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A Potent Integrin Antagonist from a Small Library of Cyclic RGD Pentapeptide Mimics Including Benzyl-Substituted Azabicycloalkane Amino Acids

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A small library of cyclic RGD pentapeptide mimics, including benzyl-substituted azabicycloalkane amino acids, was synthesized with the aim of developing active and selective integrin antagonists. In vitro binding assays established one particular compound with affinity for both the $\alpha_v\beta_3$ and the $\alpha_v\beta_5$ integrins. The synthesis in solution and the in vitro screening of these RGD derivatives, as well as the determination of the conformational properties of the integrin ligands by spectroscopic and computational methods are described.

Introduction

Integrins constitute a large family of heterodimeric cell-surface transmembrane receptors, and play a major role in cell–cell and cell–matrix adhesive interactions.[1] These receptors are composed of one α and one β subunit, both characterized by a large N-terminal extracellular domain, a transmembrane domain, and a short C-terminal intracellular tail. Eighteen α and eight β mammalian subunits are known, which assemble noncovalently into more than 24 different heterodimers.[2] Integrins are involved in fundamental cellular processes and also contribute to the genesis and/or progression of many common diseases, including neoplasia, tumor metastasis, tumor-induced angiogenesis, immune dysfunctions, viral infections, osteoporosis, inflammatory disorders, and coagulopathies.[3] The most common integrins recognize the tripeptide sequence Arg-Gly-Asp (RGD), found in many extracellular matrix adhesive proteins.[4] Although RGD peptides inhibit ligand binding to integrins with a RGD recognition specificity, these receptors can discriminate between RGD containing ligands. The context of the RGD sequence (flanking residues, 3D presentation, and individual features of the integrin binding pockets) determines specificity and efficacy of interaction.[5] It is, therefore, a major challenge to identify compounds that can discriminate between RGD-binding integrins implicated in human pathologies.

Among such RGD-dependent integrins, the vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have received increasing attention as therapeutic targets because of their critical role in tumor-induced angiogenesis and metastasis formation.[6]

Endothelial cells in the angiogenic vessels within solid tumors express several proteins that are absent or hardly detectable in established blood vessels, including integrins and receptors for certain angiogenic growth factors. For instance, $\alpha_v\beta_3$ is generally not expressed on normal endothelial cells (EC), but it is significantly upregulated on activated EC and in metastatic tumor cells. Furthermore, antagonists of $\alpha_v\beta_3$ including cyclic RGD peptides and monoclonal antibodies, significantly inhibit vessel development and tumor growth induced by cytokines and solid tumor fragments.[7] Notably, $\alpha_v\beta_3$ antagonists have very little effect on pre-existing blood vessels, indicating the usefulness of targeting this receptor for therapeutic benefit without adverse side effects. Recent studies have focused on the role of the related integrin $\alpha_v\beta_5$ in angiogenesis. Apparently, there are two major angiogenic pathways activated by two different growth factors and mediated by $\alpha_v\beta_3$ and $\alpha_v\beta_5$, respectively.[8] Therefore, selective antagonists of $\alpha_v\beta_3$

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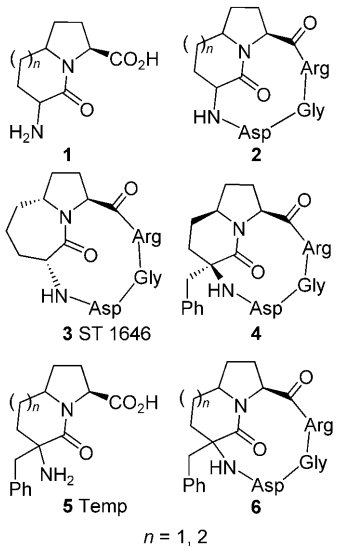
and/or $\alpha_v\beta_5$ may be useful in blocking tumor-induced angiogenesis.

In the past, one major problem in the design of potent and selective $\alpha_v\beta_3$ integrin ligands has been the lack of detailed structural information on the interaction between $\alpha_v\beta_3$ integrin receptor and its ligands. Until recently, drug design efforts have relied on pharmacophore hypotheses derived from the structures of known ligands. Three pharmacophoric regions have been identified: 1) a carboxylate group, 2) a guanidinium moiety, and 3) a hydrophobic group.[9] With this model it was possible to design highly potent ligands, but many uncertainties regarding details of the receptor–ligand interaction remained. In recent years, the crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$[10] in complex with the cyclic pentapeptide ligand cyclo(Arg-Gly-Asp-D-Phe-[*N*-Me]Val) (EMD121974) has been reported.[11] The crystal structure of the peptide–integrin complex provides the exact conformation of EMD121974 bound to $\alpha_v\beta_3$ integrin and can serve as a basis for understanding the general mode of interaction of integrins with other RGD-containing ligands.

Cyclic RGD peptides have been developed by different groups as active and selective integrin antagonists that compete with matrix molecules for specific integrin receptors.[12] The conformational constraints imposed by the cyclic template has been shown to be a valuable tool in the indirect determination of the bioactive conformation. The pioneering work in this field, by Kessler and co-workers[13] has led to a highly active $\alpha_v\beta_3$-selective first generation cyclic pentapeptide cyclo-(Arg-Gly-Asp-D-Phe-Val). Extensive modifications of this lead structure with different peptidomimetics and carbohydrate scaffolds have been performed, and new potent antagonists have been identified.[14] Systematic derivatization of the lead peptide resulted in the N-alkylated cyclopeptide cyclo(Arg-Gly-Asp-D-Phe-[*N*-Me]Val)[15] which has entered clinical phase II studies as an angiogenesis inhibitor.[16] Our group has reported a library of cyclic RGD pentapeptide mimics based on 1-aza-2-oxobicyclo[X.3.0]alkane amino acids.[17] Stereoisomeric 6,5- and 7,5-fused bicyclic lactams **1** with different reverse-turn mimetic properties were exploited as dipeptide analogues for the synthesis of a library of general formula cyclo(Arg-Gly-Asp-Temp) **2** (shown).[18] The replacement of the D-Phe-Val dipeptide in the lead structure cyclo(RGDfV) with such azabicycloalkane scaffolds, inducing different conformational preferences of the RGD sequence, provide the required activity and selectivity for integrin antagonism.[19] This library was found to contain specific high-affinity ligands for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which are presently being evaluated as very promising antiangiogenic drugs. Among the peptides tested, **3** (ST1646) showed the highest affinity to $\alpha_v\beta_3$ and a high affinity to $\alpha_v\beta_5$, inhibiting echistatin binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ with an IC_{50} value of 3.7 ± 0.6 nM and 1.39 ± 0.2 nM, respectively.[18–20]

To further address the process of spatial screening, the original library was expanded to include new members, modified at the scaffold moiety either by introducing a hydrophobic substituent or enlarging the lactam ring.[21] A preliminary work in this direction led to compound **4** showing affinity and selectivity towards the $\alpha_v\beta_5$ integrin receptor with an IC_{50} value of 4.12 ± 1.1 nM.[21] The promising preliminary data suggested us to employ the whole collection of peptidomimetic scaffolds **5**,[22,23] featuring a quaternary stereogenic center and a benzyl group for the preparation of a new library of integrin inhibitors **6**.



Herein, we report the synthesis and the results of in vitro testing of these new RGD cyclic pentapeptides to compete for the binding of [^{125}I]echistatin to purified $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. Moreover, the effects of such structural changes on the conformation of the RGD sequence and the ability of the highest affinity ligand to adopt the proper RGD orientation required for binding to the $\alpha_v\beta_3$ integrin have been investigated by means of NMR spectroscopy, molecular mechanics, and docking calculations.

Results and Discussion

Chemistry

Synthesis of the compounds of general formula **6** required the preparation of scaffolds **7**–**13** (shown). These were obtained through known synthetic procedures based on selective alkylation of suitably protected azabicycloalkanes[22] or by ring-closing metathesis protocols on suitably protected allyl prolines.[23]

With these precursors in hand we started the synthesis of the cyclic-RGD compounds. Previously, cyclic pseudopeptides have been prepared by solid-phase synthesis.[18,19] However, the use of large quantities of resins was shown to be troublesome and expensive, precluding the application of this methodology to large scale production. A solution phase method was therefore adopted.

On the basis of our previous experience, the connection of the RGD sequence to the pseudopeptides **7**–**13** was carried out by first coupling a suitably protected NH2-Arg-Gly-OH dipeptide to the carboxy function of the proline unit followed by a second coupling of an Asp residue to the N terminus of the dipeptide mimic. The resulting linear peptide sequences **14**–**20** were then cyclized to give the fully protected RGD-con-

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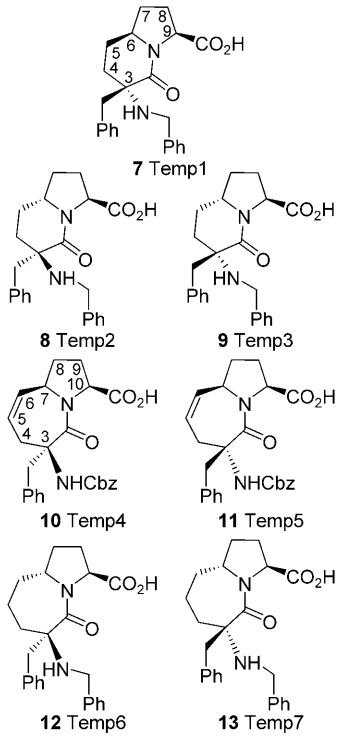
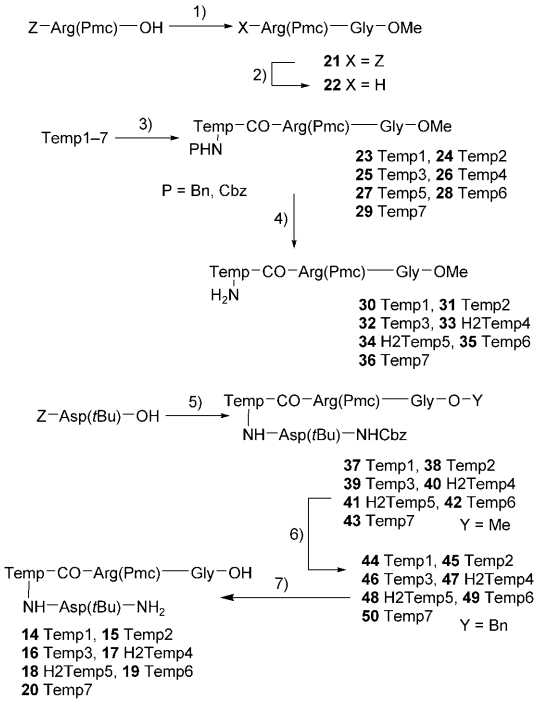
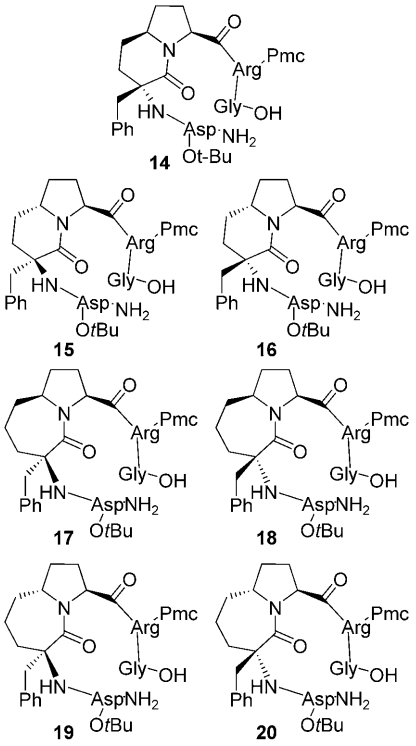


taining pentapeptides **51**–**54**. The coupling between the Gly and Asp residues was envisaged as the final cyclization step taking into account the decreased steric congestion of the Gly reacting center.

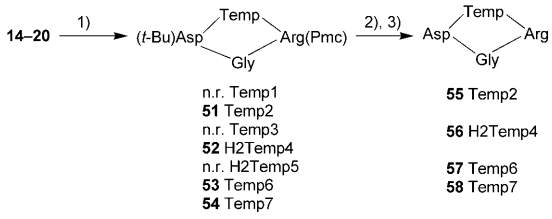
The Arg and Asp side chains were protected using the Pmc (2,2,5,7,8-pentamethylcroman-6-sulfonyl) and *tert*-butyl groups, respectively. Both these protecting groups are removed under acidic conditions and thus are compatible with the Cbz or Bn strategy. Amino acid coupling was carried out using the mixed anhydride prepared with isobutyl chloroformate, whereas HATU and HOAt were used for the final cyclization. H-Gly-OMe was condensed with Z-Arg(Pmc)-OH to obtain dipeptide **21** that was hydrogenated to give a free amino group ready for a new condensation. Iteration of this protocol gave the linear fully protected pseudo-pentapeptides **14**–**20** (Scheme 1).



Scheme 1. Reagents and conditions: 1) *i*BuOCOCl, NMM, THF, −20 °C, H-Gly-OMe·HCl, 96%; 2) H_2, Pd/C, MeOH; 3) *i*BuOCOCl, NMM, THF, −20 °C, **Temp1**–**7**, 88–98% over two steps; 4) H_2, Pd/C, MeOH; 5) *i*BuOCOCl, NMM, THF, −20 °C, **23**–**29**, 71-88% over two steps; 6) BnOH, Ti(*i*PrO)$_4$, THF, 90 °C, 79–91%; 7) H_2, Pd/C, MeOH.



The low yield observed in the Gly methyl ester hydrolysis with standard basic conditions forced us to change the carboxylic acid protecting group. Ti(O*i*Pr)$_4$-catalyzed transesterification with benzyl alcohol gave **44**–**50** with two simultaneously removable protecting groups. Hydrogenation and cyclization provided protected cyclic pseudo-pentapeptides **51**–**54** in 64–78% yield over two steps (Scheme 2). In contrast, peptides **14**,



Scheme 2. Reagents and conditions: 1) HATU, HOAt, DIPEA, DMF, 64–78% over two steps; 2) TFA, thioanisole, 1,2-ethanedithiol, anisole; 3) $HCl_{(g)}$, 71–96% over two steps.

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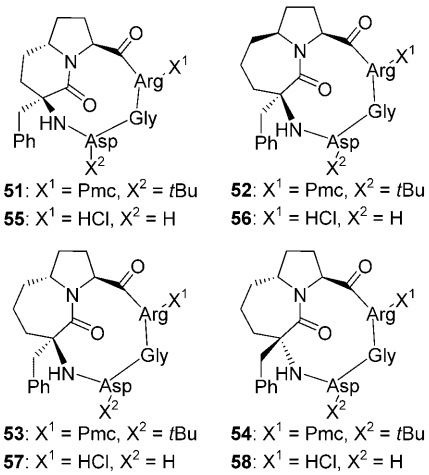
16, and **18** failed to cyclize under the above conditions. Changing the base, or warming the reaction to 40 °C did not show any beneficial effect on the reaction yield. This behavior is probably due to the unfavorable conformation adopted in solution of these compounds. The side-chain Pmc and *t*Bu protective groups were removed with TFA in the presence of ion scavengers. The resulting TFA salts were purified by crystallization and by HPLC and then transformed into the corresponding chlorides **55**–**58** with gaseous HCl (Scheme 2).



Biological evaluation

The cyclic pentapeptides **55**–**58** were examined in vitro for their ability to compete with [^{125}I]echistatin for binding to the purified $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors (Table 1). It has been demonstrated that both purified and membrane-bound integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ bind with very high affinity to echistatin, which can be inhibited efficiently by linear and cyclic RGD-containing peptides.[24] Affinities of compounds c(RGDfV), EMD 121974, and ST1646 for the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins were determined as reference in the same assays. Interestingly, the affinity of all the new compounds for the $\alpha_v\beta_3$ integrin was higher than the affinity of the previously synthesized compound **4**.

Table 1. Inhibition of [^{125}I]echistatin binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors.[a]

Entry	Compound	IC_{50} [nM] for $\alpha_v\beta_3$	IC_{50} [nM] for $\alpha_v\beta_5$
1	echistatin	0.28 ± 0.08	0.29 ± 0.02
2	c(RGDfV)	195.9 ± 16.8	0.11 ± 0.03
3	EMD 121974	18.9 ± 3.1	0.13 ± 0.01
4	ST1646	3.7 ± 0.6	1.39 ± 0.2
5	**4**	787.1 ± 54.6	4.12 ± 1.1
6	**55**	75.7 ± 1.6	325.6 ± 20.3
7	**56**	6.4 ± 0.1	7.7 ± 0.04
8	**57**	190.4 ± 19.5	221.9 ± 24.7
9	**58**	154.2 + 12.7	242.6 ± 24.6

[a] IC_{50} values were calculated as the concentration of compound required for 50% inhibition of echistatin binding as estimated by the Allfit program. All values are the mean (±SD) of triplicate determinations.

Among the five benzyl-substituted peptides, compound **56** showed the highest affinity to $\alpha_v\beta_3$ integrin and inhibited radiolabeled echistatin binding to $\alpha_v\beta_3$ with an IC_{50} value of 6.4 ± 0.1 nM. Like echistatin and compound ST1646, the cyclopeptide **56** showed similar affinities to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, whereas c(RGDfV), EMD121974, and compound **4** are over 100-fold more selective for $\alpha_v\beta_5$ integrin in this kind of assay.

To assess the activity of **56** as an integrin antagonist for endothelial cells, human umbilical vein vascular endothelial cells (HUVEC) were allowed to adhere to immobilized vitronectin in the presence of increasing concentrations of the compound (ranging between 25 nM and 200 μM). As shown in Figure S1 (Supporting Information), **56** significantly inhibited cell adhesion to vitronectin with IC_{50} value of 9.8 μM + 0.04.

Computational studies

Monte Carlo/energy minimization (MC/EM) conformational searches[25] designed to investigate the effects of the benzyl-substituted bicyclic lactam on the cyclopeptide conformation were performed on the cyclo(Ala-Gly-Ala-Temp) pentapeptide analogues **55a**–**58a** (Table 2). The principal types of backbone geometries calculated for these compounds and their relative stabilities are given in Table 2. The minimum-energy conformations of these cyclic pentapeptide mimics are characterized by the formation of peptide secondary structures, in particular β- and γ-turns, that may be stabilized by intramolecular hydrogen bonds. As shown in Table 2, the same four significant geometries (types SI-SIV) calculated for ligands of the general formula **2**[19] can be detected among the conformers within 3 kcal mol^{-1} of the global minimum of compounds **55a**–**58a**. As an example, the conformers calculated for compound **55a** are reported in Figure 1 to show the four cyclopeptide β/γ-turn arrangements.

Moreover, a strong dependence of the preferred cyclopeptide conformations on lactam ring size and stereochemistry can be observed. For instance, the SI structural type (corresponding to the strongest kinked RGD conformation and the shortest CβArg-CβAsp distance) can be detected only among the conformers of compounds **55a** and **57a**.

MC/SD simulations[26] of the cyclic RGD pentapeptide mimics **55**–**58** confirmed these results, showing the SI conformation taking part only in the conformational equilibrium of **55** and **57**. During the 10 ns MC/SD simulations in GB/SA water of compound **58** only the type SIV β/γ-turn arrangement (corresponding to the most extended RGD conformation and the highest CβArg-CβAsp distance value) was sampled, in agreement with the results provided by the MC/EM searches, whereas SII (30%), SIII (15%), and SIV (35%) conformations are populated during the simulations of compound **56**.

The molecular model of the interaction between the highest affinity ligand **56** and the $\alpha_v\beta_3$ receptor was built by means of docking calculations. The protein binding site was derived from the X-ray crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$ in complex with the cyclic pentapeptide ligand EMD121974.[11]

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Table 2. Characteristics of low-energy conformers (MC/EM, AMBER*, H_2O GB/SA) calculated for cyclic pentapeptide mimics **55a**–**58a** cyclo(Ala-Gly-Ala-Temp).[a]

Compound[b]	ΔE [kcal mol^{-1}]	Conformation	CβArg–CβAsp distance [Å]
55a (14)	0.0	Type SI; βII′(Temp)/γ(Gly)	7.6
	0.3	Type SII; γ(Gly)/βII′(Gly-Asp)[c]	8.2
	0.6	Type SIV; Inv.γ(Asp)/βI(Pro-Arg)	9.4
	1.6	Type SIII; Inv.γ(Asp)/βII′(Gly-Asp)[c]	8.6
56a (10)	0.0	Type SII; γ(Gly)/βII′(Gly-Asp)[c]	8.2
	1.0	Type SIV; Inv.γ(Asp)/βI(Pro-Arg)	9.4
	2.3	Type SIII; Inv.γ(Asp)/βII′(Gly-Asp)[c]	8.7
57a (13)	0.0	Type SII; γ(Gly)/βII′(Gly-Asp)[c]	8.2
	2.5	Type SI; βII′(Temp)/γ(Gly)	7.7
	2.6	Type SIV; Inv.γ(Asp)/βI(Pro-Arg)	9.4
58a (2)	0.0	Type SIV; Inv.γ(Asp)/βI(Pro-Arg)	9.3

[a] The lowest-energy conformer of each conformational family within 3 kcal mol^{-1} of the global minimum is described. [b] In parentheses: number of conformers <3 kcal mol^{-1}. [c] Distorted type II′ β-turn.

The crystal structure of the peptide–integrin complex provides the actual conformation of EMD121974 bound to the $\alpha_v\beta_3$ integrin active site and can serve as a basis for understanding the general mode of interaction of integrins with other RGD-containing ligands. Examination of the 3D structure of the cyclic pentapeptide ligand EMD121974 bound to the $\alpha_v\beta_3$ integrin receptor (PDB code: 1L5G) reveals a conformation characterized by an inverse γ-turn with Asp at position $(i+1)$ and by a distorted II′-βturn with Gly and Asp at the $(i+1)$ and $(i+2)$ positions, respectively. An 8.9 Å distance between the Asp and Arg Cβ atoms and an almost extended conformation of the RGD sequence are observed in this pentapeptide bound conformation.

The most important interactions in the ligand binding pocket involve the positively charged Arg guanidinium group of the ligand and the negatively charged side chains of Asp 150 and Asp 218 in the α subunit as well as one of the Asp carboxylate oxygen atoms of the ligand and the metal cation in the metal-ion-dependent adhesion site (MIDAS) region of the β subunit. Further stabilization could occur through H-bonds between the backbone NH of the Asp residue and the backbone carbonyl oxygen of Arg 216 in the β subunit as well as between the second Asp carboxyl oxygen and the backbone amides of Asn 215 and Tyr 122 in the β subunit. Moreover, the EMD121974 conformation also ensures an orientation of the central Gly residue in close contact with the integrin surface.

Starting from this complex, structural models for the interaction of compound **56** in the ligand binding site of the $\alpha_v\beta_3$ receptor were generated by automated computational docking using the ligand docking protocol of Glide[27] after removal of the EMD121974 cyclopeptide. The automated docking calculations were performed starting from the three representative cyclopeptide backbone conformations of compound **56** (γ-(Gly)/β(Gly-Asp), Invγ(Asp)/β(Gly-Asp), Invγ(Asp)/β (Pro-Arg)) obtained from computational studies (free-state molecular mechanics conformational searches and molecular dynamics simulations). Automated docking calculations starting from two cyclopeptide conformations (Invγ(Asp)/β(Gly-Asp) and Invγ-(Asp)/β(Pro-Arg)) produce poses conserving all the interactions observed in the X-ray crystal structure. As shown in Figure 2, a T-shaped interaction between the aromatic ring of the ligand **56** and the aromatic ring of the Tyr122 side chain can be observed that can enhance the binding affinity. Docking calculations starting from the cyclopeptide conformation γ(Gly)/β(Gly-Asp) result in the loss of all interactions between the ligand and the β subunit.

Moreover, difficulty in maintaining all the crystallographic key interactions with the integrin binding site is revealed by the highly preorganized cyclic pentapeptide mimic **58** in the poses generated by docking calculations starting from the Invγ(Asp)/β(Pro-Arg) conformation of the cyclopeptide backbone. Binding of this ligand, that always adopts an extended RGD conformation suitable to bind the receptor, should be enhanced for entropic reasons; unfortunately, the benzyl group on the quaternary stereogenic center of the azabicycloalkane scaffold interferes with the correct interaction of the ligand in the integrin binding site.

NMR studies

The conformational behavior of the highest affinity ligand **56** was elucidated by ^{1}H NMR experiments. We performed ^{1}H NMR analysis in 3 mM D_2O and H_2O/D_2O (9:1) solutions. The spectra were well resolved and the spectra parameters are reported in the Supporting Information. NMR experiments were conducted with the aim of detecting intramolecular hydrogen bonds by measuring the chemical shift of the N-H protons and their temperature coefficients ($\Delta\delta/\Delta T$).[28] NOESY spectra were recorded to investigate both sequential and long-range NOEs that provide evidence of preferred conformations.

In previous studies,[29] we observed that peptidomimetics incorporating a 7,5-*cis*-fused bicyclic unit are quite flexible, but in compound **56**, the benzylic substituent freezes the conformational equilibrium of the bicyclic scaffold. The analysis of chemical shifts and of the coupling constants of the protons of the scaffold (a detailed table is provided in the Supporting Information) indicates that the bicyclic moiety of cyclopentapeptide **56** exists prevalently in one rigid conformation where the carboxylic and the benzylic substituents are in a pseudo-axial position: the most relevant NOE enhancements were observed between the benzylic protons and H7, and between the aromatic protons and H7 and H10 on the bicyclic scaffold. On the contrary, the RGD moiety can exist in two different preferred conformations which are stabilized by hydrogen bonds, as reported in Figure 3 A and 3 B. The NOESY spectrum of **56** shows NOEs between Gly-NH and Arg-NH (medium) and between Gly-NH and Arg-Hα (medium). These NOE contacts and the

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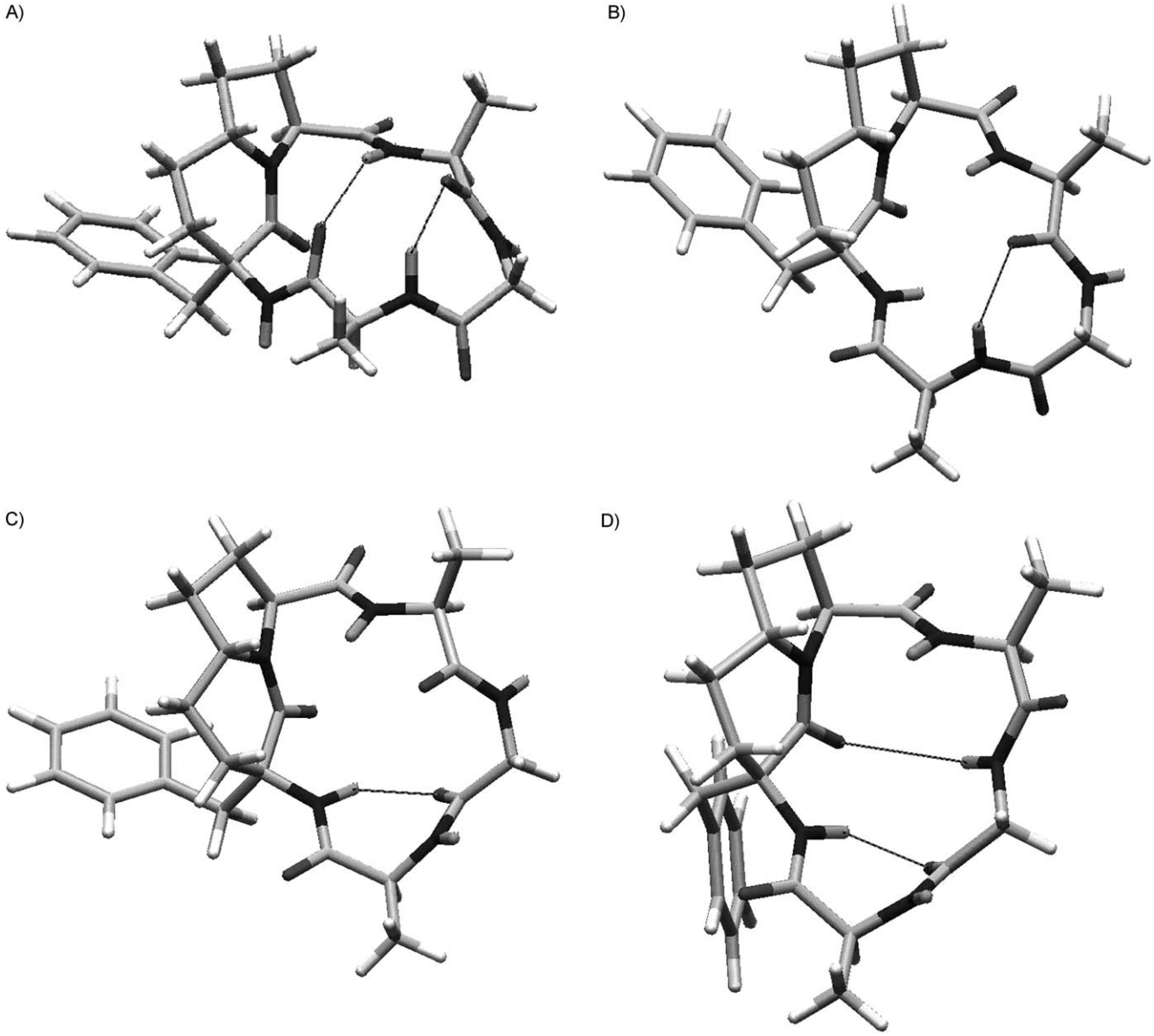

Figure 1. Minimum-energy conformations of compound cyclo(Ala-Gly-Ala-Temp) **55a**: A) Type SI, βII′(Temp)/γ(Gly); B) Type SII, γ(Gly)/distorted βII′(Gly-Asp); C) Type SIII, inverse γ(Asp)/distorted βII′(Gly-Asp); D) Type SIV, inverse γ(Asp)/βI(Pro-Arg). The formation of hydrogen bonds in which the H–O distance is <3.0 Å, the N–H–O bond angle is $>120^\circ$, and the H–O=C angle is $>90^\circ$ is indicated by a thin black line.

chemical shift values of Gly-NH are indicative of a β-turn conformation stabilized by a hydrogen bond between Gly-NH and lactamic C=O (Figure 3A). The chemical shift value (7.78 ppm) and the $\Delta\delta/\Delta T$ of the amide proton Temp-NH indicates that this proton is locked in an intramolecularly H-bonded state; a medium to weak cross-peak between GlyCαH↔Temp4ax is indicative of a close contact between the scaffold and the glycine residue and supports the γ-turn centered on the aspartic residue. The presence of β(Pro-Arg)- and γ(Asp)-turns stabilizes a cyclopeptide geometry characterized by an RGD extended conformation (Figure 3A). All these data provide experimental evidence of the participation of the calculated type SIV cyclopeptide arrangement in the conformational equilibrium of compound **56**.

Moreover, the Temp-NH amide proton can experience a hydrogen bond with Arg C=O (Figure 3B), stabilizing a β-turn with Gly and Asp at the $(i+1)$ and $(i+2)$ positions. The cross-peak Temp-NH↔AspNH supports the existence of this β-turn conformation, combined with a γ-turn centered on the glycine residue, providing experimental evidence for the calculated SII cyclopeptide arrangement.

Conclusions

Four cyclic RGD pentapeptide mimics, incorporating functionalized stereoisomeric bicyclic lactams with different ring size were synthesized to investigate the effects of the benzyl-substituted bicyclic lactams on the cyclopeptide conformations

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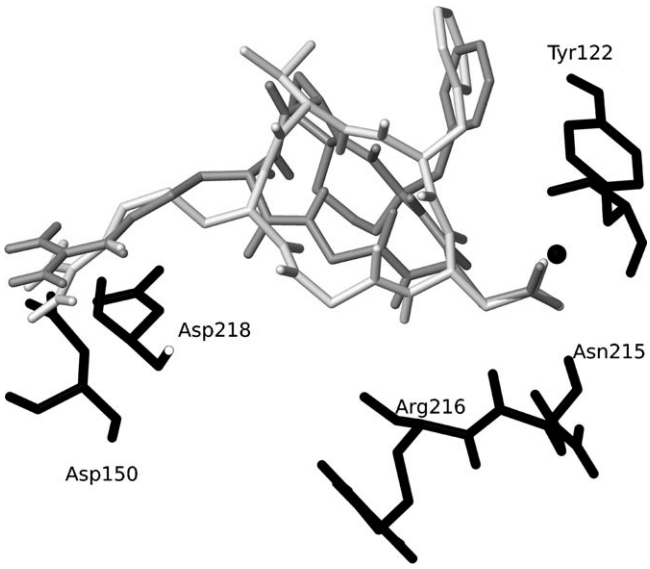

Figure 2. Top-ranked binding mode of ligand **56** (dark gray tube representation, Invγ(Asp)/β(Pro-Arg) conformation of the cyclopeptide backbone) into the crystal structure of the extracellular domain of $\alpha_v\beta_3$ integrin overlaid on the bound conformation of EMD 121974 (light gray tube representation). Selected integrin residues involved in the interactions with EMD 121974 are shown in black (tube representation). The Mn^{2+} ion at MIDAS is shown as a black CPK sphere. Nonpolar hydrogen atoms were removed for clarity.

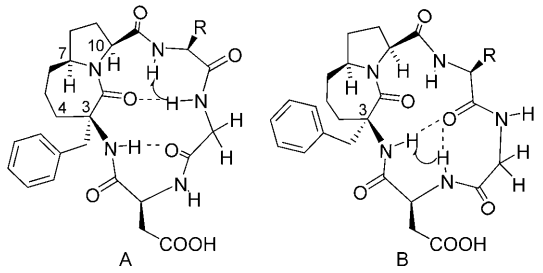

Figure 3. Preferred intramolecular hydrogen bonded patterns proposed for compound **56** on the basis of spectroscopic data. A) γ-turn centered on aspartic acid residue and β-turn at Pro-Arg. B) β-turn at Gly-Asp and γ-turn centered on the glycine residue. The arrows indicate significant NOE contacts.

and binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors. The effects of these modifications on biological activity were investigated and revealed for compound **56** a high affinity toward $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors. In particular compound **56** is slightly less active than ST1646, a potent antagonist previously synthesized in our laboratories, but slightly more active than EMD121974 toward the $\alpha_v\beta_3$ receptor. Compound **56** inhibited cell adhesion to vitronectin with an IC_{50} value similar to that of EMD121974. Structural effects of the bicyclic templates on the conformation of the RGD sequence were examined. The conformational analysis of cyclic RGD peptides containing functionalized azabicycloalkane reverse-turn inducers, performed both by computational methods and NMR studies, showed the presence of three and two main conformations respectively, characterized by β- and γ-turns stabilized by intramolecular hydrogen bonds. The docking of compound **56** in the ligand binding site of the $\alpha_v\beta_3$ receptor showed an additional interaction between the aromatic ring of the aromatic moiety and Tyr122 which can explain the activity of our compound.

Experimental Section

Solid-phase receptor binding assay

The receptor binding assays were performed as described previously.[24] Purified receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (Chemicon International Inc., Temecula, CA, USA) were diluted, respectively, to 500 ng mL^{-1} and 1000 ng well^{-1} in coating buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$). An aliquot of the diluted receptors (100 μL well^{-1}) was added to 96-well microtiter plates and incubated overnight at 4 °C. The coating solution was removed by aspiration and 200 μL of blocking solution coating buffer containing 1% bovine serum albumin (BSA)) was added to the wells, which were incubated for additional 2 h at room temperature. After incubation, the plates were rinsed with 200 μL of blocking solution (3 ×) and incubated with appropriate radiolabeled ligands for 3 h at room temperature. 0.05 nM and 0.1 nM [^{125}I]echistatin (Amersham Pharmacia) was used for the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptor binding assay, respectively. After incubation, the plates were sealed and counted in the γ-counter (Packard). Each data point is the result of the average of triplicate wells, and was analyzed by nonlinear regression analysis with the Allfit program.

Culture of human umbilical vein vascular endothelial cells

Reagents were purchased from Sigma unless indicated. Human umbilical vein vascular endothelial cells (HUVEC) were obtained from Promocell GmbH (Heidelberg, Germany) and grown in M199 supplemented with 20% FCS, 2 mM L-glutamine, penicillin (100 U mL^{-1})/streptomycin (100 μg mL^{-1}), 100 μg mL^{-1} porcine heparin, 50 ng mL^{-1} ECGF. HUVEC cells were maintained in culture and used until five passages.

Cell adhesion assay

Plates (96 wells) were coated with vitronectin (Duotech) at 10 μg mL^{-1} in PBS (Sigma), overnight at 4 °C. Cells (2.5×10^4/100 μL) were seeded in each well and allowed to adhere for 2 h at 37 °C in the presence of various concentration of compound **56**. Nonadherent cells were removed with PBS and the remaining cells were fixed with 4% paraformaldehyde for 10 min. Adherent cells were stained with 1% toluidine blue for 10 min and rinsed with water. Stained cells were solubilized with 1% SDS and quantified on a microtiter plate reader at 595 nm. Experiments were done in triplicate. Results are expressed as mean ± SE compound concentration that inhibited 50% of cell adhesion (IC_{50}). IC_{50} was calculated using GraphPad Prism 5 program. After calculation the same experiment was repeated using only the concentration of compound **56** corresponding to the IC_{50} value. Cells were seeded on vitronectin and allowed to adhere for 2 h at 37 °C in the absence or in the presence of 9.8 μM of compound **56**. Nonadherent cells were removed with PBS and the remaining cells were fixed with 4% paraformaldehyde for 10 min. After fixation cells were observed using Zeiss Axio Observer A1 inverted microscope and photographed with Zeiss AxioCam MRm.

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Computational studies

All calculations were run using the Schrödinger suite of programs (http://www.schrodinger.com) through the Maestro graphical interface.

Conformational analysis. Conformational preferences of the RGD cyclopeptides have been investigated by molecular mechanics calculations within the framework of MacroModel version 8.1,[30] using the MacroModel implementation of the Amber all-atom force field[31] (denoted AMBER*) and the implicit water GB/SA solvation model of Still et al.[32] A two-step protocol was used. Monte Carlo/energy minimization (MC/EM) conformational searches[25] of the AGA (Ala-Gly-Ala) cyclopeptide analogues containing methyl groups instead of the Arg and Asp side chains were performed as the first step. Amide bonds were included among the rotatable bonds. For each search, at least 1000 starting structures for each variable torsion angle were generated and minimized until the gradient was less than 0.05 kJ $Å^{-1}$ mol^{-1} using the truncated Newton–Raphson method[33] implemented in MacroModel. Duplicate conformations and those with an energy greater than 6 kcal mol^{-1} above the global minimum were discarded. Free simulations of the complete RGD cyclic peptides (Asp and Arg side chains were considered ionized) were then performed at 300 K using the metropolis Monte Carlo/stochastic dynamics (MC/SD) hybrid simulation algorithm,[26] starting from the cyclopeptide backbone geometries located by the previous MC/EM step. RGD side-chain dihedral angles were defined as internal coordinate degrees of freedom in the Monte Carlo part of the algorithm. A time step of 1 fs was used for the stochastic dynamics (SD) part of the algorithm. At least two 10 ns simulations were run for each RGD cyclopeptide starting from different conformations to test the convergence. Samples were taken at 2 ps intervals during each simulation, yielding 5000 conformations for analysis.

Protein setup. The recently solved crystal structure of the extracellular domain of the integrin $\alpha_V\beta_3$ receptor in complex with EMD121974 and in the presence of the pro-adhesive ion Mn^{2+} (PDB code: 1L5G)[11] was used for docking studies. As the headgroup of integrin has been identified in the X-ray structure as the ligand binding region, the docking was performed only on the globular head. The protein structure was setup for docking as follows: The protein was truncated to residue sequences 41–342 for chain α and 114–347 for chain β. As a result of the lack of parameters, the Mn^{2+} ions in the experimental protein structure were modeled by replacing them with Ca^{2+} ions. The protein-charged groups that were neither located in the ligand binding pocket nor involved in salt bridges were neutralized using the Schrödinger pprep script. The hydrogen atoms were added using the Schrödinger graphical user interface Maestro and the resulting structure was optimized using the Schrödinger impref script.

Docking. The automated docking calculations were performed using Glide (grid-based ligand docking with energetics) within the framework of Impact version 2.7. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. To begin the Glide calculation an enclosing box and a bounding box are defined starting from the center of the reference ligand. The starting poses for the ligands to be screened are generated by placing the center of the ligand in random points of the bounding box. Conformational flexibility is handled in Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates conformations deemed unsuitable for binding to a receptor, such as conformations that have long-range internal hydrogen bonds. After all the filters have been applied, the remaining best 400 poses are partially minimized in the grid field using OPLSAA and finally scored using the GlideScore scoring function. GlideScore is based on ChemScore,[34] but includes a steric clash term and adds buried polar terms to penalize electrostatic mismatches. The grid generation step started from the extracellular fragment of the X-ray structure of the $\alpha_V\beta_3$ complex with EMD121974 as described in the protein setup section and used mae input files of both the ligand and active site, including hydrogen atoms. The center of the grid enclosing box was defined by the center of the bound ligand, as described in the original PDB entry. The enclosing box dimensions, which are automatically deduced from the ligand size, fit the entire active site. For the docking step, the size of bounding box for placing the ligand center was set to 12 Å. No further modifications were applied to the default settings. The GlideScore function was used to select 30 poses for each ligand. The Glide program was initially tested for its ability to reproduce the crystallized binding geometry of EMD121974. The program was successful in reproducing the experimentally found binding mode of this compound, as it corresponds to the best-scored pose.

NMR spectroscopy

NMR experiments were performed at a temperature of 294 K on Bruker Avance 400 MHz and 600 MHz spectrometers. All proton and carbon chemical shifts were assigned unambiguously for **56**. The NMR experiments were carried out in a D_2O/H_2O (1:9) mixture, to observe amide protons. Two-dimensional experiments (TOCSY, COSY, NOESY, and HSQC) were carried out on 3 mM samples of **56**. NOESY spectra were performed with 0.2, 0.4, 0.6, and 0.8 s as mixing times. The water resonance was saturated with the excitation sculpting sequence from the Bruker library. The conformation of the pentapeptide was first analyzed with respect to hydrogen bonding of amide protons (VT-NMR spectroscopy) and NOE contacts.

Chemical procedures

All chemicals were of reagent grade and were used without further purification. Solvents were dried by standard procedures, and reactions requiring anhydrous conditions were performed under N_2. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. Optical rotations $[\alpha]_D$ were measured in a cell of 1 dm path length and 1 mL capacity with a PerkinElmer 241 polarimeter. ^1H and ^{13}C NMR routine spectra were recorded at 300 K on a Bruker AVANCE-400 or Bruker AC-200 spectrometer. Chemical shifts δ are expressed in ppm relative to internal Me_4Si as standard. Mass spectroscopy was determined with a VG 7070 EQ-HF apparatus. Thin-layer chromatography (TLC) was carried out with precoated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey–Nagel silica gel 60 (230–400 mesh). Semipreparative HPLC was carried out with Symmetry-Prep C_{18}-7 μm 7.8×300 mm Waters column using gradient mixture of CH_3CN/H_2O + 0.1% TFA. Elemental analyses were performed by the staff of the microanalytical laboratory of our department. The following abbreviations also apply: Cbz, Z: carbobenzyloxy; Bn, benzyl; Pmc: 2,2,5,7,8-pentamethylchroman-6-sulfonyl; CHA: cyclohexylamine; NMM: 4-methylmorpholine; HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt:

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1-hydroxy-7-azabenzotriazole; TFA: trifluoroacetic acid; DIPEA: *N*,*N*-diisopropylethylamine.

Synthesis of Z-Arg(Pmc)-Gly-OMe (21). HCl (2 N) was slowly added to a suspension of Z-Arg(Pmc)-OH·CHA·1/2 H_2O (5.0 g, 7.47 mmol) in H_2O/EtOAc 1:1 (300 mL), under vigorous stirring, till the solid was completely dissolved. The aqueous phase was then extracted with EtOAc (2×150 mL) and the combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure yielding the free amino acid as a white foam. NMM (3.3 mL, 29.9 mmol) and, at −20 °C, isobutyl chloroformate (1.2 mL, 8.96 mmol) were added under nitrogen atmosphere to a solution of free amino acid (7.47 mmol) in dry THF (50 mL). After 20 min H-Gly-OMe·HCl (1.4 g, 11.2 mmol) was added. The mixture was stirred overnight at room temperature. After reaction completion, the resulting suspension was filtered through a pad of celite and washed with THF. The solvent was evaporated under reduced pressure and the crude was dissolved in EtOAc (100 mL) and washed with H_2O (100 mL). The solvent, dried with Na_2SO_4, was evaporated under reduced pressure. The crude was purified by flash chromatography (CH_2Cl_2/acetone 7:3) to obtain **21** (96%) as white foam. $[\alpha]_D^{22} = -7.4$ ($c = 1.06$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.61 (m, 2H, *H*γ Arg), 1.70 (m, 1H, *H*β Arg), 1.81 (t, 2H, J = 6.7 Hz, Ar-CH_2-CH_2 Pmc), 1.91 (m, 1H, *H*β Arg), 2.11, 2.55, 2.57 (3s, 9H, CH_3 Pmc), 2.62 (t, 2H, J = 6.7 Hz, Ar-CH_2-CH_2 Pmc), 3.21 (m, 1H, *H*δ Arg), 3.31 (m, 1H, *H*δ Arg), 3.68 (s, 3H, COOCH_3), 3.89 (dd, 1H, J = 17.8 Hz, J = 5.5 Hz, *H*α Gly), 4.03 (dd, 1H, J = 17.8 Hz, J = 5.5 Hz, *H*α Gly), 4.36 (m, 1H, *H*α Arg), 5.07 (s, 2H, CH_2-Ph), 6.05 (d, 1H, J = 7.9, N*H* Arg), 6.1–6.35 (bs, 3H, (N*H*)$_2$C=N*H*), 7.31 ppm (m, 5H, *Arom.*), 7.63 (m, 1H, N*H* Gly); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 72.8$, 170.6, 156.3, 153.7, 136.1, 135.4, 134.8, 132.7, 128.4, 128.0, 127.8, 124.1, 117.9, 73.6, 66.8, 54.0, 52.2, 40.9, 40.1, 32.6, 30.0, 26.6, 25.0, 21.3, 18.4, 17.4, 12.0 ppm; MS (FAB$^+$): *m/z* calcd for $C_{31}H_{43}N_5O_8S$ 645.28, found: 646 $[M+H]^+$; Microanalysis: calcd for $C_{31}H_{43}N_5O_8S$: C 57.66, H 6.71, N 10.85, found: C 57.55, H 6.72, N 10.87.

Synthesis of H-Arg(Pmc)-Gly-OMe (22). A solution of **21** (4.8 mmol) in MeOH (48 mL) containing a catalytic amount of 10% Pd/C was stirred for 2 h under hydrogen atmosphere. After reaction completion, the mixture was filtered through a pad of celite and washed with MeOH. The collected organic phase was evaporated under reduced pressure to yield **22** (99%) as white foam that was used without any further purification. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.32$ [s, 6H, C(CH_3)$_2$ Pmc], 1.50–2.20 (m, 4H), 1.81 (t, 2H, J = 6.3 Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.52, 2.55 (3 s, 9H, CH_3 Pmc), 2.63 (m, 2H, Ar-CH_2-CH_2 Pmc), 3.21 (m, 2H, *H*δ Arg), 3.46 (m, 1H, *H*α Arg), 3.72 (s, 3H, COOCH_3), 3.97 (m, 2H, *H*α Gly), 6.38 (m, 5H, (N*H*)$_2$C=N*H*, NH_2 Arg), 7.96 (m, 1H, N*H* Gly).

General procedure A. Synthesis of Z-Temp-Arg(Pmc)-Gly-OMe. NMM (110 μL, 1 mmol) and, at −30 °C, isobutyl chloroformate (39 μL, 0.3 mmol) were added under nitrogen atmosphere to a solution of **Temp1**–**7** (0.2 mmol) in dry THF (2 mL). After 20 min a solution of **22** (205 mg, 0.4 mmol) in dry CH_2Cl_2 (0.8 mL) was added. The mixture was stirred overnight at room temperature. After reaction completion, the resulting suspension was filtered through a celite pad and washed with THF. The solvent was evaporated under reduced pressure and the crude was dissolved in EtOAc (3 mL) and washed with H_2O (3 mL). The organic phase, dried with Na_2SO_4, was evaporated under reduced pressure. The crude was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to obtain **23**–**29** (88–98%).

Temp1-Arg(Pmc)-Gly-OMe (23). Compound **23** was prepared following general procedure A (94%) as a white foam. $[\alpha]_D^{22} = -23.0$ ($c = 0.98$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.60–2.05 (m, 12H, CH_2), 1.80 (t, 2H, J = 6.7 Hz, Ar-CH_2-CH_2 Pmc), 2.10, 2.54, 2.56 (3 s, 9H, CH_3 Pmc), 2.61 (t, 2H, J = 6.7 Hz, Ar-CH_2-CH_2 Pmc), 3.02, 3.11 (2m, 2H, CH_2-Ph), 3.27 (m, 2H, *H*δ Arg), 3.66 (m, 3H, *H*-6, PhCH_2-N), 3.72 (s, 3H, COOCH_3), 3.96 (dd, 1H, J = 4.6 Hz, J = 17.6 Hz, *H*α Gly), 4.08 (dd, 1H, J = 4.6 Hz, J = 17.6 Hz, *H*α Gly), 4.51 (m, 1H, *H*-9), 4.57 (m, 1H, *H*α Arg), 6.30–6.40 (bs, 3H, (N*H*)$_2$C=N*H*), 7.20–7.35 (m, 10H, *Arom.*), 7.39 (bs, 1H, N*H* Gly), 7.68 ppm (d, 1H, J = 8.6 Hz, N*H* Arg); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 174.0$, 172.5, 172.2, 170.0, 156.4, 153.5, 140.4, 136.0, 135.3, 134.6, 133.4, 131.2, 128.3, 127.9, 126.9, 126.5, 124.1, 117.9, 111.2, 73.6, 62.3, 60.9, 59.7, 53.4, 52.2, 48.0, 44.2, 41.2, 40.4, 32.8, 31.8, 30.6, 29.7, 29.1, 28.8, 28.7, 26.8, 25.8, 21.4, 19.0, 18.5, 17.5, 13.7, 12.2 ppm; MS (FAB$^+$): *m/z* calcd for $C_{46}H_{61}N_7O_8S$ 871.43, found: 872 $[M+H]^+$; Microanalysis: calcd for $C_{46}H_{61}N_7O_8S$: C 63.35, H 7.05, N 11.24, found: C 63.39, H 7.06, N 11.25.

Temp2-Arg(Pmc)-Gly-OMe (24). Compound **24** was prepared following general procedure A (90%) as a white foam. $[\alpha]_D^{22} = -46.7$ ($c = 1.06$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.60–2.40 (m, 12H, CH_2), 1.81 (t, 2H, J = 6.6 Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.55, 2.58 (3 s, 9H, CH_3 Pmc), 2.63 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.81 (m, 1H, HC*H*-Ph), 3.28 (m, 3H, *H*δ Arg, *H*CH-Ph), 3.69 (s, 3H, COOCH_3), 3.71 (m, 2H, PhCH_2-N), 3.80 (m, 1H, *H*-6), 4.03 (m, 2H, *H*α Gly), 4.57 (m, 2H, *H*α Arg, *H*-9), 6.30–6.60 (bs, 3H, (N*H*)$_2$C=N*H*), 7.15–7.35 (m, 10H, *Ph*), 7.48 (m, 1H, N*H* Gly), 7.93 ppm (bs, 1H, N*H* Temp); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 172.4$, 170.0, 156.5, 153.5, 136.7, 134.6, 133.3, 129.9, 128.3, 126.9, 124.0, 117.9, 73.5, 62.1, 60.2, 53.2, 52.1, 47.4, 41.1, 40.3, 32.7, 29.4, 26.7, 21.3, 18.5, 17.4, 12.1 ppm; MS (FAB$^+$): *m/z* calcd for $C_{46}H_{61}N_7O_8S$ 871.43, found: 872 $[M+H]^+$; Microanalysis calcd for $C_{46}H_{61}N_7O_8S$: C 63.35, H 7.05, N 11.24, found: C 63.38, H 7.05, N 11.24.

Temp3-Arg(Pmc)-Gly-OMe (25). Compound **25** was prepared following general procedure A (98%) as a white foam. $[\alpha]_D^{22} = -35.4$ ($c = 1.04$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.33$ (s, 6H, C(CH_3)$_2$ Pmc), 1.50–2.40 (m, 12H, CH_2), 1.80 (t, 2H, J = 6.8 Hz, Ar-CH_2-CH_2 Pmc), 2.10, 2.55, 2.57 (3 s, 9H, CH_3 Pmc), 2.61 (m, 2H, Ar-CH_2-CH_2 Pmc), 3.10 (d, 1H, J = 12.1 Hz, HC*H*-Ph), 3.27 (m, 3H, *H*δ Arg, *H*CH-Ph), 3.72 (s, 3H, COOCH_3), 3.75 (m, 3H, PhCH_2-N, *H*-6), 3.97 (dd, 1H, J = 18.6 Hz, J = 5.2 Hz, *H*α Gly), 4.03 (dd, 1H, J = 18.6 Hz, J = 5.2 Hz, *H*α Gly), 4.51 (m, 1H, *H*-9), 4.59 (m, 1H, *H*α Arg), 6.10–6.50 (bs, 3H, (N*H*)$_2$C=N*H*), 7.10–7.60 ppm (m, 12H, N*H* Gly, N*H* Arg, *Arom.*); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 172.9$, 172.6, 172.0, 170.2, 156.8, 156.5, 153.8, 136.8, 135.6, 135.5, 133.8, 134.8, 133.7, 131.0, 128.5, 128.2, 128.1, 126.7, 124.3, 124.2, 118.2, 73.8, 62.7, 60.9, 60.6, 60.4, 53.4, 52.3, 48.3, 41.5, 40.6, 33.4, 33.0, 28.8, 27.1, 26.9, 26.3, 21.6, 19.2, 18.8, 17.7, 12.4 ppm; MS (FAB$^+$): *m/z* calcd for $C_{46}H_{61}N_7O_8S$ 871.43, found: 872 $[M+H]^+$; Microanalysis: calcd for $C_{46}H_{61}N_7O_8S$: C 63.35, H 7.05, N 11.24, found: C 63.34, H 7.04, N 11.24.

Temp4-Arg(Pmc)-Gly-OMe (26). Compound **26** was prepared following general procedure A (97%) as a white foam. $[\alpha]_D^{22} = -17.2$ ($c = 1.03$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.55–2.00 (m, 10H, CH_2), 1.82 (t, 2H, J = 6.8 Hz, Ar-CH_2-CH_2 Pmc), 2.12, 2.57, 2.59 (3 s, 9H, CH_3 Pmc), 2.61 (m, 2H, Ar-CH_2-CH_2 Pmc), 3.04 (d, 1H, J = 12.8 Hz, HC*H*-Ph), 3.08 (m, 1H, *H*-7), 3.20 (m, 2H, *H*δ Arg), 3.36 (d, 1H, J = 12.8 Hz, *H*CH-Ph), 3.71 (s, 3H, COOCH_3), 3.93 (dd, 1H, J = 17.6 Hz, J = 5.8 Hz, *H*α Gly), 4.10 (dd, 1H, J = 17.6 Hz, J = 5.8 Hz, *H*α Gly), 4.40 (m, 1H, *H*α Arg), 4.68 (dd, 1H, J = 7.5 Hz, J < 1 Hz, *H*-10), 4.93 (d, 1H, J = 12.3 Hz, Ph-CH_2-O), 5.08 (d, 1H, J = 12.3 Hz, Ph-CH_2-O), 5.67 (d, 1H, J = 11.7 Hz, CH=C*H*), 5.82 (m, 1H, C*H*=CH), 5.90–6.00 (bs, 3H, (N*H*)$_2$C=N*H*), 6.21 (s, 1H, N*H* Temp), 7.05–7.30 (m, 11H, N*H* Arg, *Ph*), 7.56 ppm (bs, 1H, N*H* Gly); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 173.2$, 172.7, 172.1, 171.9, 170.6,

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170.2, 157.0, 156.7, 156.4, 153.8, 153.7, 136.3, 135.6, 135.0, 134.4, 133.6, 133.4, 130.3, 128.7, 128.5, 128.3, 128.1, 127.9, 124.2, 124.1, 118.1, 118.0, 73.8, 73.7, 71.9, 71.5, 71.2, 67.1, 65.4, 64.2, 62.0, 57.9, 54.2, 53.6, 52.4, 52.3, 41.3, 33.0, 28.1, 26.9, 21.6, 19.2, 18.7, 17.6, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{48}H_{61}N_7O_{10}S$ 927.42, found: 928 $[M+H]^+$; Microanalysis: calcd for $C_{48}H_{61}N_7O_{10}S$: C 62.12, H 6.62, N 10.56, found: C 62.15, H 6.63, N 10.57.

Temp5-Arg(Pmc)-Gly-OMe (27). Compound **27** was prepared following general procedure A (94%) as a white foam. $[\alpha]_D^{22}=-93.5$ ($c=1.82$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.60–2.50 (m, 10H, CH_2), 1.78 (t, 2H, $J=6.5$ Hz, Ar-CH_2-CH_2 Pmc), 2.10, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.62 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.98 (d, 1H, $J=13.7$ Hz, HCH-Ph), 3.29 (m, 2H, $H\delta$ Arg), 3.57(d, 1H, $J=13.7$ Hz, HCH-Ph), 3.71 (s, 3H, $COCH_3$), 3.97 (dd, 1H, $J=17.8$ Hz, $J=5.1$ Hz, $H\alpha$ Gly), 4.07 (dd, 1H, $J=17.8$ Hz, $J=5.1$ Hz, $H\alpha$ Gly), 4.54 (m, 1H, $H\alpha$ Arg), 4.62 (m, 2H, *H*-10, *H*-7), 5.12 (d, 1H, $J=12.1$ Hz, Ph-CH_2-O), 5.19 (d, 1H, $J=12.1$ Hz, Ph-CH_2-O), 5.66 (d, 1H, $J=11.6$ Hz, CH=CH), 5.81 (m, 1H, CH=CH), 6.24 (m, 3H, $(NH)_2C$=NH), 6.38 (s, 1H, NH Temp), 7.04 (m, 2H, *Arom.*), 7.22 (m, 3H, *Arom.*), 7.40 (m, 6H, NH Gly, *Arom.*), 7.55 ppm (bs, 1H, NH Arg); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=172.9$, 172.5, 171.0, 170.4, 156.7, 153.9, 153.7, 136.6, 136.2, 135.6, 135.0, 134.3, 133.6, 131.7, 128.8, 128.6, 128.3, 126.8, 126.0, 124.2, 118.1, 73.7, 67.1, 63.4, 59.6, 54.5, 53.3, 52.4, 41.3, 40.8, 35.4, 33.0, 32.6, 29.5, 27.5, 27.0,25.6, 21.6, 19.2, 18.7, 17.7, 12.2 ppm; MS (FAB^+): *m/z* calcd for $C_{48}H_{61}N_7O_{10}S$ 927.42, found: 928 $[M+H]^+$; Microanalysis calcd for $C_{48}H_{61}N_7O_{10}S$: C 62.12, H 6.62, N 10.56, found: C 62.16, H 6.62, N 10.57.

Temp6-Arg(Pmc)-Gly-OMe (28). Compound **28** was prepared following general procedure A (88%) as a white foam. $[\alpha]_D^{22}=+29.8$ ($c=1.08$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.43–2.42 (m, 14H, CH_2), 1.81 (t, 2H, $J=6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.62 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.84 (d, 1H, $J=13.6$ Hz, HCH-Ph), 3.31 (m, 2H, $H\delta$ Arg), 3.45 (d, 1H, $J=13.6$ Hz, HCH-Ph), 3.73 (s, 3H, $COOCH_3$), 4.00 (m, 2H, PhCH_2-N), 3.94–4.18 (m, 2H, $H\alpha$ Gly), 4.51 (m, 1H, $H\alpha$ Arg), 4.69 (m, 1H, *H*-10), 4.89 (m, 1H, *H*-7), 6.20–6.45 (bs, 3H, $(NH)_2C$=NH), 7.15–7.45 (m, 11H, NH Gly, *Arom.*), 7.53 ppm (d, 1H, $J=6.4$ Hz, NH Temp); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=175.4$, 172.8, 172.7, 170.3, 156.8, 156.7, 153.7, 140.8, 137.9, 135.6, 135.0, 133.6, 131.4, 129.4, 128.7, 128.4, 127.2, 126.5, 125.7, 124.2, 118.1, 78.4, 73.8, 66.5, 64.5, 64.0, 63.3, 59.1, 58.2, 53.4, 52.3, 47.5, 40.8, 40.6, 40.3, 35.4, 33.3, 33.0, 32.5, 30.8, 29.8, 27.4, 27.0, 22.9, 21.6, 18.8, 17.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{47}H_{63}N_7O_8S$ 885.45, found: 886 $[M+H]^+$; Microanalysis calcd for $C_{47}H_{63}N_7O_8S$: C 63.71, H 7.17, N 11.06, found: C 63.73, H 7.16, N 11.05.

Temp7-Arg(Pmc)-Gly-OMe (29). Compound **29** was prepared following general procedure A (88%) as a white foam. $[\alpha]_D^{22}=-33.4$ ($c=1.10$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.32$ (s, 6H, $C(CH_3)_2$ Pmc), 1.45–2.25 (m, 13H, CH_2), 1.81 (t, 2H, $J=6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.10, 2.48, 2.50 (3s, 9H, CH_3 Pmc), 2.60 (m, 3H, Ar-CH_2-CH_2 Pmc, *H*-9), 2.90 (m, 1H, HCH-Ph), 3.12 (m, 2H, $H\delta$ Arg), 3.27 (m, 1H, HCH-Ph), 3.65 (m, 2H, PhCH_2-N), 3.71 (s, 3H, $COOCH_3$), 3.98 (dd, 1H, $J=16.8$ Hz, $J=6.4$ Hz, $H\alpha$ Gly), 4.08 (dd, 1H, $J=16.8$ Hz, $J=6.4$ Hz, $H\alpha$ Gly), 4.31 (m, 1H, *H*-7), 4.50 (m, 1H, $H\alpha$ Arg), 4.66 (dd, 1H, $J=8.1$ Hz, $J<1$ Hz, *H*-10), 5.70–6.30 (bs, 3H, $(NH)_2C$=NH), 7.10–7.40 (m, 11H, NH Gly, *Arom.*), 7.76 ppm (bs, 1H, NH Temp); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=175.2$, 172.9, 172.7, 170.0, 156.1, 153.5, 141.0, 135.9, 135.2, 134.6, 133.4, 131.4, 128.2, 127.8, 127.5, 126.6, 124.0, 117.9, 73.6, 71.7, 71.3, 71.0, 65.6, 63.5, 62.6, 61.8, 58.0, 54.7, 53.5, 52.2, 48.0, 43.6, 41.2, 40.3, 34.0, 32.8, 32.4, 31.7, 30.1, 29.7, 29.3, 28.4, 28.0, 26.8, 26.7, 26.0, 25.5, 21.4, 19.0, 18.5, 17.4, 13.9, 12.1 ppm; MS (FAB^+): *m/z* calcd for $C_{47}H_{63}N_7O_8S$ 885.45, found: 886 $[M+H]^+$; Microanalysis: calcd for $C_{47}H_{63}N_7O_8S$: C 63.71, H 7.17, N 11.06, found: C 63.72, H 7.16, N 11.07.

General procedure B. Synthesis of Z-Asp(*t*Bu)-Temp-Arg(Pmc)-Gly-OMe: A solution of **23**–**29** (0.2 mmol) in MeOH (2 mL) containing a catalytic amount of 10% Pd/C was stirred for 2 h under hydrogen atmosphere. After reaction completion, the mixture was filtered through a pad of celite and washed with MeOH. The collected organic phase was evaporated under reduced pressure to yield **30**–**36** as white foams that were used without any further purification. NMM (154 μL, 1.4 mmol) and, at −30 °C, isobutyl chloroformate (57 μL, 0.44 mmol) were added under nitrogen atmosphere to a solution of Z-Asp(*t*Bu)-OH (129 mg, 0.4 mmol) in dry THF (2 mL). After 20 min a solution of **30**–**36** (0.2 mmol) in dry THF (2 mL) was added. The mixture was stirred overnight at room temperature. After reaction completion, the resulting suspension was filtered through a pad of celite and washed with THF. The solvent was evaporated under reduced pressure and the crude was dissolved in EtOAc (4 mL) and washed with H_2O (4 mL). The solvent, dried with Na_2SO_4, was evaporated under reduced pressure. The crude was purified by flash chromatography (CH_2Cl_2/MeOH 96:4) to obtain **37**–**43** (71–88% over two steps).

Z-Asp(*t*Bu)-Temp1-Arg(Pmc)-Gly-OMe (37). Compound **37** was prepared following general procedure B (88% over two steps) as a white foam. $[\alpha]_D^{22}=-8.1$ ($c=1.08$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.35 (s, 9H, $(CH_3)_3$), 1.55–2.20 (m, 12H, CH_2), 1.79 (t, 2H, $J=6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.09, 2.57, 2.58 (3s, 9H, CH_3 Pmc), 2.60 (m, 3H, $H\beta$ Asp, Ar-CH_2-CH_2 Pmc), 2.86 (m, 1H, $H\beta$ Asp), 2.94 (d, 1H, $J=14.0$ Hz, CH_2Ph), 3.20 (d, 1H, $J=14.0$ Hz, CH_2Ph), 3.28 (m, 2H, $H\delta$ Arg), 3.72 (s, 3H, $COOCH_3$), 3.77 (m, 1H, *H*-6), 3.98 (dd, 1H, $J=5.3$ Hz, $J=17.8$ Hz, $H\alpha$ Gly), 4.07 (dd, 1H, $J=5.3$ Hz, $J=17.8$ Hz, $H\alpha$ Gly), 4.41 (m, 1H, $H\alpha$ Asp), 4.48 (dd, 1H, $J=9.2$ Hz, $J<1$ Hz, *H*-9), 4.54 (m, 1H, $H\alpha$ Arg), 5.11 (d, 1H, $J=12.6$ Hz, PhCH_2O), 5.17 (d, 1H, $J=12.6$ Hz, PhCH_2O), 5.91 (bs, 1H, NH Asp), 6.15–6.65 (bs, 3H, $(NH)_2C$=NH), 7.05–7.40 ppm (m, 13H, NH Gly, NH Arg, NH Temp, *Arom.*); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=$ 173.0, 172.2, 172.1, 171.3, 170.9, 170.3, 170.1, 169.8, 156.8, 156.4, 155.9, 153.5, 136.0, 135.4, 134.8, 130.6, 128.8, 128.5, 128.3, 128.2, 127.4, 123.9, 117.9, 81.7, 73.5, 71.2, 67.2, 60.3, 59.8, 58.1, 53.9, 53.0, 52.1, 51.1, 41.4, 41.1, 40.3, 36.7, 32.7, 31.8, 30.2, 29.5, 29.1, 28.9, 27.9, 26.7, 26.0, 25.4, 21.4, 19.0, 18.5, 17.5, 12.1 ppm; MS (FAB^+): *m/z* calcd for $C_{55}H_{74}N_8O_{13}S$ 1086.51, found: 1087 $[M+H]^+$; Microanalysis calcd for $C_{55}H_{74}N_8O_{13}S$: C 60.76, H 6.86, N 10.31, found: C 60.78, H 6.87, N 10.30.

Z-Asp(*t*Bu)-Temp2-Arg(Pmc)-Gly-OMe (38). Compound **38** was prepared following general procedure B (76% over two steps) as a white foam. $[\alpha]_D^{22}=-95.2$ ($c=1.02$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.92$ (m, 1H, HCH Temp), 1.20 (m, 1H, HCH Temp), 1.31 (s, 6H, $C(CH_3)_2$ Pmc), 1.38 (s, 9H, $C(CH_3)_3$), 1.63–1.97 (m, 10H, CH_2), 1.81 (t, 2H, $J=6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.12 (s, 3H, CH_3 Pmc), 2.52 (m, 1H, $H\beta$ Asp), 2.59, 2.60 (2s, 6H, CH_3 Pmc), 2.64 (t, 2H, $J=6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.81 (m, 1H, $H\beta$ Asp), 2.83 (d, 1H, $J=13.1$ Hz, CH_2Ph), 3.23 (d, 1H, $J=13.1$ Hz, CH_2Ph), 3.30 (m, 2H, $H\delta$ Arg), 3.45 (dd, 1H, $J=5.4$ Hz, $J=17.5$ Hz, $H\alpha$ Gly), 3.61 (s, 3H, $COOCH_3$), 3.61 (m, 1H, *H*-6), 4.05 (dd, 1H, $J=5.4$ Hz, $J=17.5$ Hz, $H\alpha$ Gly), 4.35 (m, 1H, $H\alpha$ Arg), 4.42 (dd, 1H, $J=7.9$ Hz, *H*-9), 5.06 (d, 1H, $J=12.3$ Hz, PhCH_2O), 5.30 (d, 1H, $J=12.3$ Hz, PhCH_2O), 4.53 (m, 1H, $H\alpha$ Asp), 6.75 (d, 1H, $J=8.4$ Hz, NH Asp), 6.22 (bs, 3H, $(NH)_2C$=NH), 7.20–7.36 (m, 10H, *Arom.*), 7.43 (d, 1H, $J=8.6$ Hz, NH Arg), 7.59 (s, 1H, NH Temp), 7.68 ppm (m, 1H, NH Gly); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=173.4$, 172.3, 172.1, 171.4, 171.3, 170.2, 157.5, 156.1, 153.6, 136.2, 135.7, 135.1, 134.6, 130.3, 129.0, 128.9, 128.6, 128.1, 128.0, 124.0, 118.0, 82.0, 67.5, 61.5, 60.4, 58.5, 53.7, 52.5,

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50.6, 43.4, 41.6, 41.0, 36.4, 33.1, 33.0, 31.1, 29.0, 28.1, 26.9, 26.5, 21.6, 18.7, 17.6, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{55}H_{74}N_8O_{13}S$ 1086.51, found: 1087 $[M+H]^+$; Microanalysis: calcd for $C_{55}H_{74}N_8O_{13}S$: C 60.76, H 6.86, N 10.31, found: C 60.77, H 6.87, N 10.32.

Z-Asp(*t*Bu)-Temp3-Arg(Pmc)-Gly-OMe (39). Compound **39** was prepared following general procedure B (80% over two steps) as a white foam). $[\alpha]_D^{22}=-20.6$ ($c=1.00$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.38 (s, 9H, $C(CH_3)_3$), 1.53–2.45 (m, 12H, CH_2), 1.80 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.08 (s, 3H, CH_3 Pmc), 2.50 (dd, 1H, $J=17.1$ Hz, $J=4.7$ Hz, $H\beta$ Asp), 2.57, 2.59 (2s, 6H, CH_3 Pmc), 2.62 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.86 (dd, 1H, $J=17.1$ Hz, $J=4.7$ Hz, $H\beta$ Asp), 3.11 (d, 1H, $J=14.0$ Hz, CH_2Ph), 3.15 (d, 1H, $J=14.0$ Hz, CH_2Ph), 3.28 (m, 2H, $H\delta$ Arg), 3.71 (s, 3H, $COOCH_3$), 3.82 (m, 1H, *H*-6), 3.97 (dd, 1H, $J=5.7$ Hz, $J=17.9$ Hz, $H\alpha$ Gly), 4.06 (dd, 1H, $J=5.7$ Hz, $J=17.9$ Hz, $H\alpha$ Gly), 4.40 (m, 1H, $H\alpha$ Asp), 4.51 (dd, 1H, $J=8.2$ Hz, *H*-9), 4.59 (m, 1H, $H\alpha$ Arg), 5.02 (d, 1H, $J=12.5$ Hz, PhCH_2O), 5.10 (d, 1H, $J=12.5$ Hz, PhCH_2O), 5.89 (bs, 1H, N*H* Asp), 6.26 (bs, 3H, $(NH)_2C$=N*H*), 7.08 (s, 1H, N*H* Temp), 7.19–7.34 (m, 11H, N*H* Gly, *Arom.*), 7.83 ppm (d, 1H, $J=8.6$ Hz, N*H* Arg); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=172.8$, 172.4, 171.5, 170.7, 170.5, 170.2, 169.7, 156.4, 156.1, 153.8, 136.3, 135.5, 135.3, 134.8, 130.8, 128.7, 128.6, 128.4, 128.3, 127.3, 124.3, 118.1, 81.8, 73.8, 67.3, 61.8, 60.2, 58.2, 53.2, 52.3, 51.8, 43.4, 41.5, 40.7, 37.1, 35.0, 33.5, 33.1, 32.9, 28.8, 28.1, 27.1, 26.9, 26.8, 26.1, 25.4, 21.6, 19.2, 18.8, 17.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{55}H_{74}N_8O_{13}S$ 1086.51, found: 1087 $[M+H]^+$; Microanalysis: calcd for $C_{55}H_{74}N_8O_{13}S$: C 60.76, H 6.86, N 10.31, found: C 60.75, H 6.85, N 10.32.

***Z*-Asp(*t*Bu)-*H*2-Temp4-Arg(Pmc)-Gly-OMe (40).** Compound **40** was prepared following general procedure B (78% over two steps) as a white foam. $[\alpha]_D^{22}=-78.9$ ($c=1.02$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.40 (s, 9H, $C(CH_3)_3$), 1.55–2.07 (m, 14H, CH_2), 1.81 (t, 2H, $J=6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.60 (m, 1H, $H\beta$ Asp), 2.63 (t, 2H, $J=6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.72 (dd, 1H, $J=16.2$ Hz, $J=8.2$ Hz, $H\beta$ Asp), 2.93 (m, 1H, CH_2Ph), 3.22 (m, 2H, $H\delta$ Arg), 3.50 (d, 1H, $J=12.9$ Hz, CH_2Ph), 3.65 (m, 1H, $H\alpha$ Gly), 3.66 (s, 3H, $COOCH_3$), 3.72 (m, 1H, *H*-7), 4.03 (dd, 1H, $J=5.4$ Hz, $J=17.5$ Hz, $H\alpha$ Gly), 4.41 (m, 1H, $H\alpha$ Arg), 4.47 (dd, 1H, $J=8.3$ Hz, $J<1$ Hz, *H*-10), 4.55 (m, 1H, $H\alpha$ Asp), 5.00 (d, 1H, $J=12.3$ Hz, PhCH_2O), 5.15 (d, 1H, $J=12.3$ Hz, PhCH_2O), 5.90–6.20 (bs, 3H, $(NH)_2C$=N*H*), 6.41 (bs, 1H, N*H* Asp), 7.07–7.30 (m, 11H, N*H* Temp, *Arom.*), 7.52 ppm (bs, 2H, N*H* Gly, N*H* Arg); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=172.9$, 172.5, 172.3, 171.1, 170.1, 170.3, 156.5, 153.6, 136.4, 135.8, 135.6, 135.0, 133.7, 129.9, 128.6, 128.5, 128.2, 128.0, 127.3, 124.1, 118.0, 81.5, 75.1, 73.7, 69.4, 67.0, 64.9, 64.7, 59.3, 52.3, 41.2, 33.6, 33.0, 28.1, 27.0, 26.9, 21.6, 21.5, 18.7, 17.6, 16.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{56}H_{76}N_8O_{13}S$ 1100.53, found: 1101 $[M+H]^+$; Microanalysis: calcd for $C_{56}H_{76}N_8O_{13}S$: C 61.07, H 6.96, N 10.17, found: C 61.09, H 6.95, N 10.18.

***Z*-Asp(*t*Bu)-*H*2-Temp5-Arg(Pmc)-Gly-OMe (41).** Compound **41** was prepared following general procedure B (82% over two steps) as a white foam. $[\alpha]_D^{22}=-84.4$ ($c=1.01$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.40 (s, 9H, $C(CH_3)_3$), 1.55–2.10 (m, 14H, CH_2), 1.80 (t, 2H, $J=7.2$ Hz, Ar-CH_2-CH_2 Pmc), 2.12, 2.58, 2.60 (3s, 9H, CH_3 Pmc), 2.62 (m, 3H, Ar-CH_2-CH_2 Pmc, $H\beta$ Asp), 2.77 (d, 1H, $J=13.6$ Hz, CH_2Ph), 2.92 (m, 1H, $H\beta$ Asp), 3.29 (m, 2H, $H\delta$ Arg), 3.60 (d, 1H, $J=13.6$ Hz, CH_2Ph), 3.71 (s, 3H, $COOCH_3$), 3.88 (m, 1H, *H*-7), 3.97 (dd, H, $J=17.8$ Hz, $J=6.0$ Hz, $H\alpha$ Gly), 4.09 (dd, H, $J=17.8$ Hz, $J=6.0$ Hz, $H\alpha$ Gly), 4.49 (m, 1H, *H*-10), 4.53 (m, 2H, $H\alpha$ Arg, $H\alpha$ Asp), 5.07 (d, H, $J=12.0$ Hz, PhCH_2O), 5.14 (d, H, $J=12.0$ Hz, PhCH_2O), 6.28 (bs, 1H, N*H* Asp), 6.30–6.60 (bs, 3H, $(NH)_2C$=N*H*), 7.00, 7.29, 734 (3m, 11H, N*H* Temp, *Arom.*), 7.39 (bs, 1H, N*H* Gly), 7.48 ppm (bs, 1H, N*H* Arg); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=173.2$, 172.6, 171.5, 170.4, 170.0, 156.6, 153.6, 136.8, 136.2, 135.6, 134.9, 134.0, 131.7, 128.8, 128.6, 128.5, 128.4, 127.4, 127.1, 126.9, 124.0, 118.0, 82.1, 75.2, 73.7, 69.5, 67.4, 66.8, 64.9, 64.3, 60.5, 59.3, 58.1, 55.4, 53.3, 52.3, 51.4, 46.4, 41.3, 41.0, 36.6, 33.7, 33.0, 28.2, 27.8, 27.0, 25.7, 21.6, 18.7, 17.6, 16.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{56}H_{76}N_8O_{13}S$ 1100.53, found: 1101 $[M+H]^+$; Microanalysis calcd for $C_{56}H_{76}N_8O_{13}S$: C 61.07, H 6.96, N 10.17, found: C 61.10, H 6.97, N 10.16.

***Z*-Asp(*t*Bu)-Temp6-Arg(Pmc)-Gly-OMe (42).** Compound **42** was prepared following general procedure B (81% over two steps) as a white foam. $[\alpha]_D^{22}=-21.1$ ($c=1.07$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.32$ (s, 6H, $C(CH_3)_2$ Pmc), 1.42 (s, 9H, $C(CH_3)_3$), 1.55–2.12 (m, 14H, CH_2), 1.82 (t, 2H, $J=6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.58, 2.60 (3s, 9H, CH_3 Pmc), 2.63 (m, 3H, Ar-CH_2-CH_2 Pmc, $H\beta$ Asp), 2.95 (d, 1H, $J=13.9$ Hz, CH_2Ph), 3.23 (m, 1H, $H\beta$ Asp), 3.33 (m, 2H, $H\delta$ Arg), 3.47 (d, 1H, $J=13.9$ Hz, CH_2Ph), 3.60 (s, 3H, $COOCH_3$), 3.77 (m, 1H, $H\alpha$ Gly), 4.23 (dd, 1H, $J=17.5$ Hz, $J=5.9$ Hz, $H\alpha$ Gly), 4.48 (m, 2H, *H*-7, $H\alpha$ Arg), 4.51 (dd, 1H, $J=7.1$ Hz, $J=7.1$ Hz, *H*-10), 4.64 (m, 1H, $H\alpha$ Asp), 4.75 (d, 1H, $J=12.4$ Hz, PhCH_2O), 4.99 (d, 1H, $J=12.4$ Hz, PhCH_2O), 6.32 (bs, 3H, $(NH)_2C$=N*H*), 7.00, 7.12, 7.32 (3m, 12H, N*H* Temp, N*H* Asp, *Arom.*), 7.73 (d, 1H, $J=8.4$ Hz, N*H* Arg), 8.08 ppm (m, 1H, N*H* Gly); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=174.7$, 173.2, 172.0, 171.2, 136.2, 131.6, 128.6, 128.4, 128.1, 127.7, 126.7, 118.2, 81.7, 67.3, 64.4, 60.8, 58.2, 52.4, 50.6, 41.3, 40.9, 33.1, 32.9, 30.6, 28.3, 27.6, 26.9, 25.1, 23.2, 21.5, 18.7, 16.6, 12.2 ppm; MS (FAB^+): *m/z* calcd for $C_{56}H_{76}N_8O_{13}S$ 1100.53, found: 1101 $[M+H]^+$; Microanalysis: calcd for $C_{56}H_{76}N_8O_{13}S$: C 61.07, H 6.96, N 10.17, found: C 61.08, H 6.95, N 10.16.

***Z*-Asp(*t*Bu)-Temp7-Arg(Pmc)-Gly-OMe (43).** Compound **43** was prepared following general procedure B (71% over two steps) as a white foam.$[\alpha]_D^{22}=-3.5$ ($c=1.04$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.30$ (s, 6H, $C(CH_3)_2$ Pmc), 1.42 (s, 9H, $C(CH_3)_3$), 1.45–2.15 (m, 13H, CH_2), 1.81 (t, 2H, $J=6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.10, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.62 (m, 4H, $H\beta$ Asp, Ar-CH_2-CH_2 Pmc), 2.91 (m, 1H, *H*CH Temp), 3.11 (m, 2H, $H\delta$ Arg), 3.51 (d, 1H, $J=14.4$ Hz, CH_2Ph), 3.65 (d, 1H, $J=14.4$ Hz, CH_2Ph), 3.74 (s, 3H, $COOCH_3$), 3.95 (dd, 1H, $J=18.2$ Hz, $J=5.2$ Hz, $H\alpha$ Gly), 4.05 (dd, 1H, $J=18.2$ Hz, $J=6.0$ Hz, $H\alpha$ Gly), 4.39 (m, 1H, $H\alpha$ Asp), 4.45 (m, 2H, *H*-7, $H\alpha$ Arg), 4.57 (m, 1H, *H*-10), 4.80 (d, H, $J=12.0$ Hz, PhCH_2O), 5.01 (d, 1H, $J=12.0$ Hz, PhCH_2O), 5.78 (bs, 1H, N*H* Asp), 5.98 (bs, 3H, $(NH)_2C$=N*H*), 6.94 (bs, 1H, N*H* Arg), 7.15, 7.29 (2m, 11H, N*H* Gly, *Arom.*), 7.71 ppm (m, 1H, N*H*-Temp); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=172.8$, 172.4, 171.9, 170.4, 170.3, 169.0, 156.5, 156.0, 153.7, 136.4, 136.0, 135.6, 135.0, 133.7, 130.6, 128.6, 128.2, 128.1, 128.0, 126.9, 124.2, 118.1, 81.5, 75.2, 73.8, 69.5, 67.0, 64.9, 64.2, 59.3, 53.2, 52.5, 52.4, 41.3, 40.6, 37.6, 36.6, 35.1, 33.4, 33.0, 32.7, 29.9, 29.1, 28.2, 26.9, 26.8, 25.5, 23.6, 21.6, 18.7, 17.6, 16.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{56}H_{76}N_8O_{13}S$ 1100.53, found: 1101 $[M+H]^+$; Microanalysis: calcd for $C_{56}H_{76}N_8O_{13}S$: C 61.07, H 6.96, N 10.17, found: C 61.10, H 6.95, N 10.17.

General procedure C. Synthesis of Z-Asp(*t*Bu)-Temp-Arg(Pmc)-Gly-OBn: Benzyl alcohol (2.1 mL, 20 mmol), molecular sieves 4 Å (0.4 g), and $Ti(iPrO)_4$ (29.6 μL, 0.1 mmol) were added to a solution of **37**–**43** (0.2 mmol) in dry THF (2 mL), under nitrogen atmosphere. The suspension was stirred at 90 °C for 36 h, then filtered through a pad of celite and washed with THF. The solvent was evaporated under reduced pressure and the crude was dissolved in CH_2Cl_2 (2 mL) and washed with 2N HCl (2×2 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude was purified by short flash chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 95:5) yielding **44**–**50** (79–91%).

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***Z*-Asp(*t*Bu)-Temp1-Arg(Pmc)-Gly-OBn (44).** Compound **44** was prepared following general procedure C (82%) as a white foam. $[\alpha]_D^{22} = -9.2$ ($c = 1.08$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (s, 6H, C(CH_3)$_2$ Pmc), 1.39 (s, 9H, C(CH_3)$_3$), 1.50–2.35 (m, 12H, CH_2), 1.78 (t, 2H, $J = 6.4$ Hz, Ar-CH_2-CH_2 Pmc), 2.08, 2.56, 2.58 (3s, 9H, CH_3 Pmc), 2.53 (m, 3H, $H\beta$ Asp, Ar-CH_2-CH_2 Pmc), 2.88 (m, 1H, $H\beta$ Asp), 2.95 (d, 1H, $J = 14.1$ Hz, CH_2Ph), 3.20 (d, 1H, $J = 14.1$ Hz, CH_2Ph), 3.25 (m, 2H, $H\delta$ Arg), 3.77 (m, 1H, *H*-6), 4.00 (dd, 1H, $J = 5.3$ Hz, $J = 18.0$ Hz, $H\alpha$ Gly), 4.12 (dd, 1H, $J = 5.3$ Hz, $J = 18.0$ Hz, $H\alpha$ Gly), 4.43 (m, 1H, $H\alpha$ Asp), 4.48 (dd, 1H, $J = 9.2$ Hz, $J < 1$ Hz, *H*-9), 4.55 (m, 1H, $H\alpha$ Arg), 5.14 (s, 4H, CH_2Ph), 5.86 (bs, 1H, N*H* Asp), 6.10–6.60 (bs, 3H, (N*H*)$_2$C=N*H*), 7.00–7.40 ppm (m, 18H, N*H* Gly, N*H* Arg, N*H* Temp, *Arom.*); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 172.2$, 172.1, 171.4, 171.0, 169.7, 169.5, 169.3, 156.4, 155.9, 153.5, 136.0, 135.5, 135.3, 134.8, 133.2, 130.6, 130.0, 128.8, 128.6, 128.3, 128.2, 127.4, 124.0, 117.8, 81.7, 73.5, 67.2, 67.0, 60.3, 59.8, 58.2, 53.0, 51.0, 41.4, 40.3, 36.8, 32.8, 31.8, 29.7, 29.3, 28.9, 27.9, 26.8, 26.0, 25.4, 21.4, 18.6, 17.5, 12.1 ppm; MS (FAB$^+$): *m/z* calcd for $C_{61}H_{78}N_8O_{13}S$ 1162.54, found: 1163 $[M+H]^+$; Microanalysis calcd for $C_{61}H_{78}N_8O_{13}S$: C 62.98, H 6.76, N 9.63, found: C 63.00, H 6.77, N 9.64.

***Z*-Asp(*t*Bu)-Temp2-Arg(Pmc)-Gly-OBn (45).** Compound **45** was prepared following general procedure C (85%) as a white foam. $[\alpha]_D^{22} = -100.4$ ($c = 1.10$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.39 (s, 9H, C(CH_3)$_3$), 1.65–2.25 (m, 12H, CH_2), 1.81 (t, 2H, $J = 6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.12 (s, 3H, CH_3 Pmc), 2.54 (m, 1H, $H\beta$ Asp), 2.58, 2.61 (2s, 6H, CH_3 Pmc), 2.64 (t, 2H, $J = 6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.81 (m, 1H, $H\beta$ Asp), 2.82 (d, 1H, $J = 13.2$ Hz, CH_2Ph), 3.21 (d, 1H, $J = 13.2$ Hz, CH_2Ph), 3.27 (m, 2H, $H\delta$ Arg), 3.44 (dd, 1H, $J = 5.6$ Hz, $J = 17.6$ Hz, $H\alpha$ Gly), 3.75 (m, 1H, *H*-6), 4.08 (dd, 1H, $J = 5.2$ Hz, $J = 17.6$ Hz, $H\alpha$ Gly), 4.36 (m, 1H, $H\alpha$ Arg), 4.43 (dd, 1H, $J = 9.2$ Hz, *H*-9), 4.54 (m, 1H, $H\alpha$ Asp), 4.89 (d, 1H, $J = 12.4$ Hz, Ph*H*CHO-), 5.06 (d, 1H, $J = 12.0$ Hz, PhCH_2O), 5.13 (d, 1H, $J = 12.0$ Hz, PhCH_2O), 5.31 (d, 1H, $J = 12.4$ Hz, PhHC*H*O-), 6.30 (bs, 3H, (N*H*)$_2$C=N*H*), 6.73 (d, 1H, $J = 8.0$ Hz, N*H* Asp), 7.19, 7.28, 7.36 (3 m, 15H, *Arom.*), 7.45 (d, 1H, $J = 8.4$ Hz, N*H* Arg), 7.58 (s, 1H, N*H* Temp), 7.71 ppm (m, 1H, N*H* Gly); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta =$ 173.4, 172.1, 171.9, 171.4, 171.3, 157.6, 155.9, 136.2, 135.9, 135.2, 134.6, 130.3, 130.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.0, 124.2, 118.1, 82.2, 73.8, 67.5, 67.4, 62.9, 61.5, 60.5, 58.6, 53.5, 50.6, 43.4, 41.8, 41.0, 36.5, 35.0, 33.2, 33.0, 31.1, 29.0, 28.2, 28.0, 27.0, 26.9, 26.4, 26.3, 21.6, 19.1, 18.7, 17.7, 14.0, 12.3 ppm; MS (FAB$^+$): *m/z* calcd for $C_{61}H_{78}N_8O_{13}S$ 1162.54, found: 1163 $[M+H]^+$; Microanalysis: calcd for $C_{61}H_{78}N_8O_{13}S$: C 62.98, H 6.76, N 9.63, found: C 62.96, H 6.76, N 9.64.

***Z*-Asp(*t*Bu)-Temp3-Arg(Pmc)-Gly-OBn (46)**. Compound **46** was prepared following general procedure C (79%) as a white foam. $[\alpha]_D^{22} = -21.0$ ($c = 1.00$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (s, 6H, C(CH_3)$_2$ Pmc), 1.39 (s, 9H, C(CH_3)$_3$), 1.54–2.42 (m, 12H, CH_2), 1.78 (t, 2H, $J = 6.6$ Hz, Ar-CH_2-CH_2 Pmc), 2.08 (s, 3H, CH_3 Pmc), 2.53 (m, 1H, $H\beta$ Asp), 2.57, 2.58 (2s, 6H, CH_3 Pmc), 2.60 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.86 (dd, 1H, $J = 17.1$ Hz, $J = 4.8$ Hz, $H\beta$ Asp), 3.13 (d, 1H, $J = 13.8$ Hz, CH_2Ph), 3.17 (d, 1H, $J = 13.8$ Hz, CH_2Ph), 3.27 (m, 2H, $H\delta$ Arg), 3.80 (m, 1H, *H*-6), 4.00 (dd, 1H, $J = 5.7$ Hz, $J = 17.9$ Hz, $H\alpha$ Gly), 4.10 (dd, 1H, $J = 5.7$ Hz, $J = 17.9$ Hz, $H\alpha$ Gly), 4.44 (m, 1H, $H\alpha$ Asp), 4.52 (dd, 1H, $J = 8.6$ Hz, *H*-9), 4.62 (m, 1H, $H\alpha$ Arg), 5.00–5.17 (m, 4H, PhCH_2O), 5.91 (bs, 1H, N*H* Asp), 6.28 (bs, 3H, (N*H*)$_2$C=N*H*), 7.04–7.38 (m, 16H, N*H* Temp, *Arom.*), 7.44 (bs, 1H, N*H* Gly), 7.80 ppm (bs, 1H, N*H* Arg); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 172.8$, 172.6, 172.5, 171.5, 170.7, 170.1, 169.9, 169.5, 169.2, 156.1, 136.3, 135.6, 135.3, 133.5, 130.8, 130.3, 130.0, 128.7, 128.6, 128.5, 128.4, 128.3, 127.3, 124.4, 118.2, 81.8, 73.8, 69.1, 67.3, 67.1, 62.9, 61.8, 60.2, 58.2, 53.2, 51.7, 43.3, 41.6, 40.8, 37.2, 33.5, 33.1, 32.9, 29.9, 29.6, 29.0, 28.7, 28.1, 27.1, 27.0, 26.9, 25.4, 22.9, 21.9, 21.6, 18.8, 17.7, 14.3, 14.0, 12.3 ppm; MS (FAB$^+$): calcd for $C_{61}H_{78}N_8O_{13}S$ 1162.54, found 1163 $[M+H]^+$; Microanalysis: calcd for $C_{61}H_{78}N_8O_{13}S$: C 62.98, H 6.76, N 9.63, found: C 62.97, H 6.77, N 9.63.

***Z*-Asp(*t*Bu)-*H*2-Temp4-Arg(Pmc)-Gly-OBn (47).** Compound **47** was prepared following general procedure C (94%) as a white foam. $[\alpha]_D^{22} = -79.6$ ($c = 1.06$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (s, 6H, C(CH_3)$_2$ Pmc), 1.40 (s, 9H, C(CH_3)$_3$), 1.50–2.35 (m, 14H, CH_2), 1.80 (t, 2H, $J = 6.7$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.65 (m, 4H, $H\beta$ Asp, Ar-CH_2-CH_2 Pmc), 2.98 (d, 1H, $J = 11.7$ Hz, HC*H*Ph), 3.20 (m, 2H, $H\delta$ Arg), 3.44 (m, 1H, HC*H*Ph), 3.71 (m, 2H, *H*-7, $H\alpha$ Gly), 4.05 (dd, 1H, $J = 6.1$ Hz, $J = 17.1$ Hz, $H\alpha$ Gly), 4.41 (m, 1H, $H\alpha$ Arg), 4.47 (dd, 1H, $J = 8.1$ Hz, $J < 1$ Hz, *H*-10), 4.54 (m, 1H, $H\alpha$ Asp), 4.92 (d, 1H, $J = 12.2$ Hz, HC*H*Ph), 5.13 (m, 3H, CH_2 Ph), 5.90–6.15 (bs, 3H, (N*H*)$_2$C=N*H*), 6.40 (bs, 1H, N*H* Asp), 7.05–7.47 (m, 16H, N*H* Temp, *Arom.*), 7.50 (bs, 1H, N*H* Arg), 7.54 ppm (bs, 1H, N*H* Gly); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 173.0$, 172.5, 172.3, 170.3, 156.6, 156.5, 153.7, 136.5, 135.8, 135.6, 135.5, 135.0, 130.1, 130.0, 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.1, 124.1, 118.0, 81.5, 75.2, 73.7, 69.5, 67.2, 67.0, 65.0, 59.3, 41.5, 33.0, 28.1, 27.0, 26.9, 21.6, 18.7, 17.7, 16.7, 12.3 ppm; MS (FAB$^+$): *m/z* calcd for $C_{62}H_{80}N_8O_{13}S$ 1176.56, found: 1177 $[M+H]^+$; Microanalysis calcd for $C_{62}H_{80}N_8O_{13}S$: C 63.25, H 6.85, N 9.52, found: C 63.22, H 6.84, N 9.51.

***Z*-Asp(*t*Bu)-*H*2-Temp5-Arg(Pmc)-Gly-OBn (48).** Compound **48** was prepared following general procedure C (91%) as a white foam. $[\alpha]_D^{22} = -82.0$ ($c = 1.06$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (s, 6H, C(CH_3)$_2$ Pmc), 1.40 (s, 9H, C(CH_3)$_3$), 1.52–2.08 (m, 14H, CH_2), 1.79 (t, 2H, $J = 6.6$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.57, 2.60 (3s, 9H, CH_3 Pmc), 2.64 (m, 3H, Ar-CH_2-CH_2 Pmc, $H\beta$ Asp), 2.78 (d, 1H, $J = 13.4$ Hz, HC*H*Ph), 2.96 (m, 1H, $H\beta$ Asp), 3.27 (m, 2H, $H\delta$ Arg), 3.56 (d, 1H, $J = 13.4$ Hz, HC*H*Ph), 3.90 (m, 1H, *H*-7), 4.00 (dd, 1H, $J = 16.5$ Hz, $J = 5.4$ Hz, $H\alpha$ Gly), 4.11 (dd, 1H, $J = 16.5$ Hz, $J = 5.4$ Hz, $H\alpha$ Gly), 4.46 (dd, 1H, $J = 7.5$ Hz, $J < 1$ Hz, *H*-10), 4.54 (m, 2H, $H\alpha$ Arg, $H\alpha$ Asp), 5.12 (m, 4H, CH_2Ph), 6.20–6.60 (bs, 3H, (N*H*)$_2$C=N*H*), 6.28 (bs, 1H, N*H* Asp), 7.00, 7.33 (2 m, 17H, N*H* Temp, N*H* Gly, *Arom.*), 7.44 ppm (bs, 1H, N*H* Arg); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 173.2$, 172.4, 171.8, 156.6, 153.6, 136.8, 135.7, 135.4, 135.0, 131.7, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 126.9, 124.1, 118.0, 82.1, 73.7, 71.8, 71.2, 67.2, 62.1, 60.2, 28.2, 27.0, 26.9, 19.5, 18.7, 17.5, 14.1, 12.3 ppm; MS (FAB$^+$): *m/z* calcd for $C_{62}H_{80}N_8O_{13}S$ 1176.56, found: 1177 $[M+H]^+$; Microanalysis: calcd for $C_{62}H_{80}N_8O_{13}S$: C 63.25, H 6.85, N 9.52, found: C 63.24, H 6.84, N 9.53.

***Z*-Asp(*t*Bu)-Temp6-Arg(Pmc)-Gly-OBn (49).** Compound **49** was prepared following general procedure C (86%) as a white foam. $[\alpha]_D^{22} = +28.4$ ($c = 1.02$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 6H, C(CH_3)$_2$ Pmc), 1.42 (s, 9H, C(CH_3)$_3$), 1.53–2.36 (m, 14H, CH_2), 1.81 (t, 2H, $J = 6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.12, 2.59, 2.61 (3s, 9H, CH_3 Pmc), 2.64 (m, 3H, Ar-CH_2-CH_2 Pmc, $H\beta$ Asp), 2.93 (d, 1H, $J = 13.8$ Hz, CH_2Ph), 3.25 (m, 1H, $H\beta$ Asp), 3.30 (m, 2H, $H\delta$ Arg), 3.47 (d, 1H, $J = 13.8$ Hz, CH_2Ph), 3.88 (dd, 1H, $J = 17.6$ Hz, $J = 5.4$ Hz, $H\alpha$ Gly), 4.30 (dd, 1H, $J = 17.6$ Hz, $J = 6.4$ Hz, $H\alpha$ Gly), 4.48 (m, 2H, *H*-7, $H\alpha$ Arg), 4.51 (dd, 1H, $J = 7.4$ Hz, *H*-10), 4.64 (m, 1H, $H\alpha$ Asp), 4.74 (d, 1H, $J = 12.5$ Hz, HC*H*Ph), 5.01 (m, 3H, CH_2Ph), 6.23 (bs, 3H, (N*H*)$_2$C=N*H*), 7.10–7.38 (m, 17H, N*H* Temp, N*H* Asp, *Arom.*), 7.74 (d, 1H, $J = 8.4$ Hz, N*H* Arg), 8.13 ppm (m, 1H, N*H* Gly); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 174.6$, 173.1, 171.8, 171.1, 170.6, 157.7, 155.9, 153.8, 136.1, 135.1, 131.5, 130.9, 128.5, 128.3, 128.1, 128.0, 127.6, 125.7, 124.2, 118.0, 111.2, 81.5, 73.7, 67.1, 64.2, 60.7, 58.1, 50.5, 41.3, 40.7, 35.2, 33.7, 33.0, 32.7, 31.9, 30.4, 29.7, 28.2, 27.5, 26.7, 25.0,

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23.1, 22.7, 21.4, 19.1, 18.9, 18.5, 17.5, 14.1, 13.9, 12.1 ppm; MS (FAB^+): *m/z* calcd for $C_{62}H_{80}N_8O_{13}S$ 1176.56, found: 1177 $[M+H]^+$; Microanalysis: calcd for $C_{62}H_{80}N_8O_{13}S$: C 63.25, H 6.85, N 9.52, found: C 63.27, H 6.86, N 9.52.

***Z*-Asp(*t*Bu)-Temp7-Arg(Pmc)-Gly-OBn (50).** Compound **50** was prepared following general procedure C (92%) as a white foam. $[\alpha]_D^{22}=-4.4$ ($c=0.95$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ (s, 6H, $C(CH_3)_2$ Pmc), 1.43 (s, 9H, $C(CH_3)_3$), 1.55–2.10 (m, 14H, CH_2), 1.80 (t, 2H, $J=6.8$ Hz, Ar-CH_2-CH_2 Pmc), 2.11, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.62 (m, 4H, Hβ Asp, Ar-CH_2-CH_2 Pmc), 2.95 (m, 1H, Hδ Arg), 3.08 (m, 1H, Hδ Arg), 3.52 (d, 1H, $J=13.2$ Hz, HCHPh), 3.65 (d, 1H, $J=13.2$ Hz, HCHPh), 4.01 (dd, 1H, $J=18.0$ Hz, $J=5.2$ Hz, Hα Gly), 4.11 (dd, 1H, $J=18.0$ Hz, $J=5.2$ Hz, Hα Gly), 4.38 (m, 1H, Hα Asp), 4.46 (m, 2H, *H*-7, Hα Arg), 4.55 (m, 1H, *H*-10), 4.92 (d, 1H, $J=12.9$ Hz, HCHPh), 5.01 (d, 1H, $J=12.9$ Hz, HCHPh), 5.17 (s, 2H CH_2 Ph), 5.80 (bs, 1H, NH Asp), 6.10 (bs, 3H, $(NH)_2C=NH$), 6.95 (bs, 1H, NH Arg), 7.14 (m, 6H, NH Gly, *Arom.*), 7.22–7.45 (m, 10H, *Arom.*), 7.71 ppm (s, 1H, NH Temp); ^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta=172.8$, 172.4, 171.9, 170.5, 169.8, 169.0, 156.3, 156.1, 153.8, 136.3, 136.0, 135.7, 135.4, 135.0, 130.6, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 126.9, 124.2, 118.1, 81.5, 73.8, 67.2, 67.0, 64.9, 64.2, 59.4, 53.1, 52.5, 41.5, 40.6, 37.6, 35.1, 33.4, 32.9, 29.9, 29.2, 28.2, 26.9, 23.6, 21.6, 18.8, 17.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{62}H_{80}N_8O_{13}S$ 1176.56, found: 1177 $[M+H]^+$; Microanalysis: calcd for $C_{62}H_{80}N_8O_{13}S$: C 63.25, H 6.85, N 9.52, found: C 63.28, H 6.86, N 9.51.

General procedure D. Synthesis of Cyclo[Arg(Pmc)-Gly-Asp(*t*Bu)-Temp]: A solution of **44**–**50** (0.2 mmol) in MeOH (2 mL) containing a catalytic amount of 10% Pd/C was stirred for 4 h under hydrogen atmosphere. After reaction completion, the mixture was filtered through pad of celite and washed with MeOH. The collected organic phase was evaporated under reduced pressure to yield **14**–**20** as white foams that were used without further purification. HATU (152 mg, 0.4 mmol), HOAt (54.4 mg, 0.4 mmol), and 2,4,6-collidine (53 μL, 0.4 mmol) were added to a solution of crude **14**–**20** (0.2 mmol) in dry DMF (200 mL), under nitrogen atmosphere. The solution was stirred at room temperature for 48–72 h, then the solvent was evaporated under reduced pressure. The crude was dissolved in CH_2Cl_2 (4 mL) and washed with a saturated solution of $NaHCO_3$ (2×4 mL) and 1 M $KHSO_4$ (4 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) yielding **51**–**54** (64–78% over two steps).

Cyclo[Arg(Pmc)-Gly-Asp(*t*Bu)-Temp2] (51). Compound **51** was prepared following the general procedure D (72% over two steps) as a white solid; mp: 178–180 °C; $[\alpha]_D^{22}=-42.2$ ($c=1.07$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.60$ (m, 1H, *H*-7), 1.13 (m, 1H, *H*-5), 1.32 [s, 6H, $C(CH_3)_2$ Pmc], 1.38 (s, 9H, $C(CH_3)_3$), 1.52 (m, 1H, Hγ Arg), 1.63 (m, 1H, Hγ Arg), 1.75 (m, 1H, *H*-8), 1.77 (m, 2H, Hβ Arg, *H*-7), 1.80 (m, 2H, Ar-CH_2-CH_2 Pmc), 2.04 (m, 1H, Hβ Arg), 2.05 (m, 1H, *H*-5), 2.07 (m, 1H, *H*-4), 2.25 (m, 1H, *H*-4), 2.10, 2.57, 2.59 (3s, 9H, CH_3 Pmc), 2.49 (m, 1H, *H*-8), 2.54 (m, 1H, Hβ Asp), 2.64 (m, 3H, Ar-CH_2 Pmc, Hβ Asp), 2.86 (m, 1H, CH_2Ph), 3.22 (m, 1H, Hδ Arg), 3.32 (m, 1H, Hδ Arg), 3.33 (m, 1H, Hα Gly), 3.38 (m, 1H, CH_2Ph), 3.78 (m, 1H, *H*-6), 4.40 (m, 1H, *H*-9), 4.53 (m, 1H, Hα Gly), 4.65 (m, 1H, Hα Asp), 4.67 (m, 1H, Hα Arg), 6.10–6.40 (m, 3H, $(NH)_2C=NH$), 6.68 (m, 1H, NH Asp), 7.01 (m, 1H, NH Temp), 7.24 (m, 1H, NH Arg), 7.10–7.40 (m, 5H, *Arom.*), 7.77 ppm (m, 1H, NH Gly); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=172.7$, 171.7, 171.2, 170.9, 169.3, 156.4, 153.7, 135.7, 135.1, 133.5, 130.2, 129.0, 127.8, 124.1, 118.1, 81.8, 73.8, 61.9, 59.8, 59.1, 52.1, 50.3, 44.8, 44.2, 41.0, 37.6, 33.2, 33.0, 30.8, 29.5, 28.6, 28.1, 27.0, 26.8, 25.3, 21.6, 18.7, 17.6, 15.4, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{46}H_{64}N_8O_{10}S$ 920.45, found: 921 $[M+H]^+$; Microanalysis: calcd for $C_{46}H_{64}N_8O_{10}S$: C 59.98, H 7.00, N 12.17, found: C 59.96, H 6.99, N 12.18.

Cyclo[Arg(Pmc)-Gly-Asp(*t*Bu)-*H*2-Temp4] (52). Compound **52** was prepared following general procedure D (78% over two steps) as a white solid; mp: 170–172 °C; $[\alpha]_D^{22}=-42.1$ ($c=1.01$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.31$ [s, 6H, $C(CH_3)_2$ Pmc], 1.50 (s, 9H, $C(CH_3)_3$), 1.59 (m, 2H, *H*-6), 1.60 (m, 1H, *H*-4), 1.61 (m, 2H, Hγ Arg), 1.70 (m, 1H, *H*-5), 1.80 (m, 1H, *H*-8), 1.81 (m, 3H, Ar-CH_2-CH_2 Pmc, *H*-5), 1.83 (m, 1H, *H*-4), 1.85 (m, 1H, Hβ Arg), 1.86 (m, 2H, *H*-8, *H*-9), 2.01 (m, 1H, Hβ Arg), 2.11, 2.58, 2.60 (3s, 9H, CH_3 Pmc), 2.21 (m, 1H, *H*-9), 2.61 (m, 3H, Ar-CH_2 Pmc, Hβ Asp), 2.97 (m, 1H, Hβ Asp), 3.27 (m, 1H, CH_2Ph), 3.28 (m, 1H, *H*-7), 3.30 (m, 2H, Hδ Arg), 3.38 (m, 1H, Hα Gly), 3.60 (m, 1H, CH_2Ph), 3.91 (m, 1H, Hα Gly), 4.12 (m, 1H, *H*-10), 4.63 (m, 1H, Hα Arg), 4.77 (m, 1H, Hα Asp), 6.10–6.40 (m, 3H, $(NH)_2C=NH$), 6.54 (m, 1H, NH Arg), 7.00–7.30 (m, 5H, *Arom.*), 7.16 (m, 1H, NH Temp), 7.79 (m, 1H, NH Asp), 8.25 ppm (m, 1H, NH Gly); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=174.0$, 173.2, 171.6, 170.2, 169.8, 156.5, 153.7, 136.4, 135.7, 135.0, 130.4, 128.5, 127.2, 124.1, 118.1, 81.4, 73.8, 71.9, 67.8, 66.1, 62.0, 56.0, 52.4, 50.7, 45.6, 40.5, 35.6, 33.0, 31.9, 31.2, 29.9, 28.3, 27.0, 26.9, 25.6, 21.6, 19.5, 18.7, 18.4, 17.7, 12.3 ppm; MS (FAB^+): *m/z* calcd for $C_{47}H_{66}N_8O_{10}S$ 934.46, found: 935 $[M+H]^+$; Microanalysis: calcd for $C_{47}H_{66}N_8O_{10}S$: C 60.37, H 7.11, N 11.98, found: C 60.35, H 7.10, N 11.99.

Cyclo[Arg(Pmc)-Gly-Asp(*t*Bu)-Temp6] (53). Compound **53** was prepared following general procedure D (66% over two steps) as a white solid; mp: 179–181 °C; $[\alpha]_D^{22}=-16.8$ ($c=1.00$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.33$ [s, 15H, $C(CH_3)_2$ Pmc $C(CH_3)_3$], 1.53 (m, 1H, Hγ Arg), 1.63 (m, 1H, Hγ Arg), 1.70 (m, 1H, *H*-5), 1.74 (m, 1H, *H*-6), 1.80 (m, 1H, Hβ Arg), 1.81 (m, 2H, Ar-CH_2-CH_2 Pmc), 1.85 (m, 1H *H*-5), 1.88 (m, 1H, *H*-4), 1.91 (m, 1H, *H*-8), 1.97 (m, 1H, *H*-9), 2.01 (m, 1H, Hβ Arg), 2.10, 2.57, 2.60 (3 s, 9H, CH_3 Pmc), 2.23 (m, 2H, *H*-8, *H*-9), 2.25 (m, 1H, *H*-6), 2.42 (m, 1H, *H*-4), 2.53 (m, 1H, Hβ Asp), 2.63 (m, 1H, Hβ Asp), 2.65 (m, 2H, Ar-CH_2 Pmc), 3.21 (m, 1H, Hδ Arg), 3.22 (m, 1H, CH_2Ph), 3.27 (m, 1H, Hδ Arg), 3.31 (m, 1H, CH_2Ph), 3.35 (m, 1H, Hα Gly), 4.43 (m, 1H, Hα Gly), 4.49 (m, 1H, *H*-10), 4.50 (m, 1H, Hα Arg), 4.59 (m, 1H, Hα Asp), 4.61 (m, 1H, *H*-7), 6.10–6.40 (m, 3H, $(NH)_2C=NH$), 6.84 (m, 1H, NH Temp), 6.93 (m, 1H, NH Asp), 7.19 (m, 1H, NH Arg), 7.20–7.40 (m, 5H, *Arom.*), 7.75 ppm (m, 1H, NH Gly); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=174.0$, 171.6, 171.2, 170.0, 169.9, 136.5, 131.3, 128.7, 127.6, 124.3, 118.3, 81.6, 73.9, 66.4, 65.5, 59.7, 50.9, 45.9, 34.8, 34.5, 32.9, 29.9, 28.2, 27.0, 23.7, 21.6, 18.8, 18.7, 17.7, 12.4 ppm; MS (FAB^+): *m/z* calcd for $C_{47}H_{66}N_8O_{10}S$ 934.46, found: 935 $[M+H]^+$; Microanalysis: calcd for $C_{47}H_{66}N_8O_{10}S$: C 60.37, H 7.11, N 11.98, found: C 60.36, H 7.11, N 11.97.

Cyclo[Arg(Pmc)-Gly-Asp(*t*Bu)-Temp7] (54). Compound **54** was prepared following general procedure D (64% over two steps) as a white solid; mp: 175–177 °C; $[\alpha]_D^{22}=-43.4$ ($c=1.03$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.33$ (s, 6H, $C(CH_3)_2$ Pmc), 1.48 (s, 9H, $C(CH_3)_3$), 1.39 (m, 1H, *H*-6), 1.50 (m, 1H, Hγ Arg), 1.61 (m, 1H, Hγ Arg), 1.67 (m, 1H, *H*-4), 1.76 (m, 1H, *H*-8), 1.80 (m, 2H, Ar-CH_2-CH_2 Pmc), 1.91 (m, 2H, *H*-5, Hβ Arg), 1.97 (m, 1H, *H*-9), 2.00 (m, 1H *H*-5), 2.06 (m, 1H *H*-5), 2.12, 2.58, 2.60 (3s, 9H, CH_3 Pmc), 2.14 (m, 1H, Hβ Arg), 2.27 (m, 1H, *H*-9), 2.48 (m, 1H, *H*-8), 2.51 (m, 1H, Hβ Asp), 2.61 (m, 1H, Hβ Asp), 2.63 (m, 2H, Ar-CH_2 Pmc), 2.72 (m, 1H, *H*-4), 3.22 (m, 1H, Hα Gly), 3.51 (m, 2H, CH_2Ph, Hδ Arg), 3.71 (m, 1H, Hα Gly), 3.77 (m, 2H, Hδ Arg, CH_2Ph), 4.19 (m, 1H, Hα Arg), 4.33 (m, 1H, *H*-7), 4.36 (m, 1H, *H*-10), 4.98 (m, 1H, Hα Asp), 6.05–6.25 (m, 3H, $(NH)_2C=NH$), 6.38 (m, 1H, NH Asp), 6.41 (m, 1H, NH Arg), 7.10–7.35 (m, 5H, *Arom.*), 7.33 (m, 1H, NH Gly), 8.00 ppm (m, 1H, NH Temp); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=173.3$, 172.8, 171.5, 170.8, 169.7, 156.2, 135.8, 135.3, 130.8, 128.9, 127.5, 124.3, 118.2, 81.6, 73.9, 65.5,

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64.2, 57.8, 53.8, 50.4, 44.7, 41.6, 40.8, 36.7, 33.0, 32.7, 28.5, 28.1, 27.3, 27.0, 25.7, 21.6, 19.4, 18.7, 17.7, 12.3 ppm; MS (FAB$^+$): *m/z* calcd for $C_{47}H_{66}N_8O_{10}S$ 934.46, found: 935 $[M+H]^+$; Microanalysis: calcd for $C_{47}H_{66}N_8O_{10}S$: C 60.37, H 7.11, N 11.98, found: C 60.34, H 7.10, N 11.98.

General procedure E. Synthesis of Cyclo[Arg-Gly-Asp-Temp] (55–58): A solution of **51**–**54** (0.2 mmol) in TFA/thioanisole/1,2-ethanedithiol/anisole 90:5:3:2 (67 mL) was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure then the crude was dissolved in H_2O (4 mL) and washed with iPr_2O (2× 4 mL). The aqueous phase was evaporated under reduced pressure and the crude purified by HPLC (C18 column; gradient from 0–100% MeCN/H_2O + 0.1% TFA to 50–50% MeCN/H_2O + 0.1% TFA over 30 min then 50–50% MeCN/H_2O + 0.1% TFA for 5 min). Excess of TFA was removed under vacuum and the residue was treated with gaseous HCl to afford pure **55**–**58** (71–96% over two steps) as white amorphous solids.

Cyclo[Arg-Gly-Asp-Temp2] (55). Compound **55** was prepared in 96% yield over two steps following general procedure E. (White solid); $[\alpha]_D^{22}=-96.8$ ($c=1.03$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta=$ 0.05 (m, 1H, *H*-5), 0.87 (m, 1H, *H*-7), 1.44 (m, 1H, Hγ Arg), 1.57 (m, 1H, *H*-4), 1.67 (m, 1H, Hβ Arg), 1.60 (m, 1H, Hγ Arg), 1.79 (m, 1H, Hβ Arg), 1.69 (m, 1H, *H*-8), 1.81 (m, 1H, *H*-4), 1.86 (m, 1H, *H*-7), 2.05 (m, 1H, *H*-5), 2.30 (m, 1H, *H*-8), 2.66 (m, 2H, Hβ Asp), 2.77 (1H, C*H*$_2$Ph), 3.20 (m, 2H, Hδ Arg), 3.30 (m, 2H, Hα Gly, C*H*$_2$Ph), 3.53 (m, 1H, *H*-6), 4.20 (m, 1H, Hα Gly), 4.31 (m, 1H, *H*-9), 4.38 (m, 1H, Hα Arg), 4.70 (m, 1H, Hα Asp), 7.0–7.20 ppm (m, 5H, *Arom.*); ^{13}C NMR-HETCOR (400 MHz, D_2O): $\delta=$130.6, 129.6, 128.5, 61.9, 60.7, 60.0, 52.8, 50.0, 44.5, 41.1, 36.5, 32.8, 31.3, 29.4, 28.4, 25.1, 24.7 ppm; MS (FAB$^+$): calcd for $C_{28}H_{39}ClN_8O_7$ 634.26, found 599 $[M-Cl]^+$. Anal. calcd for $C_{28}H_{39}ClN_8O_7$: C 52.95, H 6.19, N 17.64, found: C 52.96, H 6.18, N 17.63.

Cyclo[Arg-Gly-Asp-*H*2-Temp4] (56). Compound **56** was prepared in 96% yield over two steps following general procedure E. (White solid); $[\alpha]_D^{22}=-85.9$ ($c=0.95$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta=$ 1.54 (m, 2H, Hγ Arg), 1.61 (m, 1H, *H*-5), 1.63 (m, 1H, Hβ Arg), 1.73 (m, 1H, *H*-8), 1.74 (m, 1H, Hβ Arg), 1.86 (m, 1H, *H*-9), 1.90 (m, 1H, *H*-5), 1.93 (m, 1H, *H*-6), 2.02 (m, 1H, *H*-4), 2.07 (m, 1H, *H*-6), 2.17 (m, 1H, *H*-8), 2.18 (m, 1H, *H*-9), 2.59 (m, 1H, *H*-4), 2.69 (m, 1H, Hβ Asp), 2.90 (m, 1H, Hβ Asp), 3.15 (m, 2H, Hδ Arg), 3.23 (1H, C*H*$_2$Ph), 3.46 (1H, C*H*$_2$Ph), 3.50 (m, 1H, Hα Gly), 3.91 (m, 1H, Hα Gly), 4.01 (m, 1H, *H*-7), 4.22 (m, 1H, *H*-10), 4.31 (m, 1H, Hα Arg), 4.79 (m, 1H, Hα Asp), 6.85 (bs, 1H, NH Arg), 7.0–7.26 (5H, *Ph*), 7.80 ppm (bs, 1H, NH Temp); ^{13}C NMR-HETCOR (400 MHz, D_2O):$\delta=$130.6, 129.2, 128.0, 66.5, 59.5, 52.8, 50.6, 44.2, 40.8, 38.4, 33.9, 32.9, 32.7, 30.5, 29.2, 26.8, 24.6, 21.9 ppm; MS (FAB$^+$): calcd for $C_{29}H_{41}ClN_8O_7$ 648.28, found 613 $[M-Cl]^+$. Anal. calcd for $C_{29}H_{41}ClN_8O_7$: C 53.66, H 6.37, N 17.26, found: C 53.67, H 6.36, N 17.27.

Cyclo[Arg-Gly-Asp-Temp6] (57). Compound **57** was prepared in 82% yield over two steps following general procedure E. (White solid); $[\alpha]_D^{22}=+38.1$ ($c=0.68$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta=$ 1.49 (m, 1H, Hβ Arg), 1.53 (m, 1H, Hβ Arg), 1.63 (m, 1H, *H*-8), 1.64 (m, 1H, *H*-4), 1.72 (m, 2H, *H*-5), 1.76 (m, 2H, *H*-4, *H*-9), 1.81 (m, 2H, Hγ Arg), 1.87 (m, 1H, *H*-8), 2.06 (m, 1H, *H*-6), 2.24 (m, 1H, *H*-9), 2.33 (m, 1H, *H*-6), 2.85 (m, 2H, Hβ Asp), 3.17 (4H, C*H*$_2$Ph, Hδ Arg), 3.48 (m, 1H, Hα Gly), 4.17 (m, 1H, *H*-7), 4.24 (m, 1H, Hα Gly), 4.31 (m, 1H, Hα Arg), 4.48 (m, 1H, *H*-10), 4.67 (m, 1H, Hα Asp), 7.12–7.31 ppm (m, 5H, *Arom.*); ^{13}C NMR-HETCOR (400 MHz, D_2O):$\delta=$ 131.5, 129.1, 127.9, 65.4, 64.9, 59.5, 53.9, 50.7, 44.5, 40.9, 34.9, 33.0, 32.3, 29.0, 27.8, 27.5, 24.9, 20.9 ppm; MS (FAB$^+$): calcd for $C_{29}H_{41}ClN_8O_7$ 648.28, found 613 $[M-Cl]^+$. Anal. calcd for $C_{29}H_{41}ClN_8O_7$: C 53.66, H 6.37, N 17.26, found: C 53.65, H 6.38, N 17.27.

Cyclo[Arg-Gly-Asp-Temp7] (58). Compound **58** was prepared in 71% yield over two steps following general procedure E. (White solid); $[\alpha]_D^{22}=-54.7$ ($c=1.01$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta=$ 1.35 (m, 1H, *H*-4), 1.49 (m, 1H, Hγ Arg), 1.62 (m, 1H, Hγ Arg), 1.73 (m, 1H, *H*-8), 1.76 (m, 1H, *H*-9), 1.83 (m, 2H, Hβ Arg, *H*-6), 1.87 (m, 1H, *H*-5), 1.95 (m, 1H, *H*-6), 1.97 (m, 1H, Hβ Arg), 2.04 (m, 2H, *H*-5, *H*-4), 2.18 (m, 1H, *H*-9), 2.42 (m, 1H, *H*-8), 2.49 (m, 1H, Hβ Asp), 2.75 (m, 1H, Hβ Asp), 3.17 (m, 2H, Hδ Arg), 3.54 (m, 2H, C*H*$_2$Ph), 3.55 (m, 1H, Hα Gly), 3.68 (m, 1H, Hα Gly), 4.20 (m, 1H, Hα Arg), 4.36 (m, 2H, *H*-7, *H*-10), 4.89 (m, 1H, Hα Asp), 7.15–7.32 (m, 5H, *Arom.*), 7.73 (bs, 1H, NH Gly), 7.81 ppm (bs, 1H, NH Asp); ^{13}C NMR-HETCOR (400 MHz, D_2O):$\delta=$131.2, 129.3, 128.6, 109.4, 66.3, 60.3, 59.7, 53.7, 51.2, 45.2, 40.7, 35.4, 35.3, 33.3, 32.6, 27.5, 26.2, 26.0, 25.1, 23.1 ppm; MS (FAB$^+$): calcd for $C_{29}H_{41}ClN_8O_7$ 648.28, found 613 $[M-Cl]^+$. Anal. calcd for $C_{29}H_{41}ClN_8O_7$: C 53.66, H 6.37, N 17.26, found: C 53.64, H 6.36, N 17.25.

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