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γ-AApeptides: Design, Structure, and Applications

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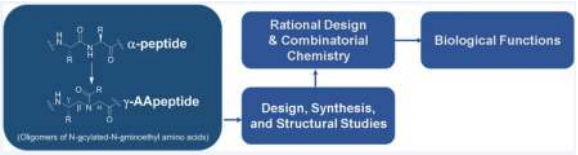
Abstract



CONSPECTUS: The development of sequence-specific peptidomimetics has led to a variety of fascinating discoveries in chemical biology. Many peptidomimetics can mimic primary, secondary, and even tertiary structure of peptides and proteins, and because of their unnatural backbones, they also possess significantly enhanced resistance to enzymatic hydrolysis, improved bioavailability, and chemodiversity. It is known that peptide nucleic acids (PNAs) are peptidic sequences developed for the mimicry of nucleic acids; however, their unique backbone as the molecular scaffold of peptidomimetics to mimic structure and function of bioactive peptides has not been investigated systematically. As such, we recently developed a new class of peptidomimetics, "γ-AApeptides", based on the chiral γ-PNA backbone. They are termed γ-AApeptides because they are the oligomers of γ-substituted-*N*-acylated-*N*-aminoethyl amino acids. Similar to other classes of peptidomimetics, γ-AApeptides are also resistant to proteolytic degradation and possess the potential to enhance chemodiversity. Moreover, in our scientific journey on the exploration of this class of peptidomimetics, we have discovered some intriguing structures and functions of γ-AApeptides. In this Account, we summarize the current development and application of γ-AApeptides with biological potential. Briefly, both linear and cyclic (either through head-to-tail or head-to-side-chain cyclization) γ-AApeptides with diverse functional groups can be synthesized easily on the solid phase using the synthetic protocol we developed. γ-AApeptides could mimic the primary structure of peptides, as they project the same number of side chains as peptides of the same lengths. For instance, they could mimic the Tat peptide to permeate cell membranes and bind to HIV RNA with high specificity and affinity. Certain γ-AApeptides show similar activity to the RGD peptide and target integrin specifically on the cell surface. γ-AApeptides with function akin to fMLF peptides are also identified. More importantly, we found that γ-AApeptides can fold into discrete secondary structures, such as helical and β-turn-like structures. Therefore, they could be rationally designed for a range of biological applications. For instance, γ-AApeptides can mimic host-defense peptides and display potent and broad-spectrum activity toward a panel of drug-resistant bacterial pathogens. Meanwhile, because of their stability against proteolysis and their

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chemodiversity, γ-AApeptides are also amenable for combinatorial screening. We demonstrate that, through combinatorial selection, certain γ-AApeptides are identified to inhibit Aβ40 peptide aggregation, suggesting their potential use as a molecular probe to intervene in Alzheimer's disease. In addition, a few γ-AApeptides identified from the γ-AApeptide library have been shown to bind to the DNA-binding domain of STAT3 and antagonize STAT3/DNA interactions. Our studies suggest that, with further studies and exploration on both structures and functions, γ-AApeptides may emerge to be a new class of peptidomimetics that play an important role in chemical biology and biomedical sciences.

1. INTRODUCTION

Sequence-specific peptidomimetics offer exciting approaches to address a variety of challenges in chemical biology.[1,2] The past decade has witnessed dramatic progress in developing biomimetic oligomers, including β-peptides,[3–5] peptoids,[6,7] α-aminoxy-peptides,[8] α/β-peptides,[9,10] azapeptides,[11] oligoureas,[12] aromatic oligoamides,[13] and so forth. Peptidomimetics can mimic primary, secondary, and even tertiary structures of peptides and proteins, and therefore they have been developed for biomolecular recognition and modulation of protein interactions. In addition, because of their unnatural backbones, these peptidomimetics display advantages over conventional peptides, including resistance to enzymatic hydrolysis, improved bioavailability, and enhanced chemodiversity.[14] Nonetheless, the development and application of peptidomimetics is still limited due to the availability of backbones and molecular frameworks. We recently developed a new class of peptidomimetics, "γ-AApeptides", based on the chiral PNA backbone. They are termed γ-AApeptides because they are oligomers of γ-substituted-*N*-acylated-*N*-aminoethyl amino acids (Figure 1).[15–17] It is demonstrated that γ-AApeptides are resistant to proteolytic degradation and possess the potential to introduce chemically diverse functional groups. In this Account, we highlight the synthesis, structure, and applications of this class of peptidomimetics.

2. SYNTHESIS OF γ-AAPEPTIDES

2.1. Synthesis of Linear γ-AApeptides

The synthesis of linear γ-AApeptides (Scheme 1a) is carried out on the solid phase using the method that combines both building-block[18] and submonomeric approaches.[19,20] The *N*-alloc γ-AApeptide building blocks are stable and could be prepared in large batches and used for a long period of time. They can be obtained by either Route 1 or Route 2 according to different R groups (Scheme 1b).

2.2. Synthesis of Cyclic γ-AApeptides

Cyclization of peptides and peptidomimetics can rigidify functional groups and endow enhanced activity as well as stability towards proteolysis.[21] As part of our continuing effort to extend the structural and functional diversity of γ-AApeptides, we also synthesized cyclic γ-AApeptides through either head-to-tail or head-to-side chain cyclization.

The head-to-tail cyclization was carried on the 2-chlorotrityl chloride (CTC) resin.[22] As ketopiperazine side products are usually formed in the Fmoc-deprotection step, which leads to the self-cleavage of building blocks on the solid support,[23] an Alloc-*N*′-Fmoc-*N*γ-AApeptide building block was employed (Scheme 2). During the synthesis, the alloc protecting groups were removed in neutral conditions to minimize the formation of ketopiperazine. After the sequence was cleaved from the resin by the cocktail (acetic acid/trifluoroethanol/dichloromethane 1:1:8), the cyclization was achieved in dichloromethane using TBTU, HOBt, and DMAP as the coupling reagents. Interestingly, the crystal structure of **γ-AA2** (Scheme 2) demonstrates its similarity to the type II β-turn (Figure 2).[24]

In contrast to the head-to-tail cyclization that was carried out in solution, the head-to-side chain cyclization was completed on the solid support (Scheme 3).[25] In this approach, a special γ-AApeptide building block with the monoallyl succinate group was introduced (**γ-AA3**), and the allyl ester was removed by $Pd(PPh_3)_4/PhSiH_3$ before cyclization. At last, the free carboxylate group reacted with the amino group of the N-terminal building block in the presence of PyBop/DIPEA to achieve cyclization.

3. THE STRUCTURE OF γ-AAPEPTIDES

The function of natural and unnatural biopolymers including proteins and nucleic acids is based on a simple dogma: the biological function is determined by the three-dimensional structure.[26] There have been significant efforts put towards the development of unnatural foldamers, which is defined as the multitude of non-natural folding oligomeric molecules with new molecular frameworks and discrete folding propensities.[27,28] Compared to native proteins, they could not only retain the structure and the function but also manifest new properties due to their unnatural backbones.[29]

One of the most attractive features of γ-AApeptides is that half of their side chains can be introduced by reacting a myriad of agents besides carboxylic acids with the secondary amines on the backbone. For instance, we recently developed sulfono-γ-AApeptides and 1:1 α/sulfono-γ-AApeptides (Scheme 4). In these sequences, the protons of the secondary amide moieties can potentially promote folding propensities through intramolecular hydrogen bonding similar to that of α-peptide. Additionally, the sulfonamido moieties are sufficiently bulky to induce intrinsic curvature in the sulfono-γ-AApeptide backbone. Furthermore, the sulfonyl groups could facilitate the folding structure by directly participating in hydrogen bonding. As such, we have studied the folding propensity of both sulfono-γ-AApeptides and 1:1 α/sulfono-γ-AApeptide hybrids using X-ray, CD, and 2D-NMR.[30,31]

3.1. Sulfono-γ-AApeptides

The crystal structure of **γ-AA4** (Scheme 5) indicates that even the sulfono-γ-AApeptide monomer possesses a preorganized β-turn-like structure. 2D-NMR studies suggest that the 8-building-block sequence **γ-AA5** (Scheme 5) adopts a right-handed helical conformation in solution with helical pitches, radius, and hydrogen-bonding pattern similar to those of α-helix (Figure 3). Circular dichroism (CD) studies reveal a clear trend that the longer

sequence forms more-defined secondary structure. Intriguingly, the helical structures have a good tolerance toward temperature change over a wide range.

3.2. 1:1 α/Sulfono-γ-AApeptides

Given that sulfono-γ-AApeptides show an α-helix-like conformation in solution, we studied the folding propensities of a series of heterogeneous peptides containing alternative α and sulfono-γ-AApeptide residues (Scheme 6). We hypothesized that additional α-amino acid residues could augment the folding propensity by potentially introducing more hydrogen bonding.

Indeed, 2D-NMR of 1:1 hybrid sequence **γ-AA6** suggests a defined helical folding structure (Figure 4). In H/D exchange studies, most of the backbone NH resonances could even be detected after 24 h, implying that the hydrogen bonds do not readily become involved in the H/D exchange. Interestingly, another 1:1 α/sulfono-γ-AApeptide, **γ-AA7**, bearing different side chains also exhibits similar right-handed helical structure, suggesting that this class of sequences may possess a general folding propensity.

4. APPLICATIONS OF γ-AAPEPTIDES

In addition to studying the folding structures of γ-AApeptides, we have also set out to study the function of γ-AApeptides, especially their capability to mimic bioactive peptides. To date, we have developed γ-AApeptides for a few biological applications through either rational design or combinatorial screening.

4.1. Rational Design

On the basis of the primary and secondary structures of some bioactive peptides, we designed γ-AApeptides that can mimic their structures and functions.

4.1.1. Mimicry of Primary Structures

Mimicking Tat Peptides: The discovery of novel molecules binding to RNA is of great importance in the biological sciences and medicine.[32,33] The Tat peptide is a cationic peptide that binds to HIV TAR RNA tightly. We designed γ-AApeptide **γ-AA8** that projects the same side chains as Tat 48-57 (**P1**, Scheme 7) and showed that this sequence can bind to HIV TAR RNA and BIV TAR RNA with similar affinity and specificity to that of the Tat peptide.[34]

It is known that, in addition to binding to HIV RNA, the Tat peptide possesses excellent cell permeability.[35] Numerous biologically active proteins have been tethered to Tat and then delivered into intact cells.[36] For exploring the capacity of γ-AApeptides for cellular translocation, the fluorescein moiety was attached to **γ-AA8** (Scheme 8).[37] Cellular uptake was evaluated by flow cytometry (Figure 5), which shows that **γ-AA9** (fluorescent **γ-AA8**, red bar) exhibits almost identical cellular uptake as **P2** (fluorescent **P1**, black bar) when both peptides were incubated at the same concentration and temperature. At lower concentrations, **γ-AA9** even resulted in a higher cellular uptake, possibly due to its tertiary amide bonds in the γ-AApeptide backbone.[38] The fact that truncated **γ-AA10** (Figure 5, blue bar) did not

exhibit cellular uptake even at the highest concentration is consistent with previous reports.[39]

Mimicking RGD Peptides: Integrin $\alpha_v\beta_3$ expresses on the surface of different cell types and plays a key role in numerous physiological processes, such as angiogenesis,[40] and therefore, it is an attractive target for several PET tracers of tumors. Because it binds proteins containing the Arg-Gly-Asp (RGD) tripeptide epitope, significant advances have been made in the development of peptides/peptidomimetics based on this motif.[40]

We thus designed γ-AApeptide **γ-AA11**, which contains guanidino and carboxyl groups to mimic the RGD motif (Scheme 9).[41] After conjugation with FITC, its binding affinity and specificity toward integrin $\alpha_v\beta_3$ on U87MG human glioblastoma cells was compared to FITC-labeled c(RGDyK) peptide **P3**. At the same concentration (Figure 6), both had similar uptake in U87MG cells at 37 °C. Blocking the receptor with 2 μM of **P3** significantly reduced the uptake of both **FITC-γ-AA11** and **FITC-P3** to a similar extent. It is noticeable that, in the following enzymatic stability study, **^{64}Cu-γ-AA12** shows much higher stability than that of **^{64}Cu-DOTA-P3**.

Next, in vivo PET imaging was carried out after intravenous injection of the tracer on a mouse model bearing a subcutaneously planted U87MG tumor (Figure 7). Tumor uptake of **^{64}Cu-γ-AA12** was visible as early as 0.5 h p.i., which remained persistent. Administering a blocking dose of **P3** reduced the U87MG tumor uptake significantly when compared with mice injected with **^{64}Cu-γ-AA12** only (Figure 7), demonstrating integrin $\alpha_v\beta_3$ targeting specificity of the tracer in vivo.

Mimicking the fMLF Peptide: The peptides bearing an *N*-formyl-methionine residue are potent chemoattractants of neutrophils.[42] Among them, the smallest formyl peptide, *N*-formyl-Met-Leu-Phe (fMLF), is the reference agonist for the G protein-coupled *N*-formyl peptide receptor (FPR) and plays a critical role in the regulation of a variety of physiological functions.[43] Significant efforts have been devoted to the development of fMLF analogues as potential molecular probes and therapeutic agents.[44] A series of γ-AApeptide were also designed, containing either *N*-formyl methionine or *N*-formyl norleucine, both of which are known to be critical for the agonistic activity of FPRs.[45] One of the most effective sequences, **γ-AA13**, is shown in Figure 8.[46]

The mimics were first assessed by testing their ability to induce calcium mobilization in human FPR1-transfected rat basophilic leukemic (RBL)-2H3 cells (Figure 8), as fMLF is a potent activator of calcium mobilization, which is the important indicator of chemotactic signaling.[45] Although at lower concentrations it was less potent, to our delight, **γ-AA13** was even more effective than fMLF at 10 μM.

It is known that, when binding to FPR1, fMLF can activate the MAP kinases ERK1 and ERK2 in human neutrophils. The corresponding activity of the lead γ-AApeptides was also investigated. At 10 μM, **γ-AA13** displayed a rapid and potent response at 1 min and peaked between 2 and 5 min. Moreover, although less potent than fMLF, **γ-AA13** effectively triggered the production of ROS and induced chemotaxis.

4.1.2. Mimicking Secondary Structure of a Peptide

Disruption of p53/MDM2 Protein–Protein Interactions: In addition to mimicking the primary structure of bioactive peptides, we also extended our effort to study γ-AApeptides for their ability to mimic secondary structure of peptides. The p53/MDM2 interaction is a testing basis for the design of helical peptidomimetics.[4,47] We therefore chose this protein–protein interaction as the modeling system to investigate if γ-AApeptides (Scheme 10) could disrupt it.[18]

Although the tested sequences were not expected to adopt a helical conformation due to their short lengths, some γ-AApeptides could effectively inhibit p53/MDM2 interactions.[48] The energy-minimized structure of the most potent sequence **γ-AA14** (Figure 9) suggests its side groups may overlap with Phe, Trp, and Leu of the p53 helical domain, which interacts with MDM2.

Antimicrobial γ-AApeptides that Mimic Host-Defense Peptides (HDPs): The increasing prevalence of pathogenic bacterial resistance toward conventional antibiotics has been emerging as a serious threat to public health.[49] The development of new generations of antibiotics with novel mechanisms has become a major focus in synthetic and pharmaceutical chemistry. Host-defense peptides (HDPs), which constitute an essential component of the innate immune system of all multicellular organisms, have demonstrated their potential as alternative therapeutic candidates to combat antibiotic resistance.[50] Although HDPs are diverse in their 3D structures, including helices, β-sheets, and extended structures, they all adopt globally amphipathic structures comprised of hydrophobic and cationic regions on the surface of bacterial membranes. One plausible mechanism of their action is that, after they are attracted to negatively charged bacterial membranes, they penetrate the membranes by their hydrophobic patches, causing membrane depolarization and cell death.[50] Because of the biophysical cell membrane interaction, which lacks defined membrane targets, HDPs are difficult for bacteria to evolve resistance. However, the development of HDPs has been hampered due to the intrinsic peptidic nature, such as proteolytic degradation, toxicity, and low activity. Peptidomimetics may be an alternative approach to overcome these drawbacks.[7,12,51,52] As γ-AApeptides exhibit limited conformational backbone flexibility capable of secondary structure formation, stability against proteolysis, and enhanced chemodiversity, a few subclasses of γ-AApeptides have been investigated for their antimicrobial activity.

Linear γ-AApeptides: The linear sequence **γ-AA15** was initially designed by linking amphiphilic building blocks (containing one hydrophobic and one cationic group in each building block) together (Scheme 11, Table 1).[53] We assumed that, on the surface of bacterial membranes, the sequence could adjust the conformation to adopt globally amphipathic structure. As expected, **γ-AA15** was found to be active against Gram-positive bacteria. The activity could also be tuned, as seen for **γ-AA16**, in which two amphiphilic building blocks in **γ-AA15** are replaced with two hydrophobic building blocks (containing two hydrophobic side chains in each building block), showing broad-spectrum activities against both Gram-positive and Gram-negative drug-resistant bacteria. The subsequent

fluorescence microscopy and drug resistance studies suggest that **γ-AA16** indeed kills bacteria through the disruption of bacterial membranes.[54]

Lipopeptides are another class of antimicrobial peptides, although their mechanism of action is sometimes different from HDPs.[55] They are composed of peptides covalently linked to aliphatic acids of variable length. The lipid chain is believed to be responsible for the lipophilicity of the peptidic chain, which facilitates bacterial membrane interactions.[56] Indeed, two lipo-linear γ-AApeptides, **γ-AA17** and **γ-AA18**, were shown to be active against both Gram-positive and Gram-negative bacteria (Scheme 12, Table 1).[57]

Recently, we have also developed lipo-linear α/γ-AApeptides (Scheme 13, Table 1) that utilize a hybrid backbone of lipidated canonical α-peptide units fused with γ-AApeptides.[58] Interestingly, these lipo-linear α/γ-AApeptides still display broad-spectrum antimicrobial activity. The heterogeneous backbone could further enhance the chemodiversity for future optimization and development.

Some natural cationic host-defense peptides (HDPs), such as magainin 2, adopt amphipathic helical structures.[59] Given that sulfono-γ-AApeptides form helical structures,[30] a series of sequences were designed to mimic the helical amphipathic structure and mechanism of action of magainin 2. Interestingly, **γ-AA22** and **γ-AA23** possess excellent antimicrobial activity[60] (Scheme 14, Table 1). The sequence **γ-AA23** lacks an acetyl group at the N-terminus, however, showing better antimicrobial activity as well as less hemolytic activity and cytotoxicity toward mammalian cells compared with those of **γ-AA22**. Small-Angle X-ray scattering (SAXS) studies suggest that the helicity of **γ-AA22** is more defined than that of **γ-AA23**, consistent with the findings that strong-helix-forming antimicrobial sequences may be more hemolytic and less active.[58] Noticeably, the stability of the **γ-AA23** is excellent; no degradation occurred after incubating with Pronase for 18 h.

Cyclic γ-AApeptides: Cyclic peptide antibiotics are widely found in nature. They are more privileged compared with their linear counterparts because the lack of N- or C-termini and reduced conformational freedom make them more stable against proteolytic degradation.[61] We thus hypothesized that cyclic γ-AApeptides could also be applicable for the development of antimicrobial agents. The design was straightforward: in these cyclic γ-AApeptides, amphiphilic building blocks were introduced, and the activity could be fine-tuned by varying the ratio of cationic/hydrophobic groups. This is seen for **γ-AA24** (Scheme 15, Table 2), which displays broad-spectrum antimicrobial activity.[25]

Similar to the development of lipo-linear γ-AApeptides, lipid tails were attached to cyclic γ-AApeptides to mimic the structure motif of daptomycin and polymyxin.[62] Interestingly, cyclic γ-AApeptides with lipid tails on the ring only showed weak antimicrobial activity. We reasoned that the lipid tail on the ring structure may have limited orientation and therefore could not position itself for membrane insertion even after the amphipathic ring contacts bacterial membranes. As such, the lipid tails were consequently moved outside the cyclic ring (Scheme 15, Table 2), leading to the identification of the lipidated sequence **γ-AA25**. Compared to nonalkylated cyclic γ-AApeptides, these lipo-cyclic γ-AApeptides are potent toward Gram-negative pathogens. More interestingly, they also harness the immune response

and inhibit lipopolysaccharide (LPS)-activated Toll-like receptor 4 (TLR4) signaling, suggesting that lipo-cyclic γ-AApeptides have dual roles as novel antimicrobial and anti-inflammatory agents. Our recent study suggests that these lipo-cyclic γ-AApeptides might be more effective in biofilm prevention than conventional antibiotics due to the existence of lipid tails that retard the growth of biofilms.[63]

4.2. Combinatorial Library of γ-AApeptides

Combinatorial chemistry provides a powerful approach for identifying molecular ligands that recognize peptides or proteins of interest with high specificity and affinity.[64] Significant efforts were extended to the development of a peptide-based library.[65] However, unnatural peptidomimetic ligand libraries are much less observed[66] even though unnatural backbones from peptidomimetics would process enhanced structural diversity and stability against proteolysis. Therefore, we have started to explore the potential of γ-AApeptides for the development of a combinatorial library.

γ-AApeptides that Disrupt Aβ Aggregation—$A\beta_{40}$ is one of the major etiological factors for Alzheimer's disease (AD) and plays a central role in the pathogenesis of AD.[67] Given the significance of the Aβ peptide, we prepared a γ-AApeptide library with 192,000 compounds using a split-and-pool method[68] and screened the library against $A\beta_{40}$ peptide binding. After screening, one hit was identified by MS/MS unambiguously (Figure 10 and Scheme 16). This lead compound, **γ-AA26**, was then resynthesized and tested for its binding activity toward the $A\beta_{40}$ peptide. The hydrophobic core of the Aβ peptide, KLVFF (**P4**, Scheme 16), was used as the control. To our surprise, **γ-AA26** was almost 100-fold as potent as **P4** in the inhibition of Aβ aggregation (Figure 11).

The ability of **γ-AA26** to prevent Aβ aggregation was further confirmed by TEM (Figure 12a,b). In fact, **γ-AA26** can even disassemble preformed Aβ fibrils (Figure 12c,d).[68]

γ-AApeptides that Disrupt STAT3/DNA Interactions—Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that regulates many biological processes.[69] Under normal physiological conditions, the activation of STAT3 is transient and tightly regulated. When activation occurs, STAT3 dimerizes and translocate to the cell nucleus, resulting in regulation of specific gene expression. Because STAT3 is constantly activated in a variety of cancers including both solid tumors and hematological cancers,[70] inhibition of STAT signaling may lead to a viable anticancer strategy. It is known that STAT3 signaling can be suppressed by inhibition of either STAT3 dimerization or STAT3–DNA binding. Although many approaches have been adopted to inhibit STAT3 dimerization, inhibition of STAT3/DNA interaction is rare.[71]

As such, a library was employed to identify ligands that potentially target STAT3–DNA binding.[72] After incubation, four sequences were identified unambiguously (Scheme 17).

The fluorescence polarization assays were then conducted to determine if these molecules disrupt the binding of STAT3 to the fluorescein-labeled GpYLPQTV phosphotyrosine peptide, which is known to bind the STAT3-SH2 domain. None of these molecules showed any inhibitory activity, suggesting that these γ-AApeptides did not bind to the STAT3-SH2

domain and failed to prevent STAT3 dimerization. However, all of the sequences disrupted STAT3–DNA binding effectively (Figure 13a,b). As GpYLPQTV was not cell permeable, S3I-1757 was used as a control to test whether these γ-AApeptides could retain STAT3–DNA binding inhibitory activity in whole cells.[73] As shown in Figure 13c, all γ-AApeptides are able to permeate cell membranes and disrupt STAT3–DNA binding.

5. CONCLUSIONS AND OUTLOOK OF γ-AAPEPTIDES

This Account summarizes the structure and applications of γ-AApeptides. Our previous studies suggest that γ-AApeptides can fold into defined structures and potentially mimic primary and secondary structures of bioactive peptides. In addition, their stability and chemodiversity enable them to be promising candidates for combinatorial development for the identification of molecular probes and potential drug leads. For expanding the scope of γ-AApeptides for biological applications in the future, a few research aspects could be broached. First, although solution structures of sulfono-γ-AApeptides are investigated, more sequences with diverse functional groups need to be explored to understand whether the folding propensity is general or diverse in this class of peptidomimetics. Obviously, more crystal structures will assist in gaining insight into their folding rules and provide a stronger basis for the future design of structural mimetics of peptides. It is also intriguing to study the tertiary structure of γ-AApeptides with the availability of secondary structural information. Second, for demonstrating the biological potential of γ-AApeptides, in vivo studies should be carried out in the near future. For example, mouse models of antimicrobial γ-AApeptides can be used to evaluate their efficacy. In addition, regarding the combinatorial development, libraries with more chemo- and structural diversity should be explored. A cyclic γ-AApeptide library can be developed and screened against molecular targets. Although stability is not an issue with γ-AApeptides, cyclization is expected to enhance their cell permeability and potential binding affinity toward protein targets.

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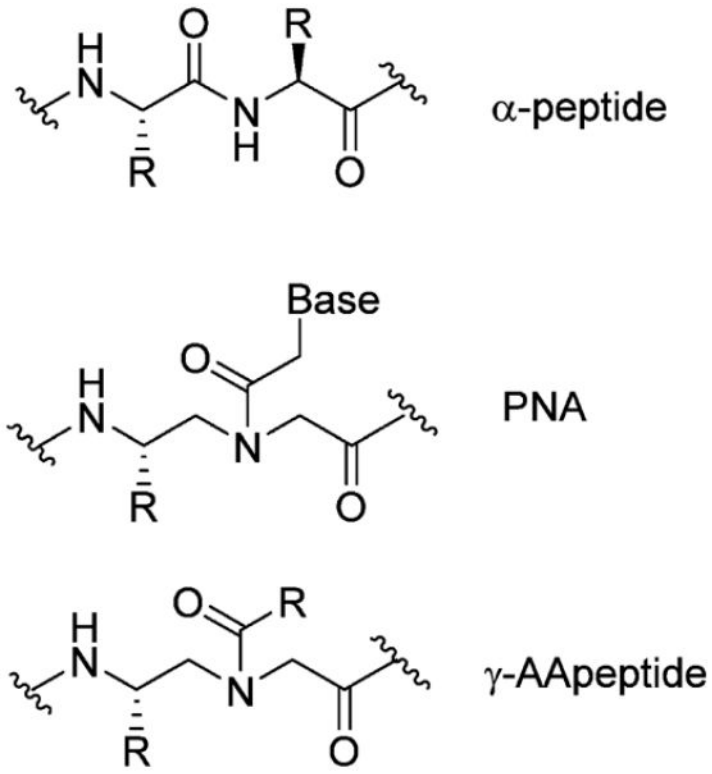


Figure 1.
Chemical structure of α-peptide, chiral PNA, and γ-AApeptide.

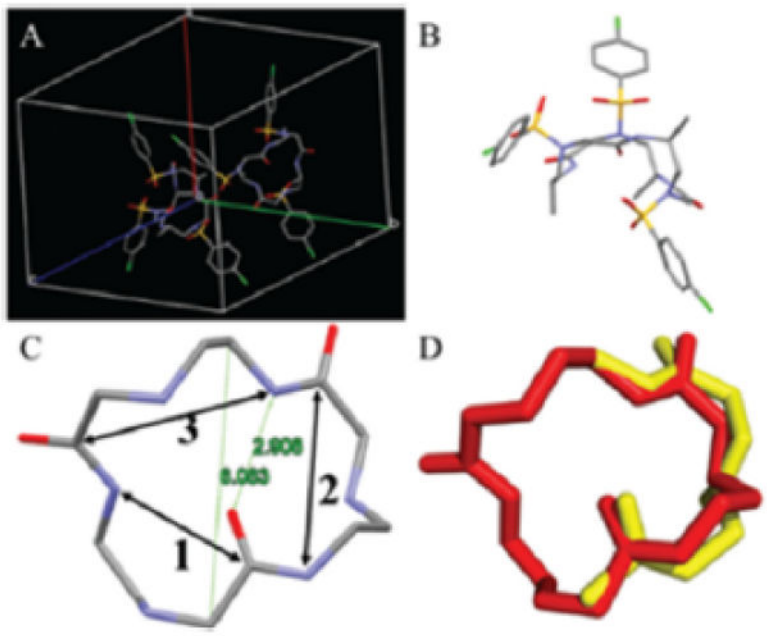


Figure 2.

Crystal structure of **γ-AA2**. Reproduced with permission from ref 17. Copyright 2015 Royal Society of Chemistry.

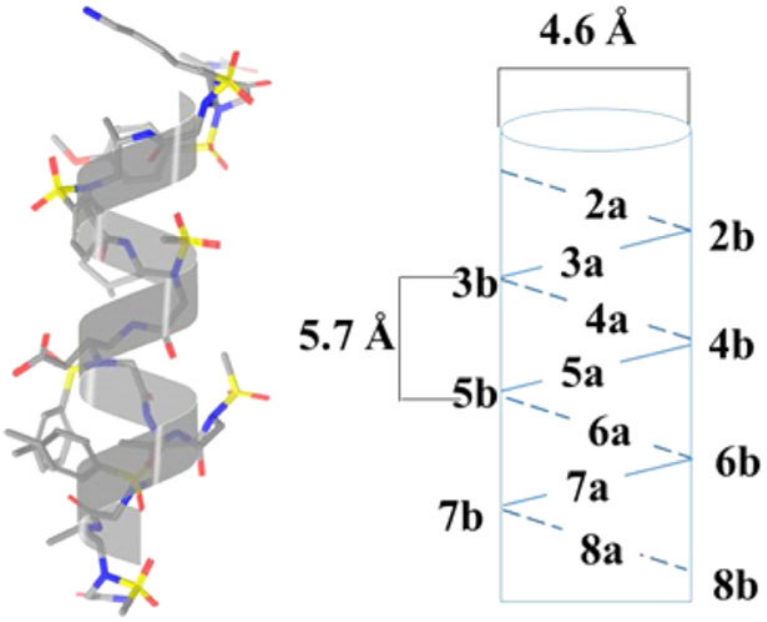


Figure 3.

Helical structure of **γ-AA5**. Reproduced with permission from ref 23. Copyright 2015 John Wiley & Sons, Inc.

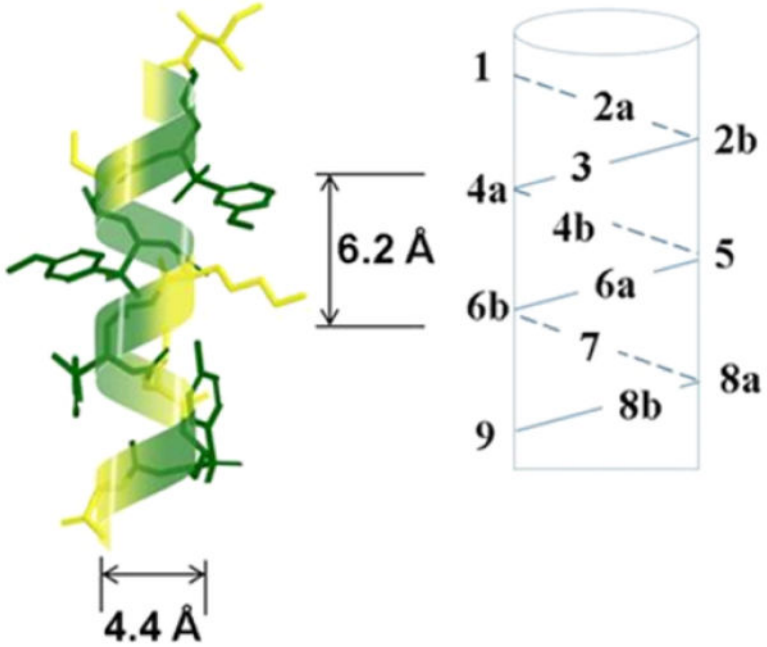


Figure 4.
Helical structure of **γ-AA6**. Reproduced with permission from ref 24. Copyright 2015 American Chemical Society.

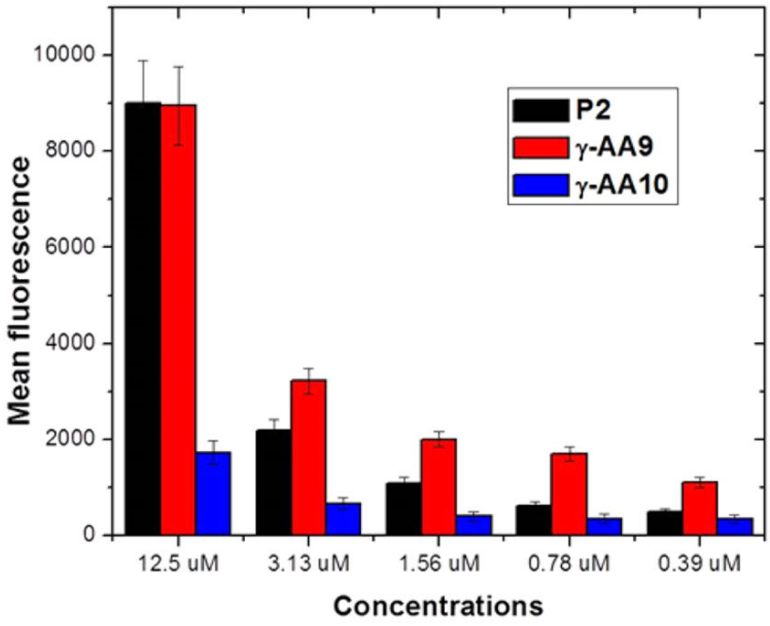


Figure 5.

Flow cytometry study of the Tat peptide analogue. Reproduced with permission from ref 30. Copyright 2012 American Chemical Society.

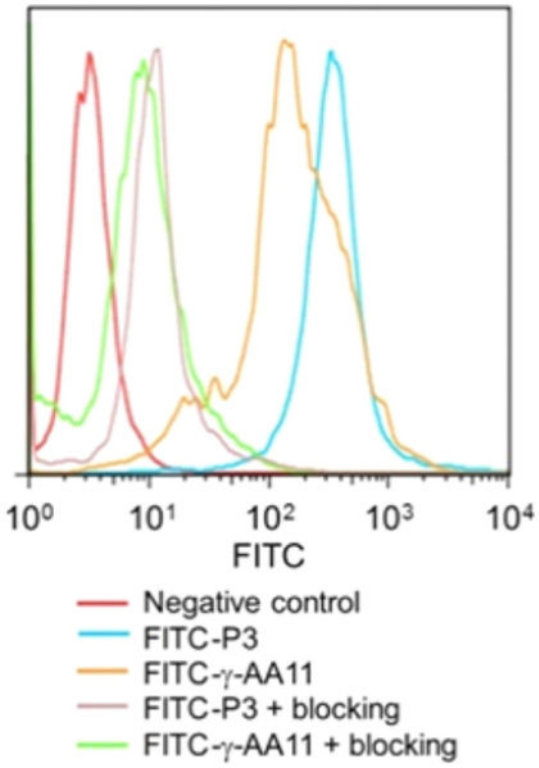


Figure 6.
Flow cytometry analysis of FITC-conjugated γ-AApeptides. Reproduced with permission from ref 33. Copyright 2012 Royal Society of Chemistry.

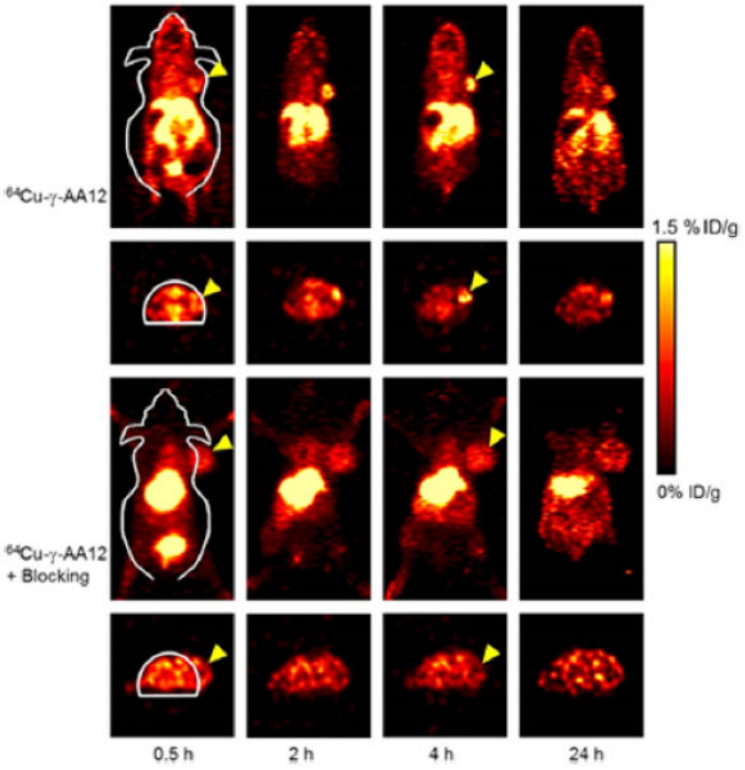


Figure 7.

Serial PET imaging and biodistribution studies of **^{64}Cu-γ-AA12**. Reproduced with permission from ref 33. Copyright 2012 Royal Society of Chemistry.

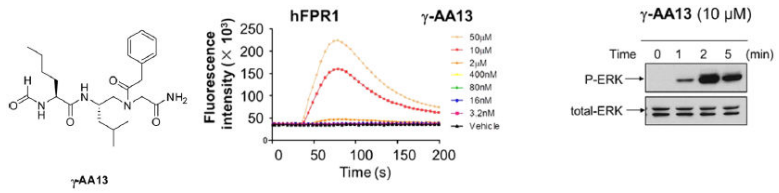


Figure 8.

Induction of calcium mobilization of **γ-AA13** and activation of ERKs. Reproduced with permission from ref 37. Copyright 2014 John Wiley & Sons, Inc.

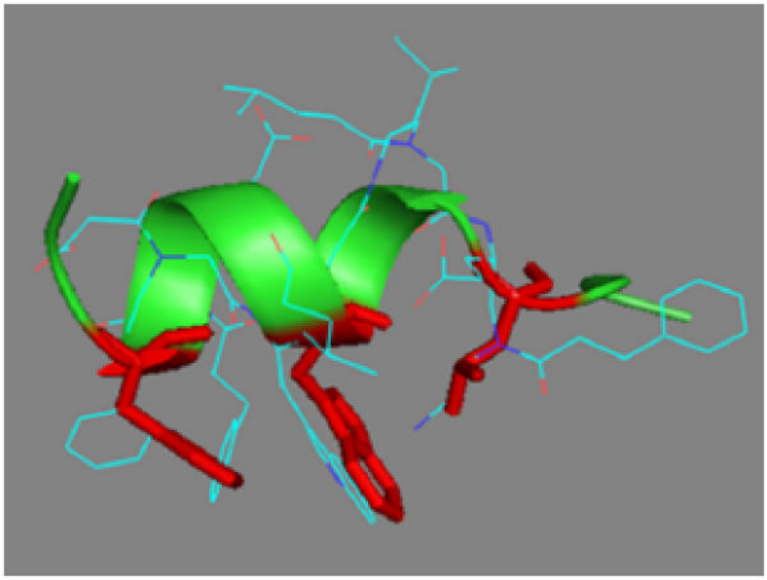


Figure 9.

Energy-minimized structure (MM2) of **γ-AA14**. Reproduced with permission from ref 14. Copyright 2011 Royal Society of Chemistry.

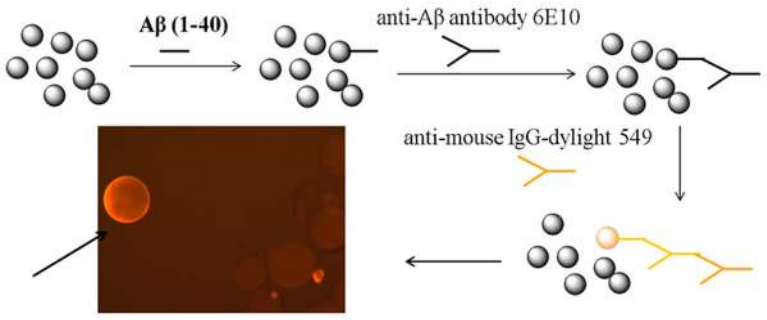


Figure 10.

Schematic representation of the on-bead screening of the γ-AApeptide library against the $A\beta_{40}$ peptide. Reproduced with permission from ref 58. Copyright 2014 Royal Society of Chemistry.

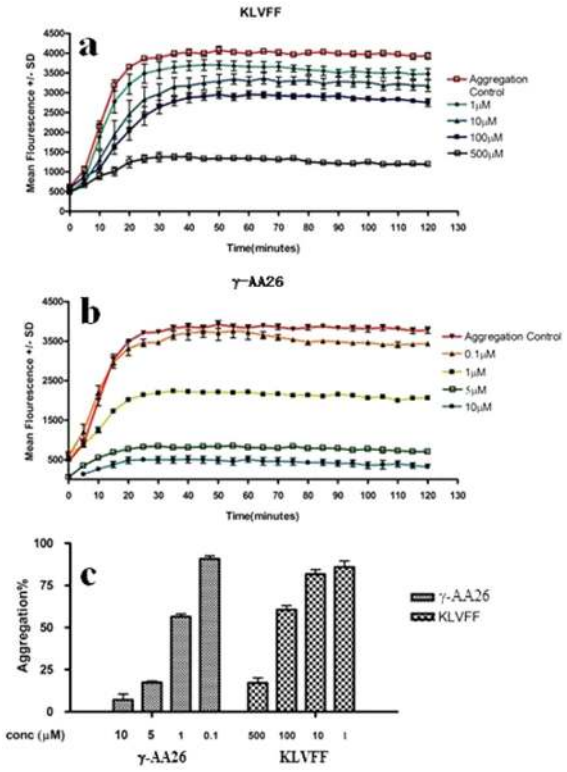


Figure 11.

ThT assay of compounds against $A\beta_{40}$. Reproduced with permission from ref 58. Copyright 2014 Royal Society of Chemistry.

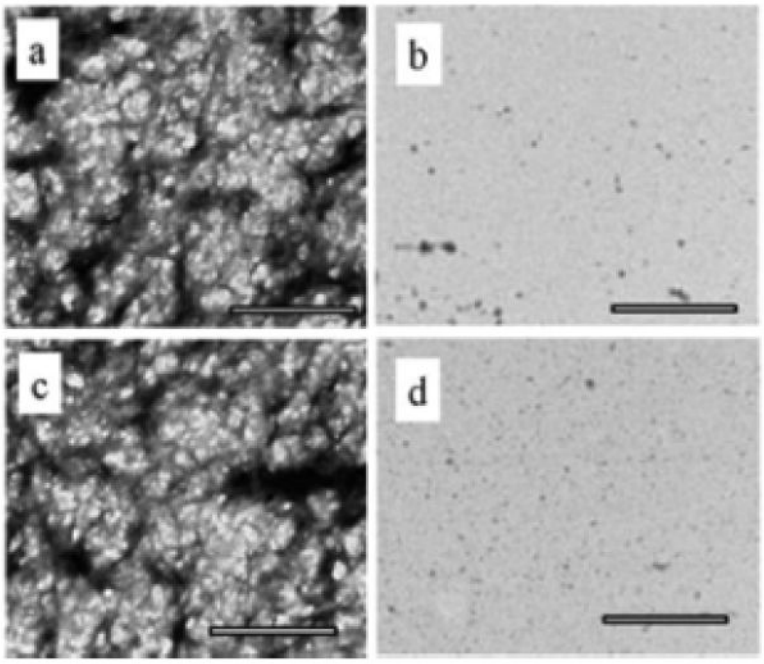


Figure 12.
TEM images. Reproduced with permission from ref 58. Copyright 2014 Royal Society of Chemistry.

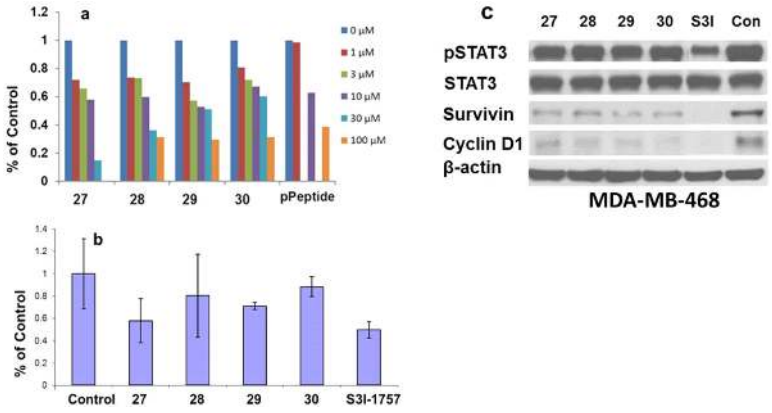
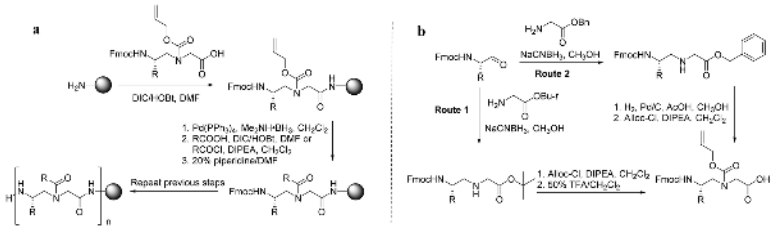
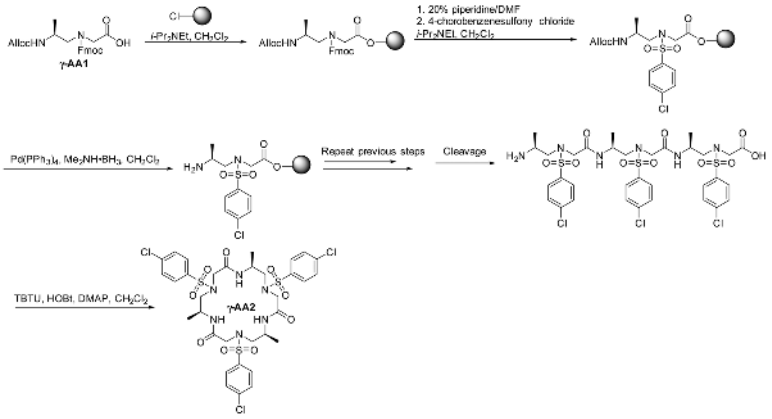


Figure 13.

DNA–STAT3 binding assay (a and b) and cell signaling assay (c). Reproduced with permission from ref 62. Copyright 2014 Royal Society of Chemistry.

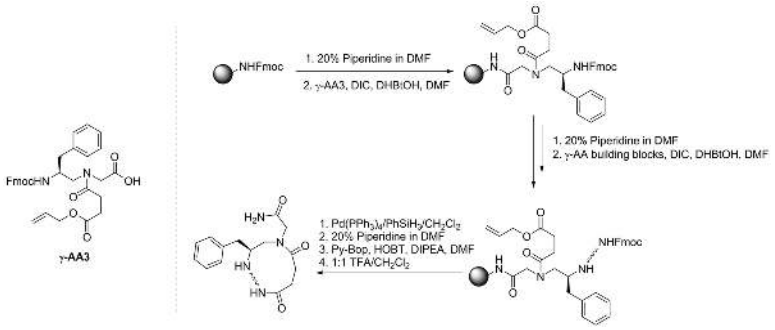


Scheme 1.
Synthesis of Linear γ-AApeptides (a) and γ-AApeptide Building Blocks (b)

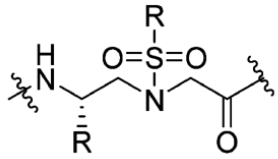


Scheme 2.

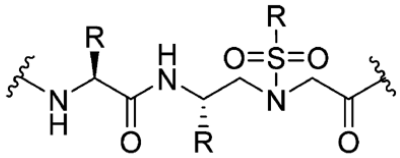
Preparation of a Head-to-Tail Cyclic-γ-AApeptide γ-AA2



Scheme 3.
Strategy of the Head-to-Side Chain Cyclization



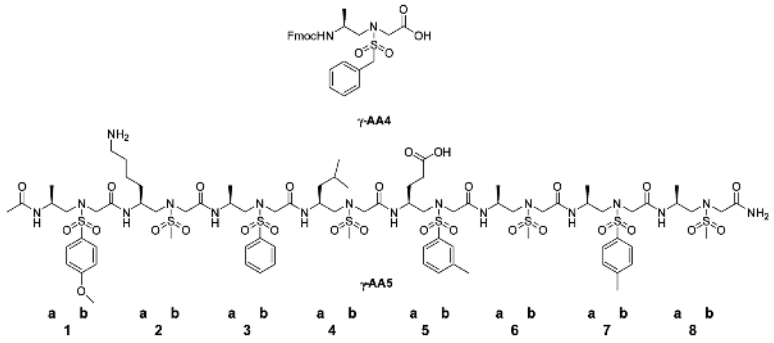
Sulfono-γ-AApeptides



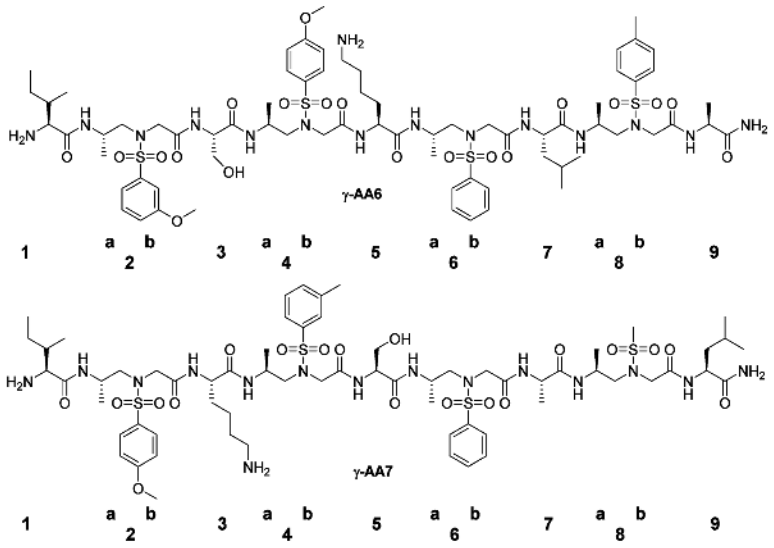
α/Sulfono-γ-AApeptides

Scheme 4.

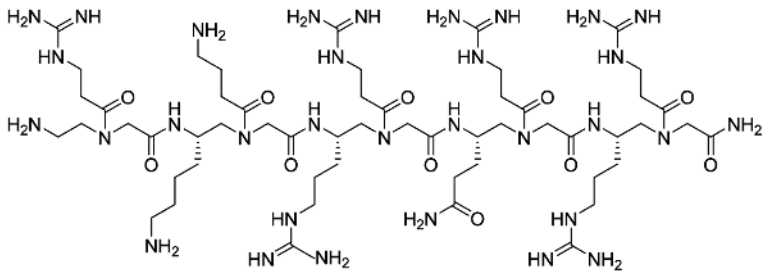
Sulfono-γ-AApeptide and 1:1 α/Sulfono-γ-AA Hybrid



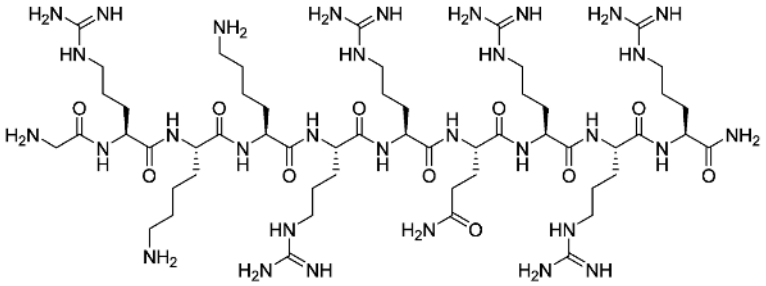
Scheme 5.
Chemical Structure of the Sulfono-γ-AApeptides



Scheme 6.
Chemical Structure of the α/Sulfono-γ-AApeptides

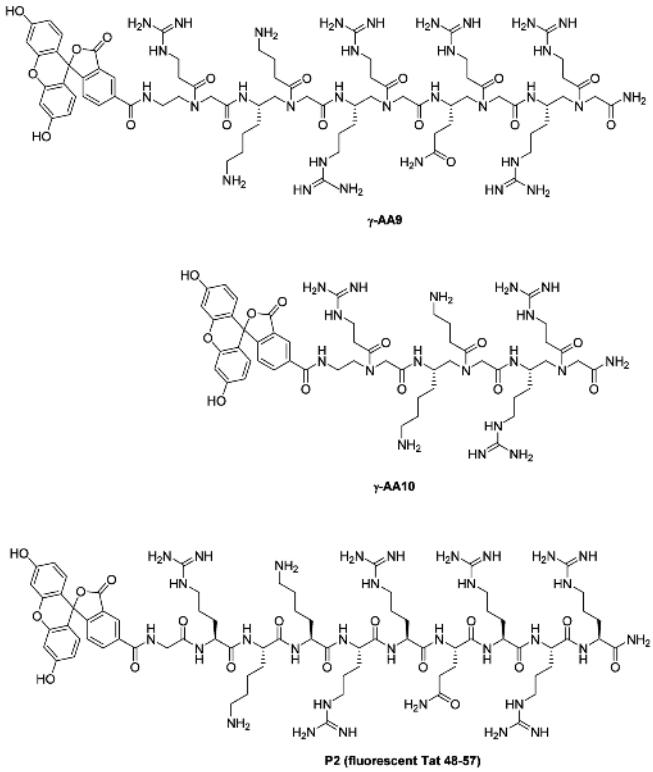


γ-AA8

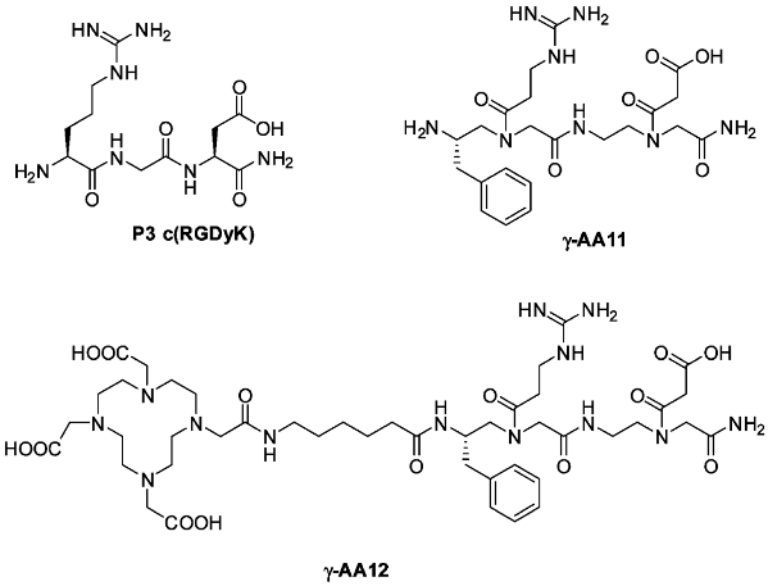


P1 (Tat 48-57)

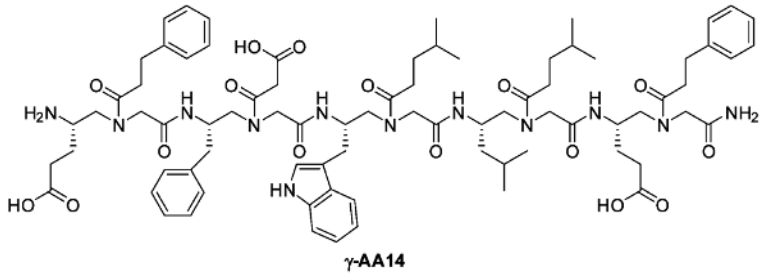
Scheme 7.
Chemical Structure of γ-AA8 and Control P1 Tat 48-57



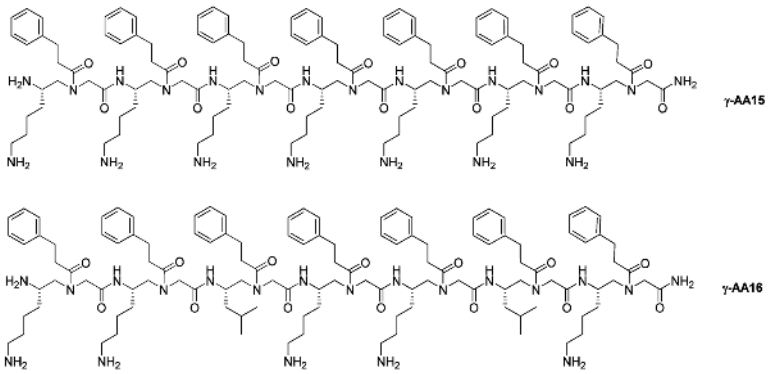
Scheme 8.
Chemical Structures γ-AA9, γ-AA10, and control P2 (Fluorescence-Labeled Tat 48-57)



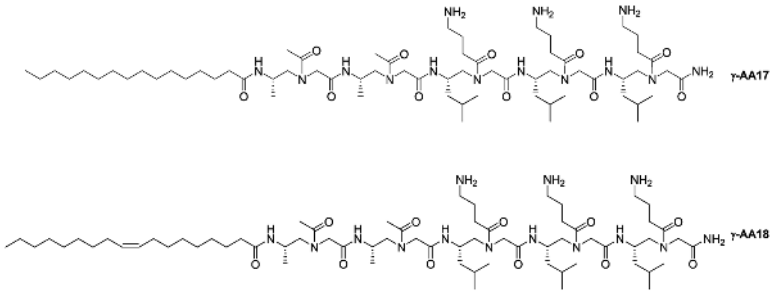
Scheme 9.
Chemical Structure of γ-AA11, γ-AA12, and Control c(RGDyK) P3



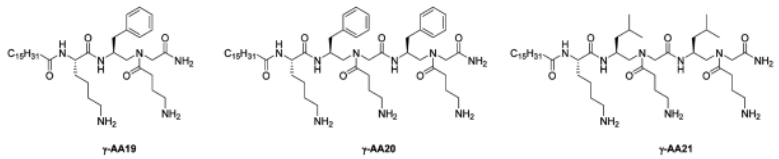
Scheme 10.
Chemical Structure of γ-AA14



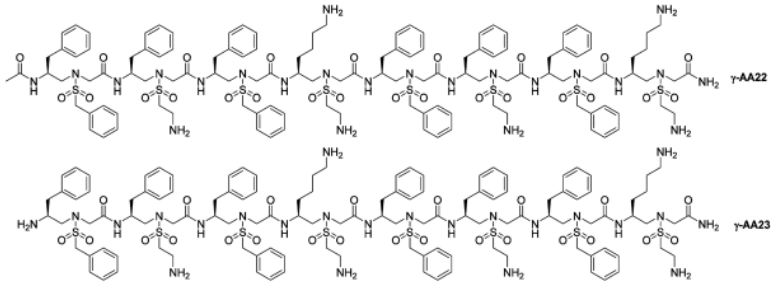
Scheme 11.
Chemical Structures of γ-AA15 and γ-AA16



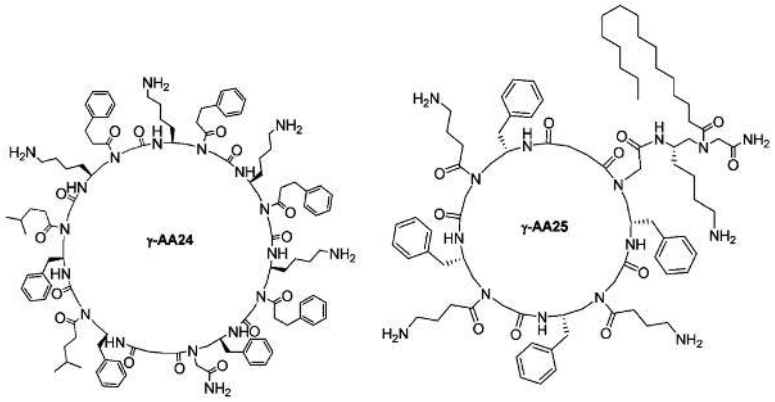
Scheme 12.
Chemical Structures of γ-AA17 and γ-AA18



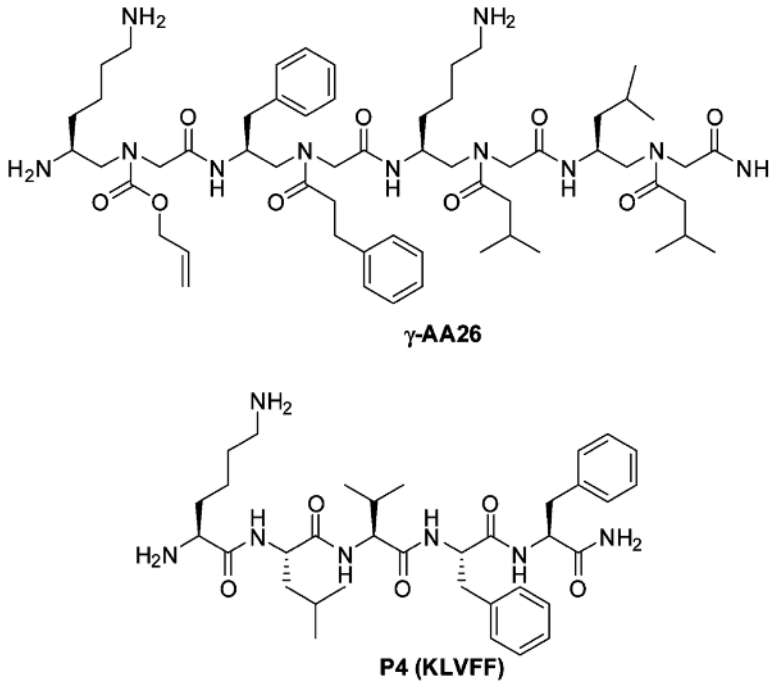
Scheme 13.
Chemical Structures of γ-AA19, γ-AA20, and γ-AA21



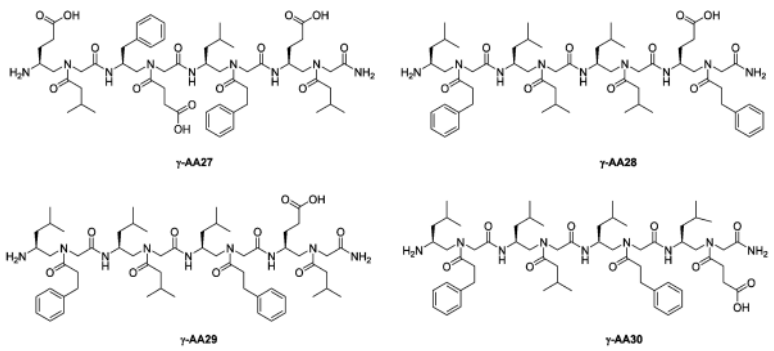
Scheme 14.
Chemical Structures of γ-AA22 and γ-AA23



Scheme 15.
Chemical Structure of γ-AA24 and γ-AA25



Scheme 16.
Chemical Structure of γ-AA26 and KLVFF P4



Scheme 17.
Chemical structures of γ-AA27, γ-AA28, γ-AA29, and γ-AA30

Table 1

Antimicrobial and Hemolytic Activities of γ-AApeptides

	MIC (μg/mL)								
organism	**γ-AA15**	**γ-AA16**	**γ-AA17**	**γ-AA18**	**γ-AA19**	**γ-AA20**	**γ-AA21**	**γ-AA22**	**γ-AA23**
Gram-negative									
E. coli	5	5	2.5	3	4	4	4	4	4
P. aeruginosa	>50	>50	5	3	2	2	2	6	4
Gram-positive									
MRSE	6	5	4	3	2	2	2	2	2
E. faecalis	15	5	5	4	2	2	2	2	2
MRSA	15	5	4	3	2	2	2	4	2
hemolysis (H50)	>500	300	>500	>500	150	250	350	75	100

Table 2

Antimicrobial and Hemolytic Activities of γ-AA24 and γ-AA25

	MIC (μg/mL)							
	MRSA	**MRSE**	***E. faecalis***	***B. subtilis***	***E. coil***	***K. pneumoniae***	***P. aeruginosa***	**hemolysis (HC_{50}, μg/mL)**
γ-AA24	1	2	5	1		8	8	100
γ-AA25	2	1	3		2	3	5	100