiling and API quality control requirements.

KEYWORDS

Vasopressin dimers, Peptide cyclization, Solid-Phase Peptide Synthesis (SPPS), Parallel, Antiparallel peptide assembly, Disulfide bond formation and 2, 2′-dithiodipyridine.

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INTRODUCTION

Sulfur-containing peptides have gained significant attention in therapeutic development owing to the distinctive chemical properties of sulfur atoms, including their presence in thiols, thioethers, thioamides, and disulfide linkages[1-3]. These functional motifs play a decisive role in imparting

structural stability, conformational rigidity, and biological specificity to peptide molecules. In numerous natural products and approved peptide drugs, disulfide bridges are particularly important, as they stabilize three-dimensional architectures essential for biological activity[4]. Recent progress in peptide synthesis encompassing site-selective protecting group strategies[5,6] late-stage functionalization[7,8] and post-translational modification techniques[9-11], has enabled the design of sulfur-rich peptides with enhanced pharmacokinetic and stability profiles. Comprehensive reviews have highlighted how disulfide, thioether, and thioamide motifs influence peptide function, while emerging sulfur-centered chemistries further expand the toolbox for peptide design and optimization[1,9,12,13]. As the generic development of sulfur-containing peptide drugs increases, the identification and control of sulfur-mediated impurities, including disulfide-linked dimers, have become increasingly critical.

Impurity profiling is a fundamental element of modern active pharmaceutical ingredient (API) development, since the safety, efficacy, and quality of drug products are directly influenced by the presence of undesired chemical entities. Regulatory authorities, including the International Council for Harmonization (ICH), mandate the identification, characterization, and control of impurities above defined thresholds, recognizing their potential to elicit toxicological, pharmacological, or stability concerns[14-16]. Peptide-based APIs are particularly prone to impurity formation due to their complex synthetic routes and labile functional groups, giving rise to sequence-related variants, oxidative by-products, racemization products, and intermolecular disulfide-linked dimers[17-20]. The European Pharmacopoeial monographs and regulatory guidelines classify such impurities as critical quality attributes that demand detailed analytical and toxicological evaluation[16, 21]. With the growing clinical use of peptide APIs, such as vasopressin and oxytocin analogues, there is a clear need for robust impurity-profiling strategies that combine advanced analytical techniques (LC–MS/MS, NMR, orthogonal chromatography) with a risk-based regulatory framework[22-24]. In this context, systematic impurity assessment supports regulatory compliance, process optimization and long-term drug safety.

Disulfide bonds, formed by the oxidation of two cysteine thiol groups, are among the most critical post-translational modifications in peptides and proteins. These covalent cross-links reduce conformational flexibility, particularly in small to medium-sized peptides, where disulfide linkages can dramatically increase thermodynamic stability, protease resistance and plasma half-life, making such peptides more suitable as therapeutic agents[25,26]. For example, clinically relevant peptides, including vasopressin, oxytocin, and octreotide, rely on intramolecular disulfide bridges to maintain their cyclic conformations and pharmacological activity. In addition to structural stabilization, disulfide bonding can influence receptor binding, selectivity, and biological response. Improper or intermolecular disulfide formation, however, may lead to dimerization or oligomerization, thereby generating parallel and antiparallel peptide dimers with altered physicochemical and biological properties compared to their monomeric counterparts[27-29]. Such species are frequently observed as process-related impurities, thus, controlling disulfide bond formation during manufacturing is not only a synthetic challenge but also a crucial factor for peptide drug design, stability and safety.

The growing global demand for peptide-based therapeutics has accelerated the pharmaceutical industry toward the development of generic peptide drugs[30,31]. Regulatory agencies require that generic products demonstrate impurity profiles and safety data comparable to those of the reference listed drug (RLD)[14,15]. Even minor deviations in impurity composition may necessitate independent toxicological evaluation of process-related and degradation impurities.

Among sulfur-containing peptides, parallel and antiparallel disulfide-linked dimers are commonly observed impurities, particularly in peptides such as vasopressin, oxytocin, octreotide, calcitonin, somatostatin, and lanreotide. Pharmacopoeial

monographs, including that of oxytocin, explicitly report such dimeric species arising from intermolecular disulfide formation rather than the intended intramolecular cyclization. However, reproducible and scalable synthetic methods for the deliberate preparation of these dimeric impurities remain largely underreported, limiting their availability for toxicological assessment and regulatory studies. Consequently, the development of practical and controlled synthetic routes for such disulfide-linked peptide impurities is of considerable importance to support regulatory compliance and quality assurance in peptide API manufacturing.

MATERIAL AND METHODS

Reagent and chemicals

All chemicals and solvents used for the synthesis of vasopressin dimers were obtained from commercial sources and used directly without further purification. NMR spectra were recorded on a JEOL 500 MHz spectrometer, using deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent, with tetramethylsilane (TMS) serving as the internal standard, chemical shifts (δ) are reported in parts per million (ppm) relative to TMS and high-resolution mass spectrometry was performed using an Agilent 6545 XT LC-QT of system.

Experimental Section

General procedure for synthesis of Vasopressin Parallel dimer

Step-1: Synthesis of resin bound parallel linear Vasopressin monomer (1)

Cys(Trt)-Tyr(tBu)-Phe-Gln(Trt)-Asn(Trt)-Cys(Acm)-Pro-Arg(Pbf)-Gly-Rinkamide Resin

Rink amide resin (50.0g, substitution degree 0.6mmol/g) was accurately weighed and charged into a solid-phase peptide synthesizer. The resin was washed twice with dimethylformamide (DMF) and allowed to swell in DMF for 30 min. Fmoc deprotection was carried out by treating the swollen resin with a premixed solution of 20% piperidine in DMF and agitating for 30 min. The resin was subsequently washed three times each with DMF and dichloromethane (DCM).

For initial amino acid loading, a premixed solution containing Fmoc-Gly-OH (22.29g, 75mmol), 1-hydroxybenzotriazole (HOBt, 10.13g) and N, N′-diisopropylcarbodiimide (DIC, 11.83mL) in DMF was added to the synthesizer and agitated for 2 h at room temperature. After completion, the resin was washed three times each with DMF and DCM, followed by capping of unreacted amine sites using a mixture of acetic anhydride/pyridine/DMF (15mL:15mL:470mL) for 30 min. The resin was then drained, washed, and shrunk using methanol. After removal of methanol under reduced pressure, Fmoc-Gly-Rink amide resin was obtained with a measured substitution degree of 0.5mmol/g.

The Fmoc-Gly-Rink amide resin (0.5mmol/g) was transferred to the reaction vessel, washed with DMF, swollen in DMF for 30 min. The Fmoc protecting group was removed using 20% piperidine in DMF, followed by washing five times with DMF. Coupling of Fmoc-Arg(Pbf)-OH was performed by dissolving Fmoc-Arg(Pbf)-OH (40.54g, 62.5mmol), HOBt (8.4g, 62.5mmol) and DIC (7.8g, 62.5mmol) in DMF and adding the solution to the synthesizer. The reaction was allowed to proceed at room temperature for 2–3 h and completion was confirmed by a negative ninhydrin test.

Subsequent Fmoc deprotection and amino acid coupling steps were carried out following the standard protocol, according to the vasopressin peptide sequence. The amino acids were coupled sequentially in the following order: Fmoc-Pro-OH, Fmoc-Cys(Acm)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Phe-OH, Fmoc-Tyr(tBu)-OH, and Boc-Cys(Trt)-OH. Each coupling step was monitored using the ninhydrin test to ensure completion of reaction. Upon completion of the full peptide chain assembly, the resin was washed, shrunk using methanol, and dried under vacuum to afford approximately 120g of resin-bound linear vasopressin peptide 1.

Step-2: Global deprotection of resin bound parallel linear vasopressin monomer

Cys-Tyr-Phe-Gln-Asn-Cys(Acm)-Pro-Arg-Gly-NH_2

A cleavage reagent (840 mL) composed of TFA/phenol/TIS/water (92.5:2.5:2.5:2.5, v/v) was prepared in a three-necked flask and cooled to 15 ±

2 °C. The resin-bound linear vasopressin peptide (120 g) was added, and the deprotection was carried out at 15 ± 2 °C for 3.5 h with stirring. After completion, the resin was removed by filtration and the filtrate was added to isopropyl ether to precipitate the peptide. The precipitate was collected, washed twice with isopropyl ether, and dried under vacuum to yield approximately 45g of parallel linear monomer vasopressin 2 as a white solid.

Step-3: Synthesis of pyridyl adduct of parallel linear vasopressin monomer (3)

Cys(Pyridyl)-Tyr-Phe-Gln-Asn-Cys(Acm)-Pro-Arg-Gly-NH$_2$

Parallel linear vasopressin monomer (30.0g, 25.92mmol) was dissolved in trifluoroacetic acid (TFA, 210mL) under stirring. To this solution, 2,2′-dithiodipyridine (11.42g, 51.84mmol) was added, and the reaction mixture was stirred at 25 ± 2 °C for 3-4 h. Reaction progress was monitored by in-process HPLC until complete consumption of the starting material. Following completion, TFA was removed completely under reduced pressure. Diethyl ether was then added to the residue, and the mixture was stirred at 25 ± 2°C for 30 min to induce precipitation. The resulting solid was isolated by filtration, washed as required, and dried under vacuum to afford 29.0 g of the pyridyl adduct of parallel linear vasopressin monomer 3 as a white solid.

Step-4: Synthesis of Vasopressin Parallel Dimer via Intermolecular Disulfide Bond Formation (4)

Cys-Tyr-Phe-Gln-Asn-Cys(Acm)-Pro-Arg-Gly-NH $_2$
|
Cys-Tyr-Phe-Gln-Asn-Cys(Acm)-Pro-Arg-Gly-NH $_2$

Parallel linear vasopressin monomer (30.0g, 25.92mmol), obtained in step-2, was dissolved in 10% TFA (210mL) under stirring. To this solution, the pyridyl adduct of parallel linear vasopressin monomer obtained in step-3 (32.83g, 25.92mmol) was added, and the reaction mixture was stirred at 25 ± 2°C for 3-4 h. Reaction progress was monitored by in-process HPLC until complete consumption of the starting materials. Upon completion, TFA was removed under reduced pressure and the residue was diluted with water to obtain a 0.5% TFA solution. The solution was then loaded onto a preparative C18 column and purified using a gradient system consisting of 0.1% TFA in water (buffer A) and acetonitrile (buffer B). Fractions containing the desired product were collected and lyophilized to afford the first disulfide bond of vasopressin parallel dimer as a solid (yield: 20 g).

Step-5: Synthesis of Vasopressin Parallel Dimer via Sequential Second Disulfide Formation (**5**)

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH $_2$
| |
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH $_2$

The first cyclized vasopressin parallel dimer (20.0g, 8.64mmol), obtained in step-4, was dissolved in 10% aqueous acetic acid (7.5L) under stirring, and 10% aqueous HCl (2.5L) was added. To this reaction mixture, 0.1M iodine in methanol was added dropwise and the reaction was stirred at 25 ± 2°C for 2 h. Reaction progress was monitored by in-process HPLC until complete consumption of the starting material. After completion, the reaction was quenched with an ascorbic acid solution, followed by neutralization using aqueous sodium bicarbonate. The reaction mixture was then loaded onto a preparative C18 column and purified using 0.1% TFA in water (buffer A) and acetonitrile (buffer B). Fractions containing the desired product were pooled, and solvents were removed under reduced pressure. The residue was further purified by a second preparative C18 chromatography using aqueous ammonia solution (buffer A) and acetonitrile (buffer B). The final product fractions were collected and lyophilized to afford pure vasopressin parallel dimer as a solid (yield: 8.0g, HPLC Purity: 95.88).

The calculated molecular mass of the product was 2166.8699 and ESI–MS analysis showed an ion at m/z 2168.8782 $[M+H]^+$, consistent with the expected structure.

General procedure for synthesis of Vasopressin AntiParallel dimer

Step-1: Synthesis of Resin-Bound Antiparallel Linear Vasopressin Monomer (6)

Cys(Acm)-Tyr(tBu)-Phe-Gln(Trt)-Asn(Trt)-Cys(Trt)-Pro-Arg(Pbf)-Gly-Rink amide resin

Rink amide resin (50.0g, substitution degree 0.6mmol/g) was charged into a solid-phase peptide synthesizer, washed twice with DMF and swollen in DMF for 30 min. Fmoc deprotection was performed using a premixed solution of 20% piperidine in DMF for 30 min, followed by washing three times each with DMF and DCM.

Initial amino acid loading was carried out using a solution of Fmoc-Gly-OH (22.29g, 75mmol), HOBt (10.13g) and DIC (11.83mL) in DMF, with agitation for 2 h at room temperature. The resin was then washed with DMF and DCM, and unreacted amine groups were capped using acetic anhydride/pyridine/DMF (15:15:470mL) for 30 min. After washing and methanol shrinkage, the resin was dried to afford Fmoc-Gly-Rink amide resin with a substitution degree of 0.5mmol/g.

The resin was reswollen in DCM, washed with dimethylformamide (DMF) and the Fmoc group was removed using 20% piperidine in DMF, followed by washing five times with DMF. Coupling of Fmoc-Arg(Pbf)-OH was performed using Fmoc-Arg(Pbf)-OH (40.54g, 62.5mmol), HOBt (8.4g, 62.5mmol), and DIC (7.8g, 62.5mmol) in DMF for 2–3 h at room temperature. Completion was confirmed by a negative ninhydrin test.

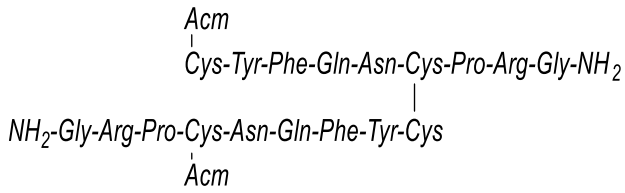
Subsequent Fmoc deprotection and amino acid coupling cycles were repeated following the vasopressin backbone sequence, using Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Phe-OH, Fmoc-Tyr(tBu)-OH, and Boc-Cys(Acm)-OH. After completion of peptide chain assembly, the resin was washed, shrunk with methanol and dried under vacuum to afford approximately 125g of resin-bound antiparallel linear vasopressin peptide.

Step-2: Global Deprotection to Obtain Antiparallel Linear Vasopressin Monomer (7)

Cys(Acm)-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

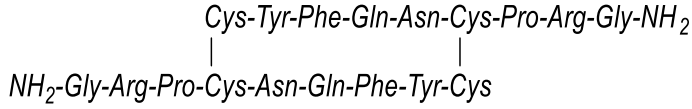
A cleavage cocktail (875mL) consisting of TFA/phenol/TIS/water (92.5:2.5:2.5:2.5, v/v) was prepared in a three-necked flask and cooled to 15 ± 2°C. The resin-bound antiparallel linear vasopressin peptide (125g) was added portion-wise, and the reaction was maintained at 15 ± 2 °C for 3.5 h with stirring. Upon completion, the resin was removed by filtration and the filtrate was slowly added to cold isopropyl ether to precipitate the crude peptide. The precipitate was collected by filtration and washed twice with isopropyl ether. Final drying under vacuum afforded 47g of antiparallel linear vasopressin monomer as a white solid.

Step-3: Synthesis of Vasopressin Antiparallel Dimer via Intermolecular Disulfide Bond Formation (8)



A stirred solution of antiparallel linear vasopressin monomer (30g, 25.92mmol; step-2) in 10% TFA (210mL) was treated with the pyridyl adduct of parallel linear vasopressin monomer (32.83g, 25.92mmol; compound 3) and stirred at 25 ± 2°C for 2-4 h. Reaction progress was monitored by in-process HPLC until complete consumption of the starting material. Following completion, TFA was completely removed under reduced pressure, and the residue was diluted with water to obtain a 0.5% TFA solution. The crude product was purified by preparative reversed-phase HPLC using a C18 column, with 0.1% TFA in water (Buffer A) and acetonitrile (Buffer B) as the mobile phases. Fractions containing the desired product were pooled and lyophilized to afford the first disulfide bond of vasopressin antiparallel dimer (19 g) as a solid.

Step-4: Synthesis of Vasopressin Antiparallel Dimer via Sequential Second Disulfide Formation (9)



A solution of first cyclized vasopressin antiparallel dimer (19g, 8.21mmol; Example 6) in 10% acetic acid in water (7.125L) and 10% hydrochloric acid in water (2.375L) was stirred at 25 ± 2°C. To this solution, 0.1M iodine in methanol was added dropwise and the reaction was continued for 3-4 h.

Reaction progress was monitored by in-process HPLC until complete consumption of the starting material. The reaction mixture was quenched with an ascorbic acid solution, followed by neutralization with aqueous sodium bicarbonate. The resulting solution was purified by preparative reversed-phase HPLC on a C18 column using 0.1% TFA in water (Buffer A) and acetonitrile (Buffer B) as the mobile phases. Solvents were removed under reduced pressure, and the product was further purified by a second C18 preparative HPLC using aqueous ammonia solution (Buffer A) and acetonitrile (Buffer B). Fractions containing the desired product were pooled and lyophilized to afford the vasopressin antiparallel dimer as a solid (Yield: 7g; HPLC Purity: 98%).

RESULTS AND DISCUSSION

Dekan *et al*, developed a sophisticated orthogonal protection strategy for the synthesis of *parallel* and *antiparallel* vasopressin dimers[32], employing a two-stage oxidation sequence. The first cyclization was carried out in 6M guanidinium hydrochloride, a strong chaotropic medium that maintains peptide solubility and the second disulfide bridge was introduced through iodine-mediated deprotection of Cys(Acm) residues. Although this method demonstrated proof of concept at the milligram scale, it is unsuitable for industrial application due to multiple operational and environmental drawbacks. The use of Gdn·HCl introduces toxicity, corrosivity and high-salinity waste-disposal issues, while the coexistence of Cys(–SH) and Cys(Acm) moieties during oxidation promotes disulfide mis-pairing and formation of scrambled or oligomeric by-products. In addition, the iodine-based oxidation often yields over-oxidized or halogenated impurities and the process relies on dilute reaction conditions, long oxidation times, and multiple RP-HPLC purifications, making it solvent-intensive and impractical for large-scale manufacturing.

Aanning *et al*, earlier reported the synthesis of the *parallel* oxytocin dimer[33] by coupling a bis(t-butyloxycarbonyl)–L-cysteine derivative to a peptide already containing the second cystine bridge through a mixed-anhydride coupling route. The approach depends on strongly reducing and oxidizing reagents, including sodium in liquid ammonia for benzyl-group removal and potassium ferricyanide for oxidative disulfide formation. These reagents pose serious safety and environmental hazards-metallic sodium is pyrophoric and violently reactive with moisture and ferricyanide can release toxic hydrogen cyanide gas under acidic or thermal conditions. Moreover, the process is structurally restrictive, being applicable only to peptides with an N-terminal cysteine and involves multiple harsh deprotection and oxidation steps with limited yields and difficult product isolation. Such features make the method impractical for reproducible or safe bulk-scale synthesis.

Chen *et al*, proposed a two-step directed disulfide-bond-formation approach to construct *parallel* and *antiparallel* heterodimers of oxytocin and vasopressin analogues[34]. The first linkage was achieved by nucleophilic attack of a free thiol on an activated cysteine surrogate (e.g., Snm or Pmp group) in the complementary monomer, and the second disulfide bridge was generated by iodine oxidation of Cys(Acm) residues. While conceptually elegant, this strategy suffers from major practical limitations: the Snm protecting group is non-commercial and synthetically cumbersome, its on-resin deprotection using DTT in mixed aqueous–organic media is operationally challenging, and it frequently leads to complex impurity profiles and variable yields. The dependence on custom protecting-group chemistry, multiple oxidation steps and non-standard reagents severely restricts scalability and reproducibility in an industrial context.

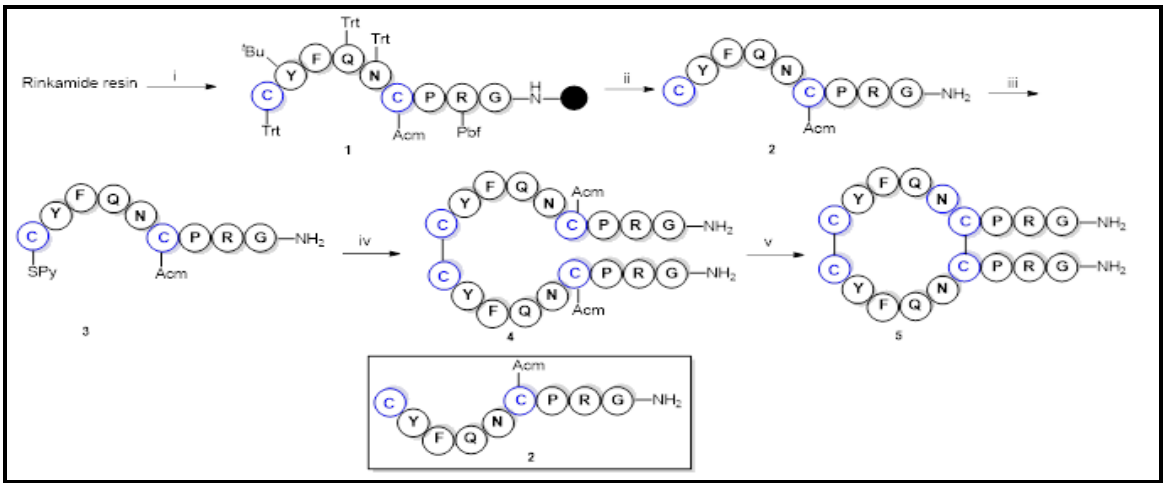
In light of these challenges and limitations, the present work focuses on developing a safer, more efficient, and industrially scalable process for the synthesis of parallel and antiparallel vasopressin dimers. The synthesis of parallel and antiparallel vasopressin dimers was achieved through an orthogonally protected solid-phase peptide synthesis (SPPS) strategy, employing conventional Fmoc chemistry with N, N′-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) as

coupling reagents. The designed sequence incorporated two cysteine residues protected with distinct thiol-protecting groups-S-triphenylmethyl (Trt) and S-acetamidomethyl (Acm)-to direct the selective formation of disulfide bridges and ultimately control the orientation of the dimeric peptides.

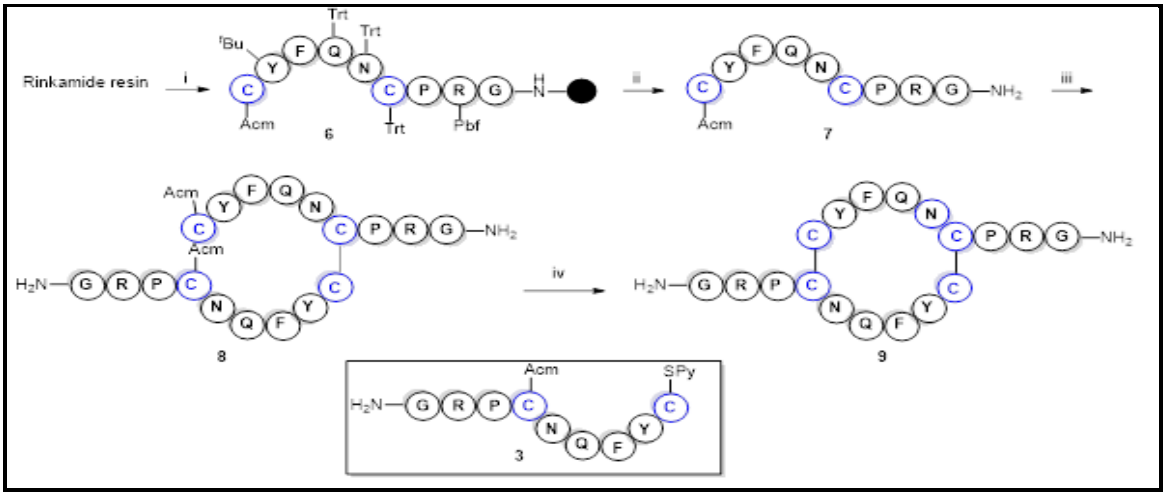
For the parallel vasopressin dimer, the linear precursor peptide 1 was assembled on Rink amide resin, wherein the terminal cysteine was protected with trityl (Trt) group and the internal cysteine with acetamidomethyl (Acm). Following chain elongation, the fully protected peptide-resin was subjected to global acidolytic cleavage using Reagent B (TFA: phenol: TIS: water, 92.5:2.5:2.5:2.5, v/v) to afford the linear vasopressin monomer 2 bearing one free thiol (Cys–SH). This free thiol was then selectively converted to a pyridyl disulfide derivative via reaction with 2,2′-dithiodipyridine (DTDP), yielding the corresponding activated intermediate 3. The first disulfide linkage was formed by coupling this pyridyl adduct with an equimolar amount of the deprotected linear vasopressin monomer 2 under mild conditions, producing the first disulphide bond peptide derivative 4. Subsequent oxidation of the Acm-protected cysteine residues using a dilute iodine solution in an aqueous acetic acid medium completed the second disulfide bridge formation, furnishing the fully cyclized parallel vasopressin dimer 5 in high purity and yield (Scheme No.1).

The antiparallel vasopressin dimer was prepared analogously, except that the orthogonal protection pattern of cysteine residues was reversed: the terminal cysteine was protected with Acm and the internal cysteine with Trt 6. After global deprotection with Reagent B, the resulting linear peptide 7 possessing an internal free thiol was activated with DTDP to form the corresponding pyridyl adduct 3. This intermediate 3 was then coupled with the deprotected parallel monomer 7 to form the first disulfide bond peptide intermediate 8, followed by iodine-mediated oxidation of the Acm-protected cysteines to afford the antiparallel vasopressin dimer 9 (Scheme No.2).

Our approach replaces hazardous and non-commercial reagents with robust, orthogonally protected cysteine chemistry (Trt/Acm/SPy) and employs a two-stage, directionally controlled disulfide-formation sequence under mild, precisely quenched conditions. This design minimizes mispairing, eliminates halogenated waste, and enables gram-scale production of highly pure dimers with excellent reproducibility, addressing both the synthetic and safety shortcomings of earlier methods and providing a scalable route suitable for industrial and regulatory applications. Moreover, the workflow is fully compatible with GMP standards and supports the generation of high-purity dimers suitable for toxicological evaluation and regulatory compliance in generic peptide drug development. Overall, this method bridges the gap between laboratory synthesis and commercial production, establishing a robust, safe and environmentally sustainable route for large-scale vasopressin dimer synthesis.



Scheme No.1: Synthetic process for the preparation of parallel vasopressin dimer Reaction Conditions: i) SPPS; ii) Reagent-B; iii) 2, 2'-dithiodipyridine; iv) 2; v) Iodine solution in aq. Acetic acid



Scheme No.2: Synthetic process for the preparation of anti-parallel vasopressin dimer Reaction Conditions: i) SPPS; ii) Reagent-B; iii) 3; iv) Iodine solution in aq. Acetic acid

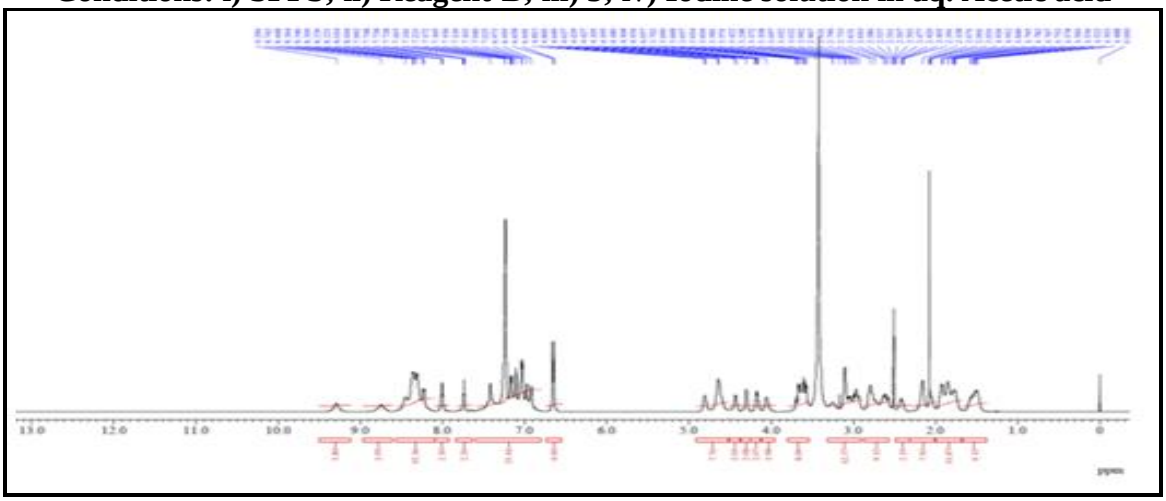


Figure No.1: ^{1}H NMR of Vasopressin parallel dimer

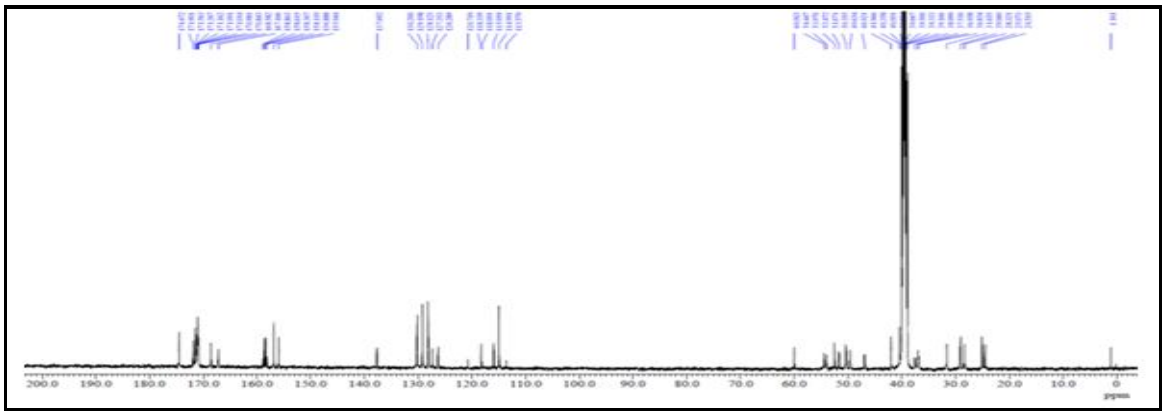


Figure No.2: ^{13}C NMR of Vasopressin parallel dimer

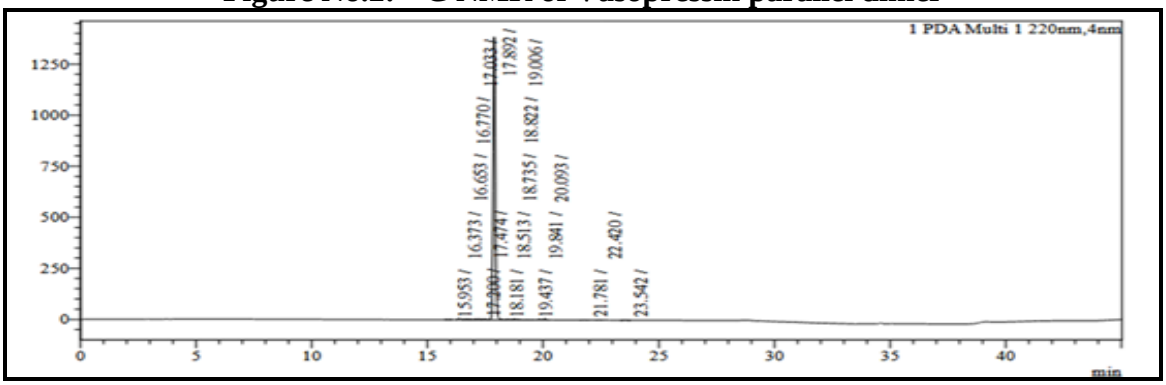


Figure No.3: HPLC Spectrum of Vasopressin parallel dimer

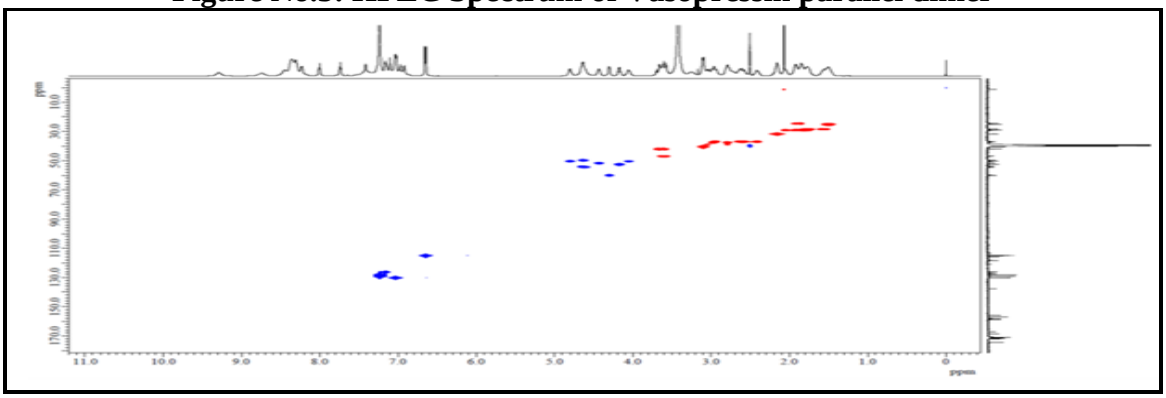


Figure No.4: Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin parallel dimer

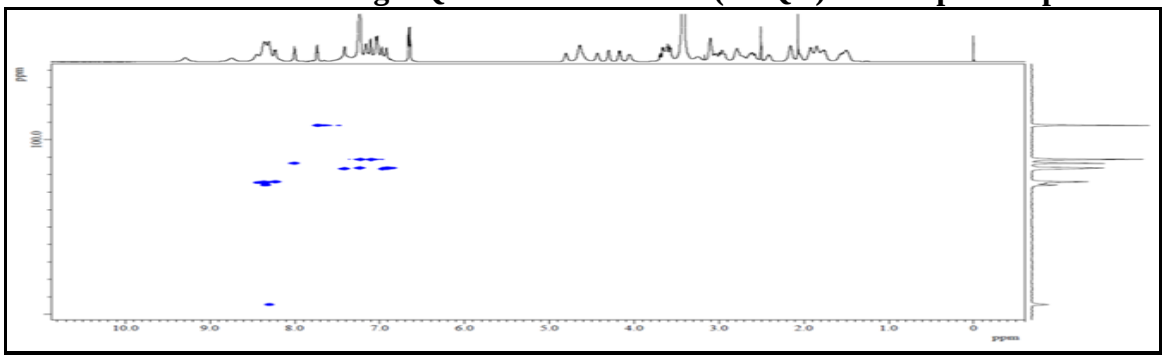


Figure No.5: ^{15}N Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin parallel dimer

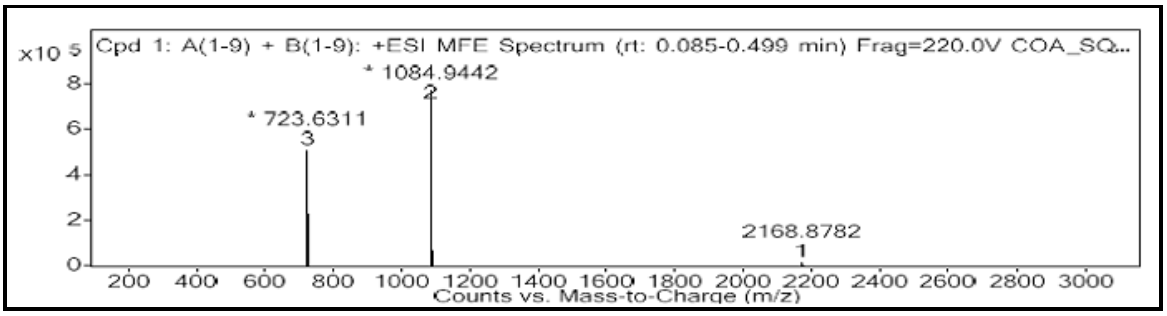


Figure No.6: Mass Spectrum of Vasopressin parallel dimer

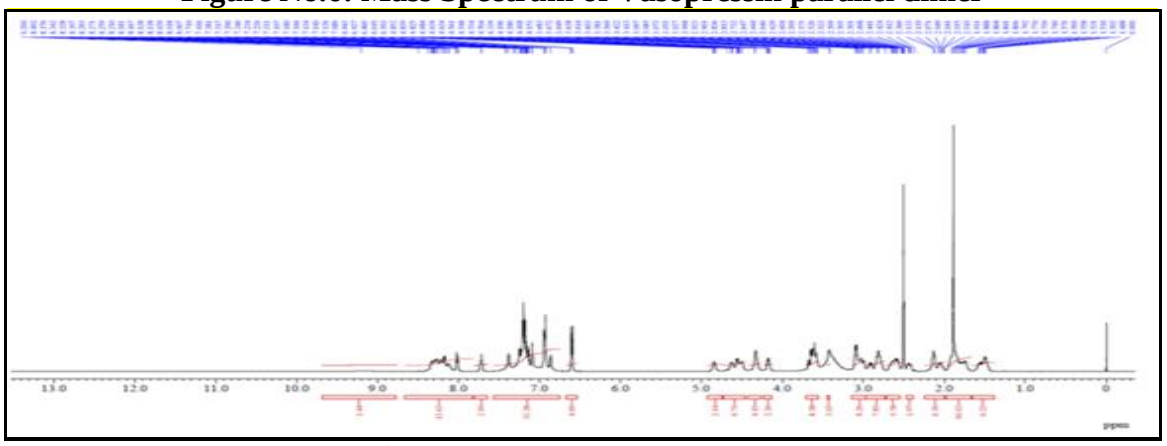


Figure No.7: ^{1}H NMR of Vasopressin antiparallel dimer

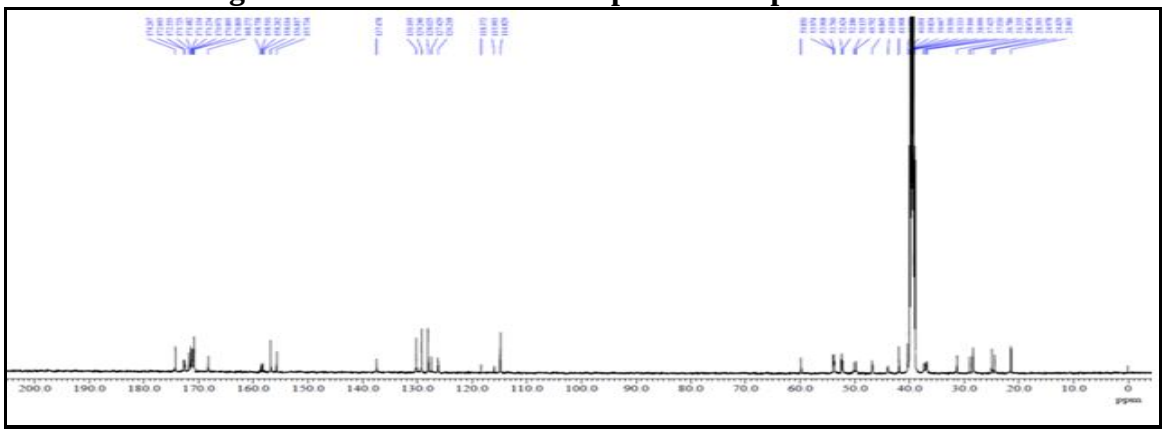


Figure No.8: ^{13}C NMR of Vasopressin Antiparallel dimer

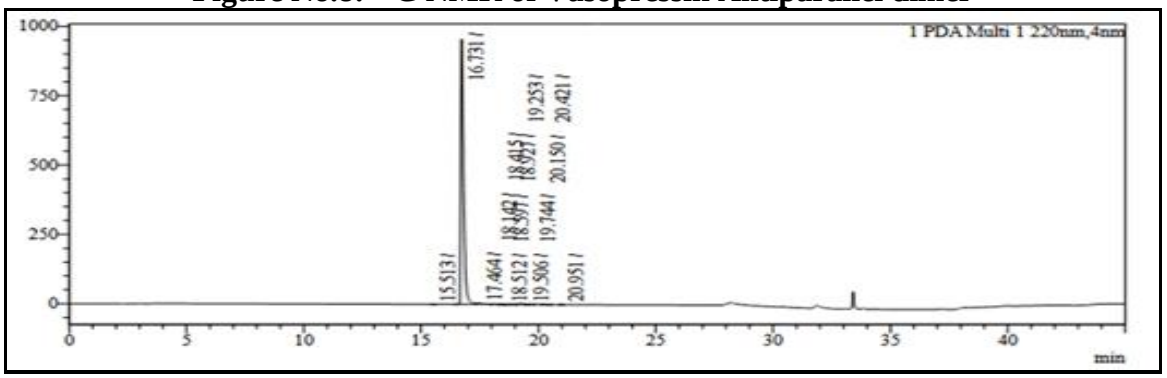


Figure No.9: HPLC Spectrum of vasopressin antiparallel dimer

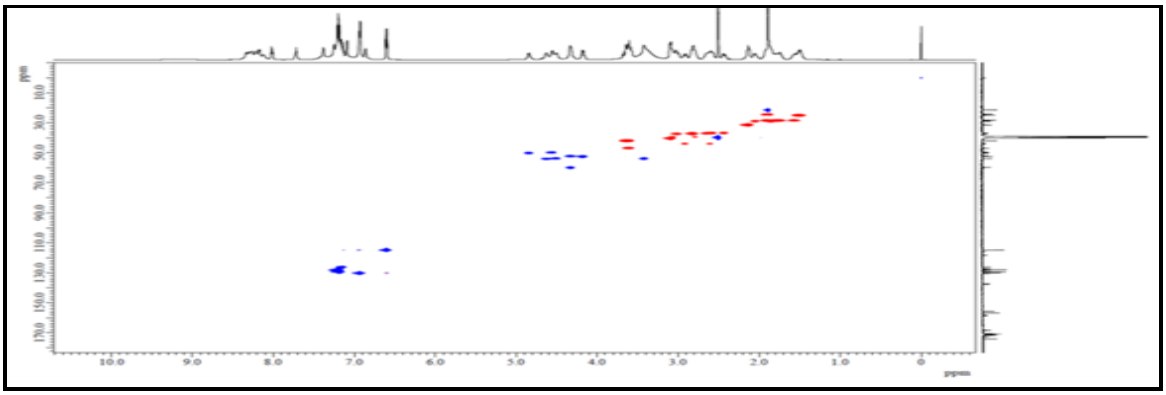


Figure No.10: Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin antiparallel dimer

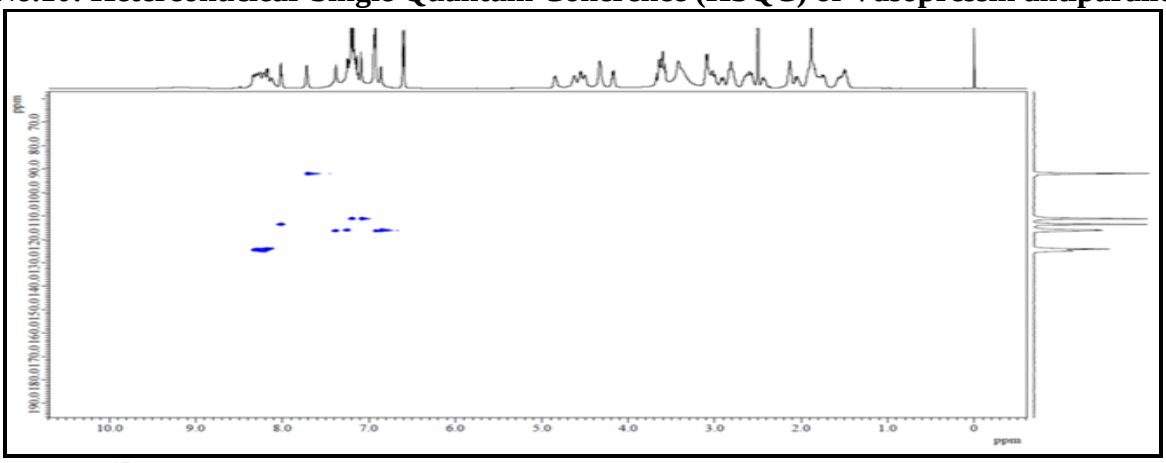


Figure No.11: ^{15}N Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin antiparallel dimer

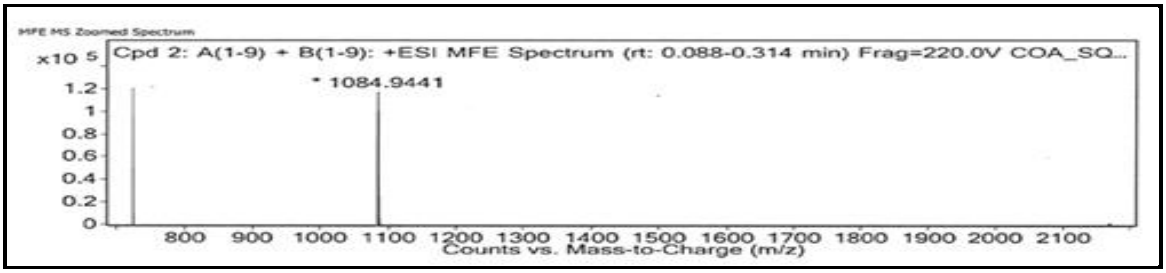


Figure No.12: Mass Spectrum of Vasopressin antiparallel dimer

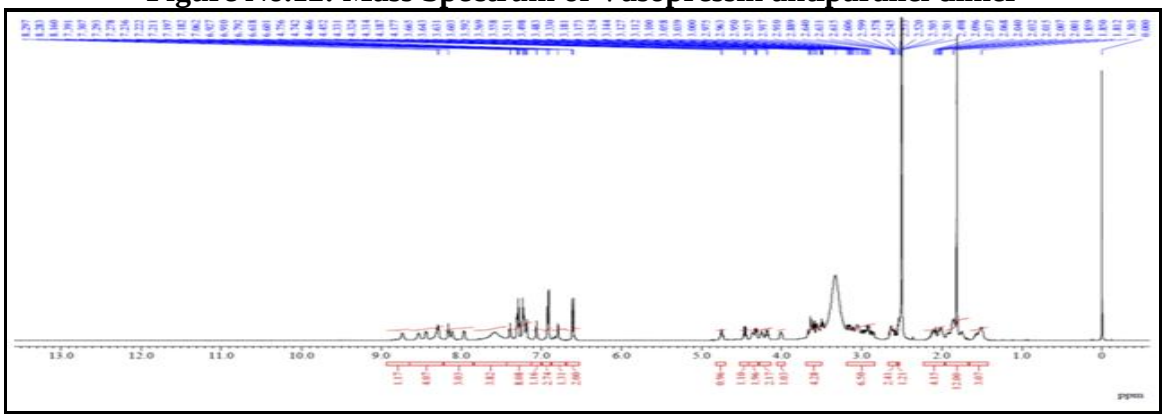


Figure No.13: ^{1}H NMR of Vasopressin API

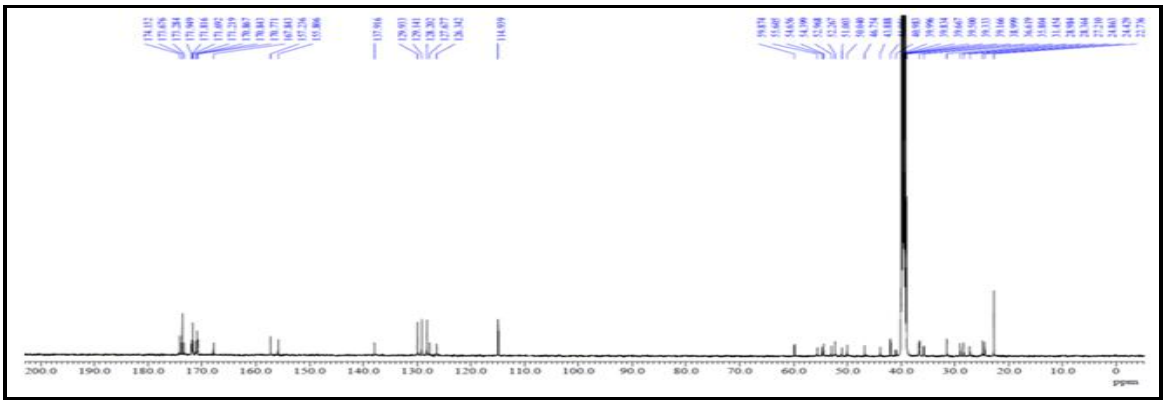


Figure No.14: ^{13}C NMR of Vasopressin API

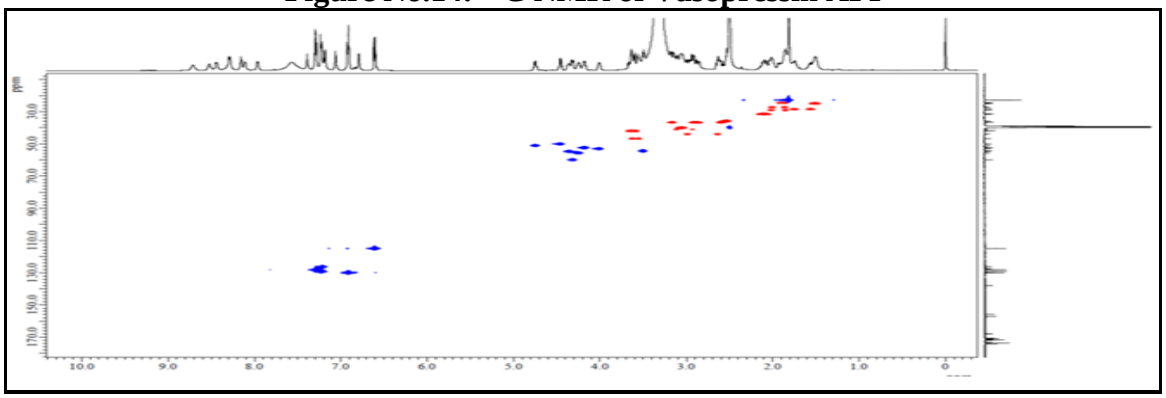


Figure No.15: Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin API

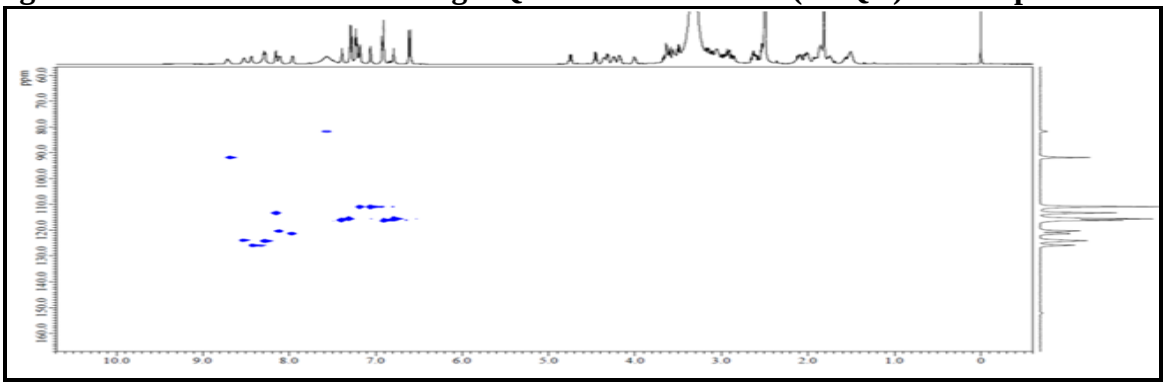


Figure No.16: Perfect Hetereonuclear Single Quantum Coherence (HSQC) of Vasopressin API

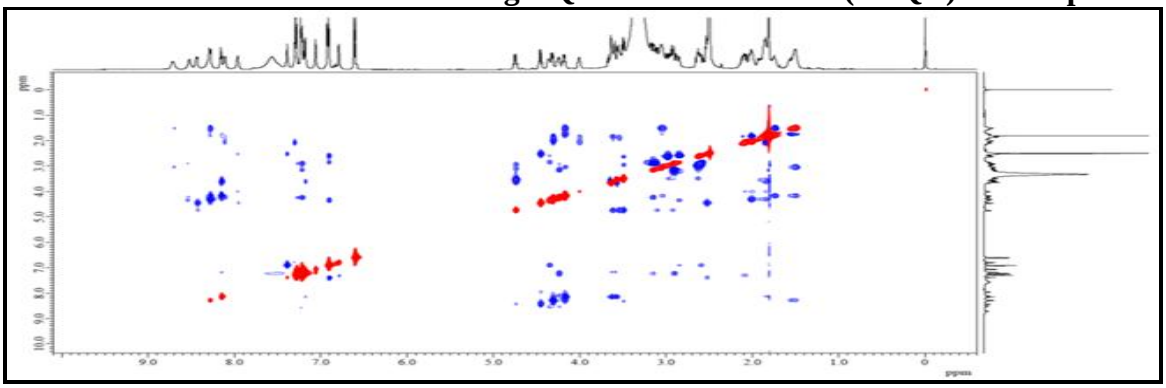


Figure No.17: Rotating Frame Overhauser Effect Spectroscopy (ROESY) of Vasopressin API

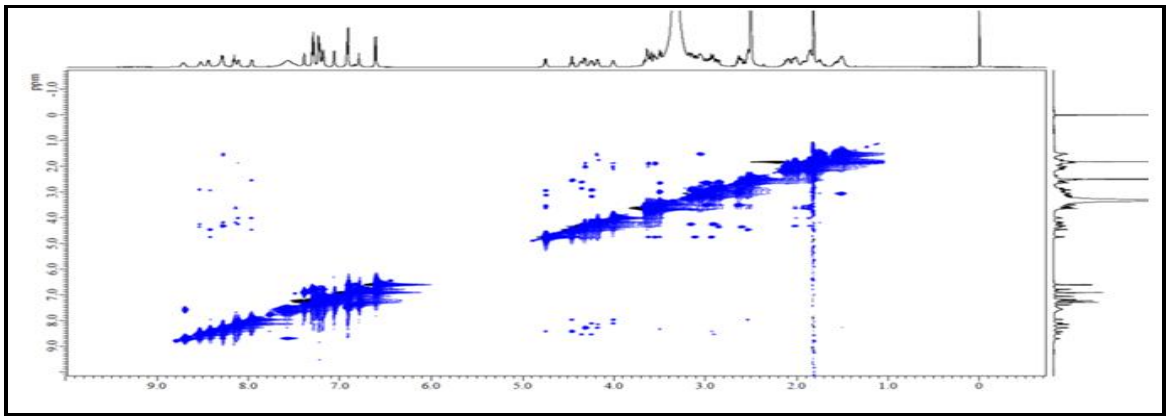


Figure No.18: Total Correlation Spectroscopy (TOCSY) of Vasopressin API

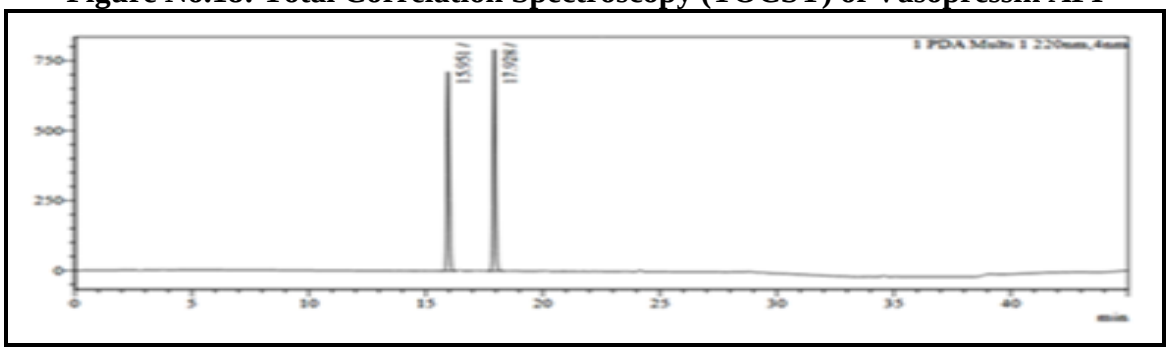


Figure No.19: Merged HPLC of Vasopressin parallel dimer and Vasopressin API

CONCLUSION

We developed a scalable and generalizable method for synthesizing parallel and antiparallel cysteine-bridged peptide dimers using DPDS. This method is efficient, allows monitoring of disulfide exchange and yields gram-level material suitable for impurity profiling and biological evaluation. Beyond regulatory relevance, this approach also highlights potential therapeutic applications of dimeric peptides.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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