Design, Synthesis, and Biological Activities of Four Angiotensin II Receptor Ligands with γ-Turn Mimetics Replacing Amino Acid Residues 3−5

Boris Schmidt,[†,#] Susanna Lindman,[†] Weimin Tong,[†] Gunnar Lindeberg,[‡] Adolf Gogoll,[§] Zhennan Lai,[▽] Madeleine Thörnwall,[▽] Barbro Synnergren,[▽] Annika Nilsson,[⊥] Christopher J. Welch,[†] Morgan Sohtell,[⊥] Christer Westerlund,[∥] Fred Nyberg,[▽] Anders Karlén,[†] and Anders Hallberg*[,†]

Departments of Organic Pharmaceutical Chemistry and Medical and Physiological Chemistry, Biomedical Centre, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden, Department of Organic Chemistry, Uppsala University, SE-751 21 Uppsala, Sweden, Department of Biological Research on Drug Dependence, Uppsala University, SE-751 24 Uppsala, Sweden, and Department of Pharmacology and Medicinal Chemistry II, Astra Hässle AB, SE-431 83 Mölndal, Sweden

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Disulfide cyclization is a powerful method for reducing the conformational space of a peptide. This in turn may enable the study of its bioactive conformation. Several analogues of angiotensin II (Ang II) containing a disulfide bridge between amino acids 3 and 5 have been reported. Among these the cyclic octapeptides c[Hcy^{3,5}]-Ang II, c[Cys^{3,5}]-Ang II, and c[Pen^{3,5}]-Ang II showed significant activity at Ang II receptors. We have performed conformational analysis studies using theoretical calculations and ^{1}H-NMR spectroscopy on tripeptide model compounds of these cyclic octapeptides which show that the cyclic moieties of c[Cys^{3,5}]-Ang II and c[Pen^{3,5}]-Ang II preferentially assume an inverse γ-turn conformation. On the basis of these results, we substituted amino acid residues 3−5 in Ang II with two different γ-turn mimetics giving four diastereomeric Ang II analogues. Interestingly, two of these are equipotent to Ang II in binding to AT_1 receptors. In the contractile test using rabbit aorta rings, one of the analogues is an agonist with full contractile activity approximately equipotent to c[Pen^{3,5}]-Ang II but 300-fold less potent than Ang II. This low potency may suggest that Ang II does not adopt a γ-turn in the 3−5 region when interacting with the receptor.

Introduction

Small peptide hormones as well as peptide neurotransmitters have become important candidates for the development of new chemical entities. However, peptides are seldom directly usable as drugs due to low oral absorption and/or rapid metabolism. Therefore, a major challenge is to devise strategies for the transformation of peptides into nonpeptides. A hypothesis of a bioactive conformation can serve as a starting point for the design of such analogues. Restriction of the conformational freedom by cyclization provides one tool with which to gain valuable information on the bioactive conformation(s).[1] We have an interest in angiotensin II (Ang II; Chart 1) which plays an important role in blood pressure regulation. The structure−activity relationships (SAR) of Ang II are well established[2−4] making this peptide a suitable and challenging target for the design and synthesis of nonpeptide analogues.

Several models of the bioactive conformation of Ang II have been proposed. Among these, a possible receptor-bound conformation was identified by Nikiforovich and Marshall by searching for a common spatial arrangement of functionally important groups in low-energy conformers of Ang II and its active analogues.[5,6] This model allowed for the design of [D-Tyr^4,Pro^5]-Ang II which was found to exhibit good binding affinity.[5] On the basis of conformational analysis and cluster analysis, Joseph et al.[7] proposed putative models of the Ang II receptor-bound conformations, one of which is closely related to that proposed by Nikiforovich and Marshall. Samanen et al.[8] also recently proposed a refined conformational model of Ang II. The models above were all partly based on the pioneering work of Spear et al.[9] and Sugg et al.[10] who synthesized and evaluated 3,5-disulfide-bridged Ang II analogues.

Among the compounds synthesized by Spear et al.[9] (Chart 1), c[Hcy^{3,5}]-Ang II (**1**) exhibited high contractile activity in isolated rabbit aortic rings ($pD_2 = 8.48$) and high binding affinity to rat uterine membrane preparations ($IC_{50} = 2.1$ nM). The related peptides c[Cys^{3,5}]-Ang II (**2**) and c[Pen^{3,5}]-Ang II (**3**) were reported to be essentially devoid of, or to have only weak, contractile activity. Despite the low contractile activity of **2** and **3**, they showed excellent binding affinity (43 nM for **2** vs 2.6 nM for **3**). The authors concluded that these results may indicate the presence of some partial antagonist character, although this was not explicitly examined.[11]

Our objective is to develop rational and general strategies for the transformation of peptides to nonpeptides by iterative incorporation of well-defined secondary-structure mimetics in the target peptides. In this approach conformational restriction through side chain disulfide formation constitutes the first step to reduce the conformational complexity of the peptides. If the cyclized peptide is active at the given receptor, conformational analysis techniques can be used to study if the cyclic moiety prefers certain turn geometries (γ-turns, β-turns, etc.) which at a later stage can be substituted for relevant secondary-structure mimetics. This is an iterative process and should optimally lead to receptor selective and potent nonpeptidic ligands. The apparent

* Author to whom correspondence should be addressed.
† Department of Organic Pharmaceutical Chemistry.
‡ Department of Medical and Physiological Chemistry.
§ Department of Organic Chemistry.
▽ Department of Biological Research on Drug Dependence.
⊥ Department of Pharmacology.
∥ Medicinal Chemistry II.
Current address: Institut für Organische Chemie, Universität Hannover, Schneiderberg 1B, D-30167 Hannover, Germany.
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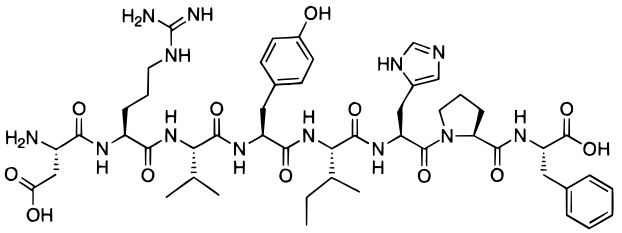
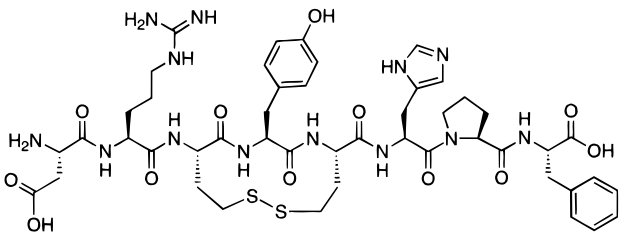
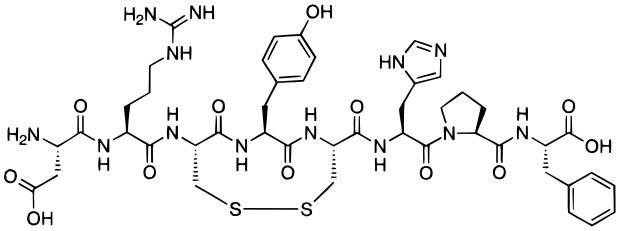
Chart 1



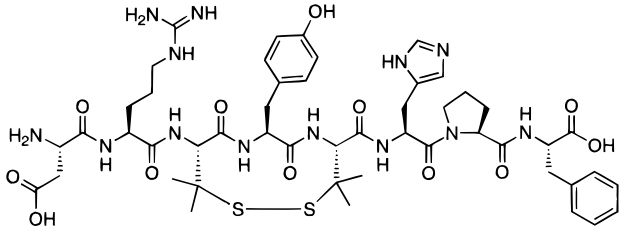
Angiotensin II



c[Hcy^{3,5}]Ang II (**1**)

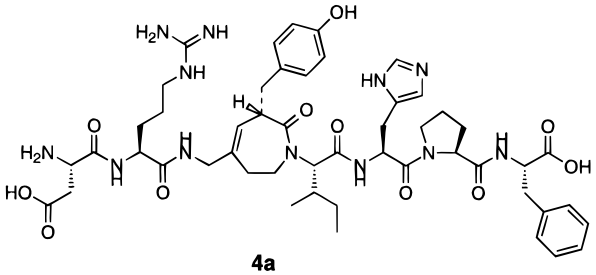


c[Cys^{3,5}]Ang II (**2**)

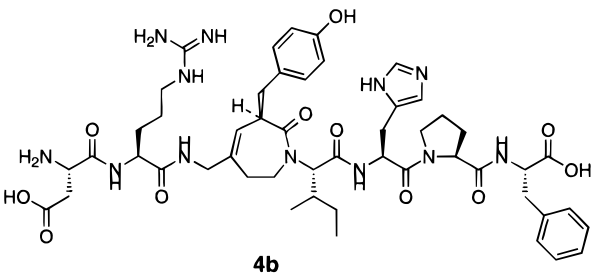


c[Pen^{3,5}]Ang II (**3**)

Chart 2



4a



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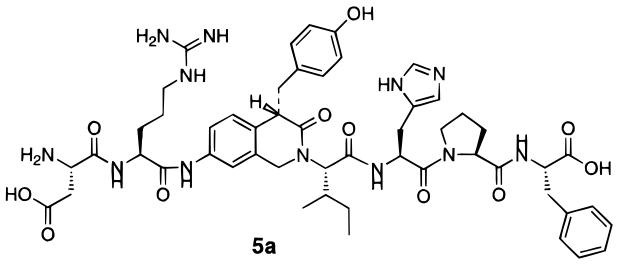
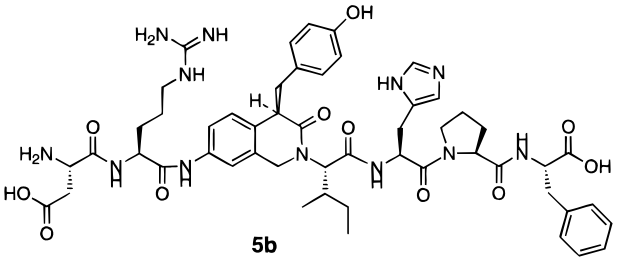


5a



5b

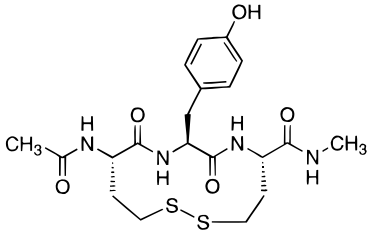
impact of the conformation in the 3−5 region of Ang II for the biological response encouraged us to synthesize secondary-structure mimetics based on the anticipated conformational preferences of the cyclic moiety of **1**−**3**.

We herein describe the conformational characteristics as deduced by ^{1}H-NMR spectroscopy and theoretical calculations of tripeptide model compounds of **1**−**3**. Furthermore, we report the synthesis of four diastereomers of two γ-turn mimetics incorporated into Ang II, **4a**,**b** and **5a**,**b** (Chart 2), as well as the affinities of these compounds for rat pituitary AT_1 receptors and the contractile activity of **4a**,**b** on rabbit aorta strips. (**a** and **b** denote single diastereomers of unassigned absolute stereochemistry. We use the convention for graphic presentation of enantiomerically pure compounds with unknown absolute configuration, wedge outlines, and broken lines, as suggested by Maehr.[12])

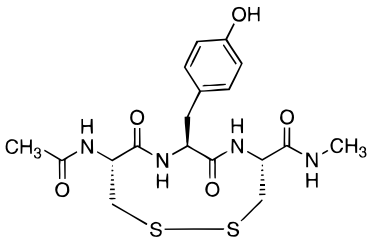
Results

Conformational Analysis. (a) ^{1}H-NMR Spectroscopy of 6−8. The tripeptides **6**−**8** (Chart 3) were selected as suitable models for the cyclic region in **1**−**3** and were used in the NMR spectroscopic investigation and theoretical calculations. The assignment of all ^{1}H-NMR resonances of peptides **6**−**8** is based on the use of the *N*-methyl or the tyrosine β-protons as unambiguous starting points and was achieved with standard 2D NMR techniques (see Supporting Information for ^{1}H-NMR chemical shifts for **6**−**8**). Peptides **6** and **7** showed only one set of signals over the monitored temperature range (25−45 °C). Peptide **8** showed two main sets of signals (A and B) of relative intensity 1:0.9 and a minor set (relative intensity 0.2). The two major components were in equilibrium with each other. Signals of the minor component could not be assigned, since most of them were obscured by those of the major components. Temperature coefficients of the NH proton chemical shifts varied between 0.0 and 8.0 ppb/K and are provided in Table 1. In **7** and **8**, the low temperature coefficient for the NH proton of residue 3 indicates its involvement in an intramolecular hydrogen bond, whereas this is not the case in **6**. Instead, in that

Chart 3



6



7

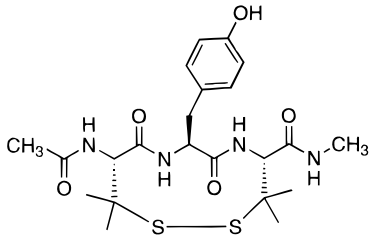


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Table 1. Temperature Coefficients of NH Chemical Shifts[a] of **6**−**8** in DMSO-d_6

peptide	residue 1	residue 2	residue 3	CH_3NH
6	4.7	4.0	8.0	0.0
7	6.4	4.0	0.4	4.8
8A[b]	5.5	3.5	0.0	4.4
8B[b]	6.9	3.5	0.0	4.5

[a] $\Delta\delta/\Delta T$ (ppb/K). [b] Peptide **8** showed three sets of signals, of which two are assigned to the main conformers **8**A,B.

Table 2. $^3J_{NH-C\alpha H}$ Coupling Constants of **6**−**8** in DMSO-d_6 and the Possible Values for the Corresponding Backbone Angles Φ[a]

peptide	residue 1	residue 2	residue 3
6	8.0 (−147,−93)	9.2 (−132,−108)	8.0 (−147,−93)
7	7.2 (−154,−86)	6.5 (−159,−81,45,75)	9.1 (−133,−107)
8A[b]	9.4 (−127,−113)	7.6 (−150,−90)	8.8 (−138,−102)
8B[b]	9.4 (−127,−113)	7.9 (−148,−92)	8.0 (−147,−93)

[a] In parentheses Φ angles (degrees) obtained from Ludvigsen's Karplus equation (Ludvigsen et al.[24]). *J* values are given in hertz. [b] Peptide **8** showed two sets of signals corresponding to the two conformations in equilibrium.

compound the NH proton of the C-terminal aminomethyl group has a low temperature coefficient.

Vicinal NH−CαH coupling constants were measured in the range of 6.5−9.4 Hz (Table 2). The coupling constants in residue 3 are rather similar for all peptides. Residue 2 shows a considerably larger value (9.2 Hz) for peptide **6** as compared to **7** and **8**. For residue 1, peptide **8** has a significantly larger coupling constant than the other peptides. The two major conformers of peptide **8** (A and B) show no significant difference. For the Tyr^2 side chain, the coupling constants between CαH and the two β-protons were determined as **8**, 4.0 and 9.6 Hz (signals for A and B overlap); **7**, 5.6 and 9.1 Hz; and **6**, 7.8 and 5.9 Hz. NOE data were evaluated semiquantitatively from cross-peak volumes in ROESY spectra but could not be assigned to a single conformer (data not shown).

(b) Conformational Energy Calculations and Turn Classification of 6−8. During the course of this study different theoretical conformational analysis studies have been presented on tri- and octapeptide analogues of Ang II, i.e., c[Hcy-Ala-Hcy],[8,13] c[Cys-Ala-Cys],[8,13] c[Cys-Tyr-Cys],[14] c[$Cys^{3,5}$]-Ang II,[7] and c[$Hcy^{3,5}$]-Ang II.[7] In addition, a ^{1}H-NMR spectroscopic investigation of Boc-Cys-Ala-Cys-$NHCH_3$ has been presented.[15] We have performed a conformational analysis, using the Amber* all atom force field and the GB/SA water solvation model[16] in Macromodel version 4.5,[17] on blocked model tripeptides **6**−**8** (Chart 3) in order to study their conformational characteristics, especially if preferred turn geometries exist.[18] To help us classify the conformations into γ-turns and β-turns, we used the XCluster program[19] in Macromodel. Families with similar conformational characteristics were classified into clusters based on the torsional rms (root-mean-square) difference. To cluster γ-turns, the Φ_{i+1} and Ψ_{i+1} angles were used, and to cluster β-turns, the Φ_i, Ψ_i, Φ_{i+1}, and Ψ_{i+1} or the Φ_{i+1}, Ψ_{i+1}, Φ_{i+2}, and Ψ_{i+2} angles were used. By displaying the different clusters in Macromodel, the conformations forming γ-turns and β-turns can be studied. Twenty-two out of the 87 low-energy conformations identified for **6** assumed an inverse γ-turn conformation (Table 3). The ranges of Φ_{i+1} and Ψ_{i+1} angles in the inverse γ-turn conformations were −74−(−91)° and 60−125°, respectively. When the distance from the Cα of the first residue to Cα of the fourth residue is less than 7 Å and the tetrapeptide sequence is not in an α-helical region, it is considered to be a β-turn.[20] Only one of the 87 conformations identified, a type I β-turn, fulfilled this distance criterion. All the inverse γ-turn conformations of **6** had energies more than 11.2 kJ/mol above the lowest energy minimum and are therefore not significantly populated according to a Boltzmann distribution. In the XCluster analysis of the 19 conformations identified for **7** using the Φ_{i+1} and Ψ_{i+1} angles, three clusters were identified. Two of the three clusters, containing 18 out of the 19 conformations, contained inverse γ-turn conformations, and the third cluster contained one type I β-turn conformation. The ranges of Φ_{i+1} and Ψ_{i+1} angles in the inverse γ-turn conformations were −73−(−124)° and 52−89°, respectively. The XCluster analysis of **8** also shows a strong preference for the inverse γ-turn conformation. Twelve out of the 16 conformations identified contained an inverse γ-turn conformation. One conformation corresponded to a type III β-turn. The ranges of Φ_{i+1} and Ψ_{i+1} angles in the inverse γ-turn conformations were −75−(−113)° and 44−78°, respectively.

(c) Possible Solution Conformations of 6−8. A temperature coefficient for the amide proton chemical shift of <3 ppb/K is indicative of intramolecular hydrogen bond formation.[21] Thus, the temperature coefficients of the residue 3 amide protons in **7** and **8** (0.4 and 0.0, respectively) and the CH_3NH proton in peptide **6** (0.0) indicate that these protons are intramolecularly hydrogen bonded. There are two possible hydrogen-

Table 3. Comparison of Results Obtained from Theoretical Calculations[a] and Experimental ^{1}H-NMR Spectroscopic Data

peptide	no. of low-energy conformations[a]	no. of inverse γ-turn conformations[b]	no. of β-turn conformations[c]	preferred NMR conformation[d]
6	87	22	1	no preference[e]
7	19	18	1	inverse γ-turn
8	16	12	1	inverse γ-turn

[a] The number of conformations identified within 20 kJ/mol of the lowest energy conformation. [b] The number of inverse γ-turn conformations identified within 20 kJ/mol of the lowest energy conformation. [c] The number of β-turn conformations identified within 20 kJ/mol of the lowest energy conformation. [d] Preferred turn conformation around Tyr^2 in solution as determined by ^{1}H-NMR spectroscopy. [e] Does not have a preferred turn geometry around Tyr^2.

bonding arrangements for residue 3 in **7** and **8**: a γ-turn to residue 1 or a β-turn to the acetyl group. For the CH_3NH group in **6**, there are at least three hydrogen-bonding possibilities. Using SYBYL,[22] we extracted and examined those conformations for each peptide (within 20 kJ/mol of the lowest energy minimum) in which the identified NH group participated as a hydrogen bond donor.[23] The Φ values of these conformations were systematically compared to those Φ values calculated from the $^3J_{NH-C\alpha H}$ coupling constants using Ludvigsen's Karplus equation (Table 2).[24] In trying to determine the solution conformation, we allowed for differences in the Φ values of $\pm 15°$. The conformation with the lowest energy that fulfilled these criteria is shown in Figure 1. In **7** we had to search for conformations above 20 kJ/mol to identify one that fulfilled the predefined criteria. Using these principles, one conformation of each peptide was identified that possessed the required intramolecular hydrogen bond, had dihedral angles as indicated by $^3J_{NH-C\alpha H}$ coupling constants, and also fit reasonably well, but not completely, to the NOE data (Figure 1). The energy differences between these conformers and the lowest energy conformation identified were $\Delta E = 18.9$ kJ/mol (**6**), 32.9 kJ/mol (**7**), and 8.1 kJ/mol (**8**). The relatively high energy of these conformations may reflect that calculations were conducted in a water solvent model, while NMR spectroscopy was performed in DMSO. These conformers, however, most likely represent an average peptide conformation, due to the presence of a conformational equilibrium. This is indicated in the case of **8**, where separate subspectra for at least three conformations were observed, most probably caused by the bulky methyl groups. The main conformers A and B are similar and may represent different conformations of the $C(Me)_2S-S(Me)_2C$ chain. In peptide **6**, containing the larger ring structure, no hydrogen bond in the cyclic part of the molecule seems to be present.

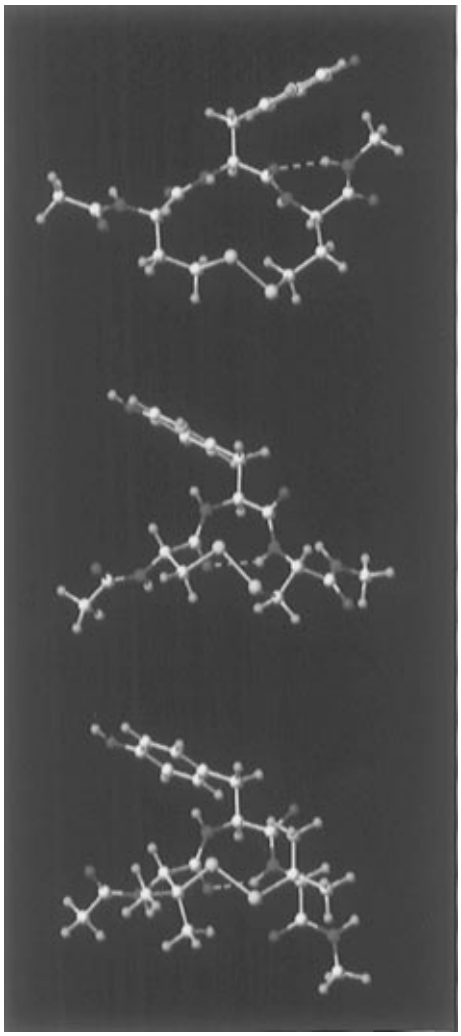
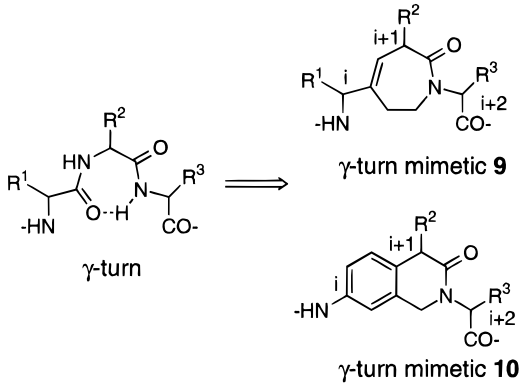


Figure 1. Conformations of **6** (top), **7** (middle), and **8** (bottom) derived from energy calculations and which are consistent with NMR data. The Φ_i, Ψ_i, Φ_{i+1}, Ψ_{i+1}, Φ_{i+2}, and Ψ_{i+2} values in these conformations are $-160°$, $127°$; $-131°$, $116°$; $-86°$, $68°$ (**6**); $-163°$, $137°$; $-74°$, $76°$; $-112°$, $16°$ (**7**); $-143°$, $134°$; $-80°$, $52°$; $-153°$, $140°$ (**8**).

Chemistry. (a) Selection and Design of Secondary-Structure Mimetics to Incorporate in Ang II. Compound **6** is highly flexible with no regular secondary structure. In contrast, **7** and **8** preferentially adopt the inverse γ-turn conformation (Table 3). Although these results may not reflect the actual conformation of the octapeptides when binding to the receptor, we decided to focus initially on γ-turn mimetics to replace amino acids 3−5 in Ang II. Only a few γ-turn mimetics have been reported in the literature.[25−27] We decided to use the seven-membered azepine system, reported by Huffman et al.,[25] represented by structure **9** (Scheme 1). In this mimetic the amide bond between the i and $i + 1$ residues is replaced with a double bond and the oxygen and hydrogen involved in the hydrogen bond are each replaced by an ethylene group. Huffman et al. have incorporated the template γ-turn mimetic **9** in both linear[25] and cyclic enkephalin analogues,[28] in fibrinogen receptor antagonists,[29] and in a model HIV-protease substrate.[30] X-ray crystallography of this template alone revealed torsional angles quite similar to those of an idealized inverse γ-turn,[28] with the R^2 substituent in a pseudoequatorial orientation.

Synthetic features to be incorporated into the γ-turn mimetic **9**, besides Tyr in position $i + 1$, are the i and $i + 2$ side chains. The lipophilic side chains of Val^3 and Ile^5 in Ang II are primarily considered to have a conformation stabilizing role.[31] However, it cannot be excluded that they contribute to binding by favorable hydrophobic interactions with the Ang II receptor. Assuming that the side chains in Val^3 and Ile^5 have a conformational stabilizing role only, it would be suf-

Scheme 1

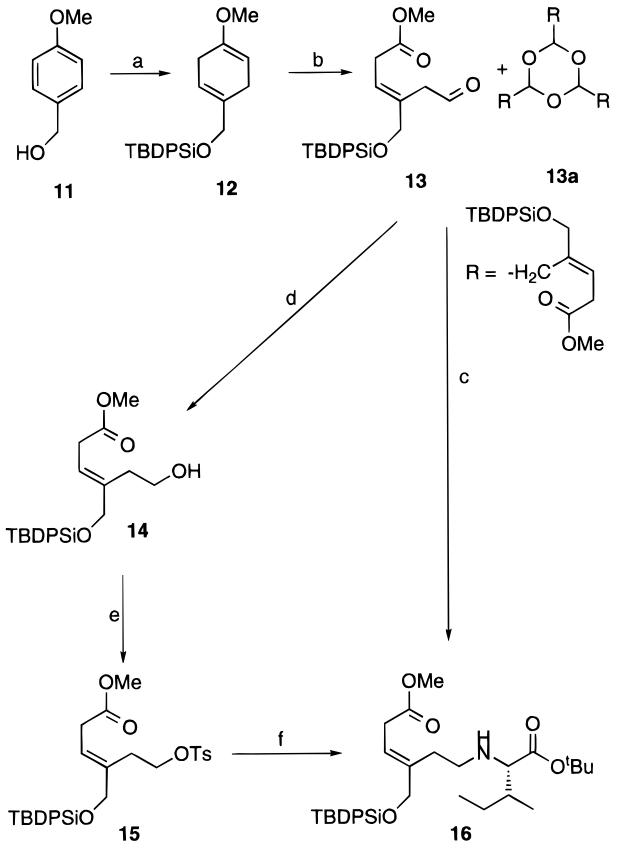


ficient to introduce Gly in these positions, but since we believe that these side chains also contribute to binding, we desired a lipophilic side chain in either of these positions. We preferred the lipophilic side chain attached to the 5 rather than to the 3 position, since Spear and co-workers have shown that c[Cys^3,Pen^5]-Ang II exhibited higher binding affinity than c[Pen^3,Cys^5]-Ang II.[9] In addition, the lipophilic side chain attached to the imidazole ring of the nonpeptidic Ang II receptor antagonist DuP 753 has been suggested to correspond to Ile^5 in Ang II.[32,33]

The γ-turn mimetic represented by **10**, which is structurally similar to compounds currently under investigation in our laboratory as potential β-turn mimetics, was used as an alternative to the γ-turn mimetic **9** (Scheme 1). In the bicyclic mimetic **10** the amide bond connecting the i and $i + 1$ residues is replaced by part of an aromatic ring. The characteristic feature of this mimetic is the aromatic π-electron system and the anilide amide. Isoleucine was introduced in the $i + 2$ position based on the same criteria that were described for γ-turn mimetic **9**.

(b) Synthesis. The synthetic approach to the benzylated azepine γ-turn mimetic incorporated into **4** is based on the strategy outlined by Huffman et al.[25,29,30] utilizing anis alcohol **11** in a Birch reduction (Schemes 2 and 3).[34] Protection of the alcohol[35] was followed by selective ozonolysis[36,37] to give **13**. The unstable aldehyde **13** underwent reductive amination[38] with isoleucine *tert*-butyl ester to afford the diester **16**, which after selective saponification and cyclization with diphenyl phosphorazidate (DPPA)[39] provided the lactam **17**. The reductive amination also resulted in the formation of some isomerized product which contaminated the desired compound and caused a reduced yield in the following step. In an alternative approach, the cyclic trimer **13a**, spontaneously formed from the aldehyde **13** regardless of conditions used for storage or separation of the latter, was reduced to give the homoallylic alcohol **14** in quantitative yield. The same alcohol was also obtained quantitatively by reduction of **13** or reduction of a mixture of aldehyde and trimer under the same reaction conditions. The alcohol function was then replaced by the amino group of the *tert*-butyl ester of isoleucine in a two-step procedure. First, the tosylate ester **15** was prepared using standard conditions; thereafter the product was used to alkylate the amino acid ester to provide, after cyclization, the lactam **17** in high purity. The silyl protecting group was removed with TBAF prior to the alkylation reaction in order to avoid

Scheme 2[a]

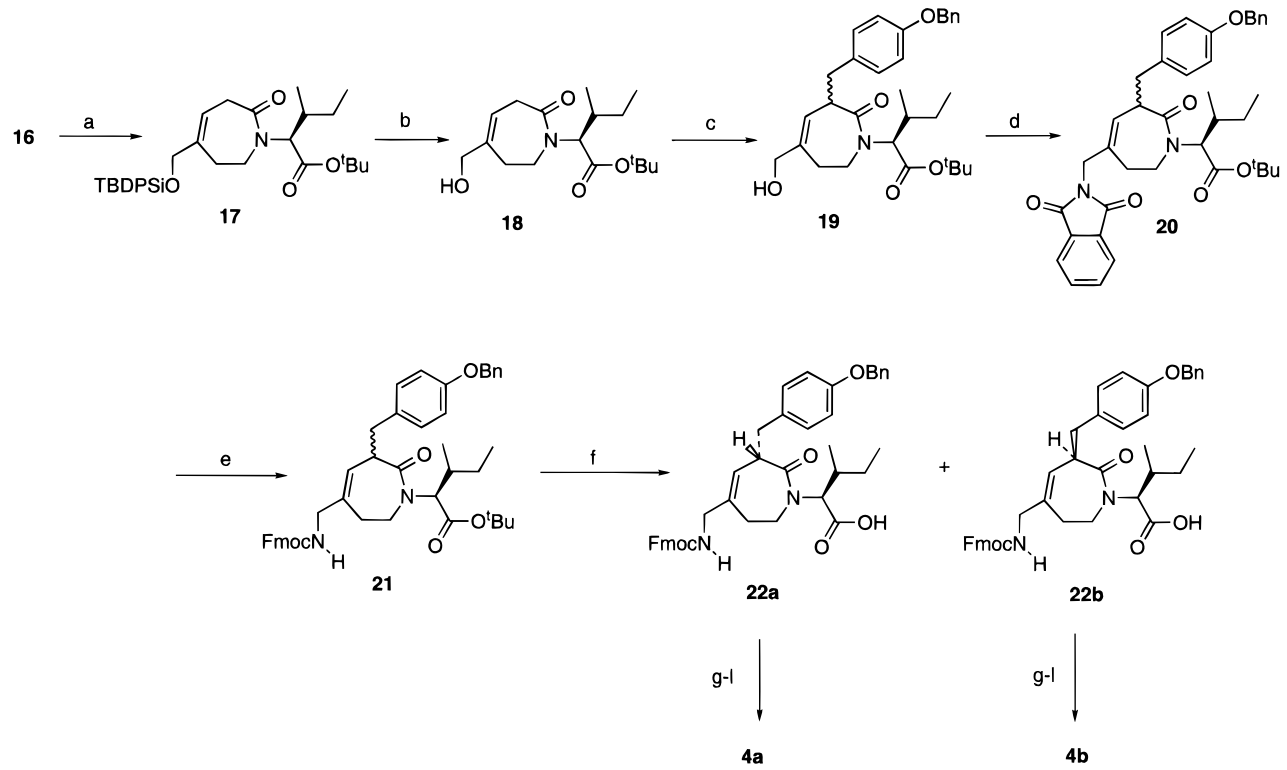


[a] Reagents: (a) (i) Na, NH_3, THF, EtOH, (ii) TBDPSiCl, imidazole, DMF, 50%; (b) (i) O_3, MeOH, Et_2O, CH_2Cl_2, (ii) Me_2S, 67%; (c) IleO^{t}Bu·HCl, NaOAc, $NaBH_3CN$, MeOH, 55%; (d) $NaBH_4$, MeOH, >95%; (e) TsCl, Et_3N, DMAP, CH_2Cl_2, 90%; (f) IleO^{t}Bu·HCl, THF, 90%.

side reactions. Double lithiation, as described by Huffman et al.,[25] followed by benzylation with 4-(benzyloxy)-benzyl bromide allowed regioselective introduction of the protected tyrosyl side chain to give the diastereomers **19** in a 5:3 ratio. Mitsunobu and Gabriel reactions[40] followed by treatment with Fmoc-Cl afforded the fully protected diastereomers **21**. Cleavage of the *tert*-butyl ester by TFA yielded the acids **22a**,**b** in a 3:2 ratio, which were easily separated, but no attempt was made to assign their absolute stereochemistry. The Fmoc derivatives were then submitted to manual SPPS, coupled to a His(Boc)-Pro-Phe-resin, and elaborated to the final peptides using Fmoc-Arg(Pmc)-OH[41] and Fmoc-Asp(O^{t}Bu)-OH. As debenzylation of tyrosine is particularly troublesome for Ang II analogues,[42] a two-step soft acid/hard acid deprotection was used. It furnished the peptides **4a**,**b**, but in a rather poor overall yield. Catalytic hydrogenolysis[43,44] was also unsuccessful. Classical HF treatment, however, which was applied for the deprotection of one isomer, afforded **4a** in 8.6% overall yield.

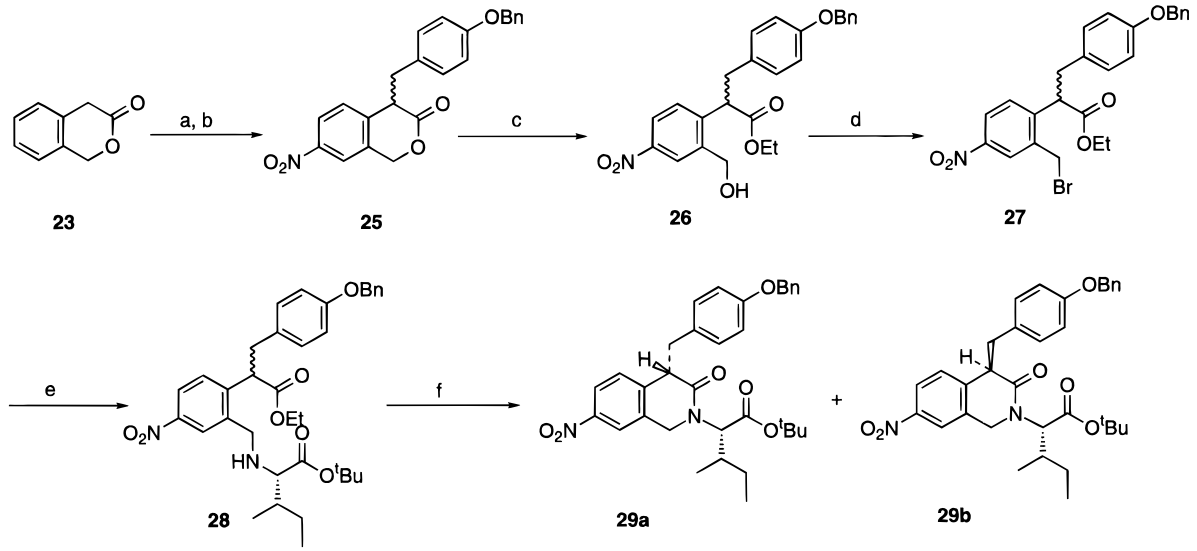
The γ-turn mimetics **32a**,**b**, required for incorporation in the pseudopeptides **5a**,**b**, were prepared from 3-isochromanone, **23** (Schemes 4 and 5). Nitration using ammonium nitrate and trifluoroacetic anhydride[45] gave a mixture of 6- and 7-nitro-3-isochromanones **24**, which were separable only with great difficulty and loss of material. The mixture was therefore used without further purification for the subsequent alkylation reaction where the aromatic portion of the tyrosine was introduced using 4-(benzyloxy)benzyl bromide and LDA

Scheme 3[a]



[a] Reagents: (a) (i) 1 N NaOH, aq dioxane, 3 N HCl, (ii) DPPA, Na_2CO_3, DMF, 32%; (b) TBAF·$3H_2O$, THF/CH_2Cl_2, 71%; (c) LDA, 4-BnOBnBr, THF, 62%; (d) DEAD, PPh_3, phthalimide, THF, 98%; (e) (i) H_2NNH_2·H_2O, EtOH, (ii) Fmoc-Cl, TBAF, dioxane, Na_2CO_3, 47%; (f) TFA, CH_2Cl_2, anisole, 95%; (g) His(Boc)-Pro-Phe-Wang resin, TBTU, HOBt·H_2O, DIEA, DMF; (h) Fmoc-Arg(Pmc), TBTU, HOBt·H_2O, DIEA, DMF; (i) Fmoc-Asp(O^{t}Bu), TBTU, HOBt·H_2O, DIEA, DMF; (j) TFA; (k) TFMSA, TFA, Me_2S, *m*-cresol; (l) HF.

Scheme 4[a]

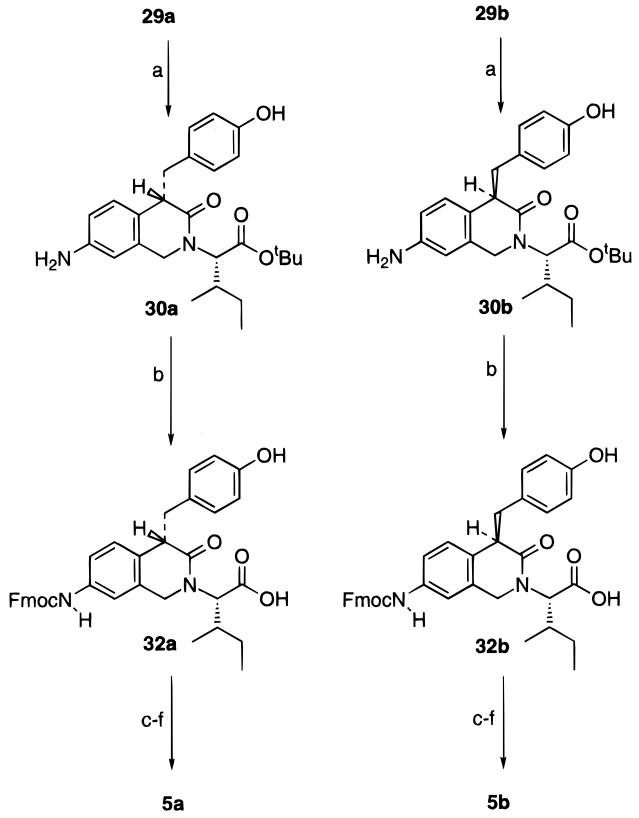


[a] Reagents: (a) NH_4NO_3, $(CF_3CO)_2O$, 81%; (b) LDA, 4-BnOBnBr, THF, 24%; (c) H_2SO_4, EtOH, 48%; (d) CBr_4, PPh_3, THF, 58%; (e) Et_3N, IleO^{t}Bu, THF, 91%; (f) toluene, AcOH.

to give pure racemic **25** after a relatively easy separation. Refluxing **25** in ethanolic sulfuric acid opened the lactone[46] to give the hydroxy ester **26,** which was readily converted into the bromide **27**.[47] This reaction should be performed as soon as possible in order to avoid the reformation of the lactone. Substitution of the bromide with isoleucine *tert*-butyl ester gave the diastereomeric mixture of **28**, which cyclized to form the δ-lactams **29a**,**b** upon treatment with acetic acid in refluxing toluene (Scheme 4). These diastereomers were separated by chromatography on silica gel. All subsequent reactions were then performed as a parallel series on the pure compounds. Removal of the benzyl group, to reveal the tyrosine phenolic function, was performed simultaneously with reduction of the nitro function, using palladium-catalyzed reduction,[48] to give **30a**,**b**. Standard reaction conditions were used to introduce the Fmoc group in **31a**,**b** and to cleave the *tert*-butyl ester giving the partially protected dipeptide analogues **32a**,**b** for use in solid phase peptide synthesis (Scheme 5).

Pharmacology. (a) In Vitro Binding Affinity. Compounds **1**−**5** were analyzed in a radioligand binding

Scheme 5[a]



[a] Reagents: (a) 10% Pd/C, HCO_2NH_4, HCOOH, CH_2Cl_2; (b) (i) Fmoc-Cl, pyridine, (ii) TFA, CH_2Cl_2; (c) His(Boc)-Pro-Phe-2-chlorotrityl resin, PyBOP, HOBt·H_2O, DIEA, DMF; (d) Fmoc-Arg(Pmc), PyBOP, HOBt·H_2O, 4-methylmorpholine, DIEA, DMF; (e) Fmoc-Asp(O^tBu), PyBOP, HOBt·H_2O, 4-methylmorpholine, DIEA, DMF; (f) TFA/H_2O/triethylsilane (90:5:5).

Table 4. Binding Affinities for the AT_1 Receptor and Agonistic Activity

	IC_{50} (nM)[a]		
compd	AT_1[b]	AT_1[c]	EC_{50} (nM)[d]
Ang II		2.0	2.6
DuP 753		1.8	
1	15.4	1.7	
2	28	2.9	
3	3.8	0.9	217
4a		2.0	
4b		2.8	830
5a		$>10^5$	
5b		$>10^5$	

[a] Concentration of the analogue needed to produce 50% inhibition in specific binding of [^{125}I]Ang II to rat pituitary membranes (AT_1). Data represent the mean of duplicate determinations. [b] BSA (0.2%) included in the assay mixture. [c] No BSA included in the assay mixture. [d] The concentration of the peptide which produces 50% of the maximal contractile response at rabbit aorta strips.

assay involving displacement of [^{125}I]Ang II from rat pituitary membranes, mainly containing AT_1 sites (Table 4). It has been reported that BSA (bovine serum albumin) may affect the binding affinity of certain Ang II receptor antagonists.[49,50] Therefore, two slightly different versions of the assay were performed for compounds **1**−**3**. In one version, the assay mixture contained 0.2% BSA, while in the other, no BSA was present. The IC_{50} values obtained from the assay where BSA was omitted were 4 −10 times lower as compared to the assay where BSA was included. Since BSA affected the binding of **1**−**3**, and since it has influenced

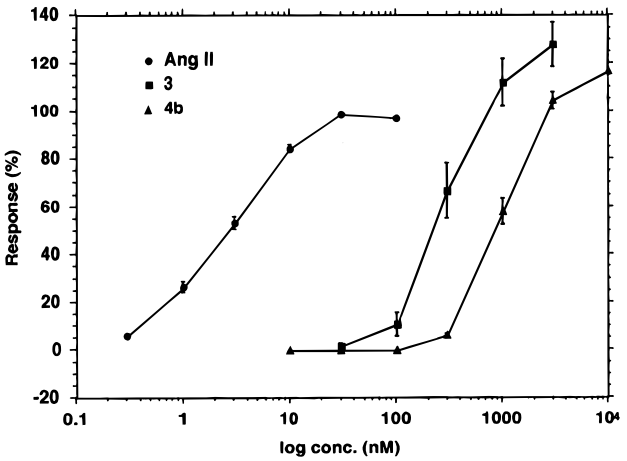


Figure 2. Concentration−response curves in isolated rabbit aortic strips for Ang II (●), **3** (■), and **4b** (▲). Values represent the mean ± SEM ($n = 42$ for Ang II, $n = 5$ for **3**, and $n = 4$ for **4b** except at lowest and second lowest concentrations where $n = 1$ and 2, respectively).

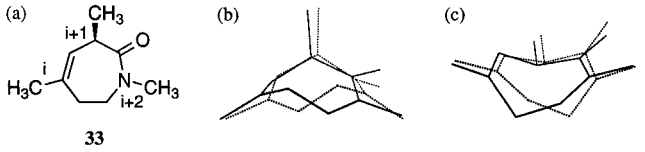
the binding activity of other Ang II ligands markedly, we decided to omit it in the binding assays of **4a**,**b** and **5a**,**b**.

The IC_{50} values of compounds **1**−**3** using displacement of [^{125}I]Ang II have previously been reported[9] using a rat uterine membrane preparation in which both AT_1 and AT_2 receptors are present.[51] In the present study the high affinities of **1**−**3** observed by Spear et al. were confirmed. Compound **4a** was equipotent to Ang II in binding to AT_1 receptors, and a similar observation was made for compound **4b**. In contrast, compounds **5a**,**b** lacked any affinity for the AT_1 receptor ($IC_{50} > 10^{-4}$ M).

(b) Functional Data. Compounds **3** and **4a**,**b** were evaluated for agonistic and antagonistic properties on rabbit aorta (Table 4). **3** and **4b** behaved as full agonists (Figure 2). Compound **4b** was slightly less potent than **3** and about 300 times less active than Ang II itself. In both **3** and **4b** the concentration−response curve was potentiated with a maximum effect more than 30% higher than Ang II. Compound **4a** showed no agonistic effect at 10 μM. In the antagonist model no effect was observed for the compounds tested. Compounds **5a**,**b** were not tested in the functional assay since they lacked affinity for the AT_1 receptor.

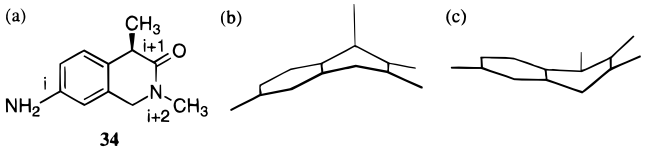
Discussion

Introduction of γ-turn mimetic **9** (Scheme 1) in Ang II resulted in two diastereomeric peptidomimetics, **4a**,**b**, which bind to the AT_1 receptor with equal or almost equal affinity compared to Ang II. Interestingly, one diastereomer is an agonist with full contractile activity but 300 times less potent than Ang II. The other diastereomer had no contractile activity, nor did it antagonize Ang II contraction. A rationale for this finding is at present unclear to us. When the γ-turn mimetic **10** was incorporated in Ang II, affording peptidomimetics **5a**,**b**, no receptor binding affinity was observed. At least three reasons for the loss of binding affinity in compounds **5a**,**b** as compared to **4a**,**b** can be suggested as follows: (a) γ-turn mimetic **10**, in contrast to **9**, does not effectively mimic the γ-turn conformation, (b) the extra steric bulk of the aromatic ring of the bicyclic mimetics may interact negatively with the receptor, and (c) the pharmacophore groups after incor-



33

Figure 3. (a) Compound **33**, a model compound of **4b**. (b) Conformations identified from the conformational search of **33** which adopt a classic γ-turn conformation. Relative steric energies are as follows: $\Delta E = 10.6$ kJ/mol (dotted lines) and $\Delta E = 31.8$ kJ/mol (solid lines). (c) Conformations identified from the conformational search of **33** which adopt an inverse γ-turn conformation. Relative steric energies are as follows: $\Delta E = 0$ kJ/mol (dotted lines) and $\Delta E = 19.3$ kJ/mol (solid lines).



34

Figure 4. (a) Compound **34**, a model compound of **5b**. (b) Conformations identified from the conformational search of **34** which adopt a classic γ-turn conformation. The relative steric energy is $\Delta E = 0$ kJ/mol. (c) Conformations identified from the conformational search of **34** which adopt an inverse γ-turn conformation. The relative steric energy is $\Delta E = 3.4$ kJ/mol.

poration of **10** in Ang II are oriented in an unfavorable position for interacting with the receptor.

Huffman et al. compared the torsion angles for the *i* + 1 residue of a γ-turn in an ideal conformation,[18] the same angles derived from X-ray crystallography[28] and theoretical calculations[26] of γ-turn mimetic **9**. The torsion angles compared favorably, and interestingly the X-ray structures assumed the inverse γ-turn conformation. We have studied the conformational preferences of **33** (Figure 3a), a model compound of **4**, and **34** (Figure 4a), a model compound of **5**, by use of a Monte Carlo search using the MM2* force field in Macromodel (no solvation model was used in these calculations). Four different conformations within 50 kJ/mol of the global minimum were identified for **33**. Two of these conformations corresponded to the classic γ-turn (Figure 3b) and the other two to the inverse γ-turn (Figure 3c). These ring conformations differ mainly in the position of the two methylene groups; however, the direction of the incoming and outgoing bonds from the azepine ring are very similar in the classic and inverse γ-turn conformation. In **34**, two conformations within 50 kJ/mol of the lowest energy conformation were identified which correspond to the classic γ-turn and the inverse γ-turn (Figure 4b,c, respectively).

The conformations identified were compared to experimentally determined γ-turns in proteins[52] in order to evaluate how well they mimicked the γ-turn conformation (Table 5). In the comparison we focused on two parameters: the distance between $C\alpha_i$ and $C\alpha_{i+2}$ and the rms distance between $C\alpha_i$, $C\alpha_{i+1}$,$C\beta_{i+1}$, N_{i+2}, and $C\alpha_{i+2}$ in the crystal γ-turn and the corresponding atoms in **33** and **34**. N_i was not included in the fitting procedure since the position of this atom is not defined by the rest of the cyclic system in **33**. The $C\alpha_i-C\alpha_{i+2}$ distance is almost exactly the same in the classic and inverse γ-turn conformations in **33** as found in the crystal structures. The 5-atom rms deviation in the inverse γ-turn conformation is 0.15 Å, making **33** a good geometrical mimic of an inverse γ-turn. Compound **33** is also a fairly good geometrical mimetic of the classic γ-turn conformation (rms = 0.35 Å). In **34** the $C\alpha_i-C\alpha_{i+2}$ distance in the classic and inverse γ-turns is somewhat longer than the experimentally determined distances. The rms fit is also slightly worse than for **33**. Taken together, our theoretical calculations support the previous findings[25,26,28] that the template **9** (Scheme 1) is a good mimetic of a γ-turn. The γ-turn template **10** also shows a reasonable good fit to the experimental γ-turn.

When **33** and **34** are superimposed using the atoms described in the legend to Figure 5, the larger ring skeleton of **34** is evident. In this overlay the extra van der Waals volume of **34** compared to **33**, as depicted in Figure 5, could be deleterious for binding affinity. The different orientation of the $N_i-C\alpha_i$ bond vector in **33** as compared to **34** also becomes evident in this superposition which may also be a reason for the difference in activity of **33** and **34**.

The pharmacophore elements of Ang II have been suggested to correspond to Tyr^4, His^6, Phe^8, and the C-terminal carboxyl group.[2] Inspection of Figure 5 and Chart 2 indicates that residues 4−8 should be able to assume the same conformations in **4** and **5**. In **5**, some Tyr^4 side chain rotamers may be energetically disfavored due to unfavorable interactions between the pseudoequatorial Tyr^4 side chain and the aromatic ring hydrogen. Therefore, the inactivity of **5** could be due to an incorrect position of Tyr^4 in respect to the other pharmacophore groups. The hydroxyl group in Tyr^4 is necessary for agonistic activity, and a methylated hydroxyl group produces antagonists.[53] Joseph et al. also recently implicated the hydroxyl group of the Tyr^4 side chain in a postulated mechanism for Ang II receptor activation.[54]

In one of the proposed models of the bioactive conformations of Ang II, a γ-turn was suggested to be centered at Tyr^4.[55] If Ang II interacts with its receptor in a γ-turn conformation, an increase in activity of **4** compared to Ang II would be expected. This would be explained by the fact that the loss of internal rotational entropy upon binding is smaller in the more rigid analogue **4**. The considerable loss in agonistic activity of **4b** can therefore be interpreted as that another conformation corresponds to the bioactive conformation. However, the structural features of γ-turn mimetic **9** are somewhat different from the native γ-turn, which may also influence the activity, i.e., the substitution of the peptide bond for an alkene group and/or the replacement of the hydrogen bond for two methylene units. Khosla et al.[56] introduced an *N*-methyl group in the 4 position of the Ang II antagonist sarile ([Sar^1,Ile^8]-Ang II) producing antagonists with reduced activities. Conformational studies suggested that the N-methylation changed the conformation of the peptide backbone and limited the rotation of the Tyr^4 side chain, which makes it difficult to draw any conclusions regarding the importance of the Tyr NH for receptor binding. Regarding the ethylene bridge, Huffman et al.[28] concluded that the steric bulk introduced can change the conformational preferences of the backbone and side chains on either side of the mimetic. The fact that [Pro^5]-Ang II,[57] [Sar^1,Dtc^5]-Ang II,[58] and also the bicyclic analogue c[Hcy^3,MPt^5]-Ang II,[59] where the amide hydrogen involved in the proposed γ-turn conformation has been replaced by a methylene group, still show agonistic

Table 5. Comparison of Distances and rms Values in the Model γ-Turn Mimetics **33**[a] and **34** to the Classic[b] and Inverse[c] γ-Turns Found in the Crystal Structure

	classic γ-turn		inverse γ-turn	
system/conformer	$C\alpha_i - C\alpha_{i+2}$ (Å)[d]	rms fit (Å)[e]	$C\alpha_i - C\alpha_{i+2}$ (Å)[d]	rms fit (Å)[e]
33	5.7	0.35	5.6	0.15
34	6.0	0.36	5.9	0.21
experimental γ-turn	5.6[b]		5.5[c]	

[a] The classic and inverse γ-turn conformations of **33** with lowest energy were used in the comparisons. [b] From hemotaxis protein CheY-*Escherichia coli* (PDB code name 3CHY). [c] From rat oncomodulin (PDB code name 1RRO). [d] The distance (Å) between $C\alpha_i$ and $C\alpha_{i+2}$. [e] Best fit between calculated and experimental γ-turn geometry (atoms fitted are $C\alpha_i$, $C\alpha_{i+1}$, $C\beta_{i+1}$, N_{i+2}, $C\alpha_{i+2}$).

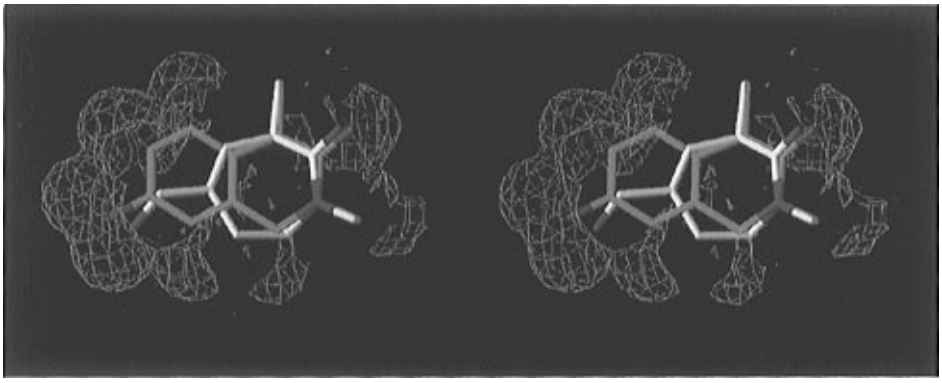


Figure 5. Stereorepresentation of the best fit of **33** (white) and **34** (green) in the inverse γ-turn conformation. $C\alpha_i$, $C\alpha_{i+1}$, $C\beta_{i+1}$, N_{i+2}, and $C\alpha_{i+2}$ were included in the fitting procedure. Mean distance between fitted atoms = 0.16 Å. The excess van der Waals volume of **34** when compared to **33** is shown in yellow. The excess volume was generated in SYBYL using the MVOLUME command. N_i was added to **33** in order to depict the $N_i - C\alpha_i$ bond vector.

activity does not support the γ-turn as a probable bioactive conformation of Ang II.

Spear et al.[9] measured the binding affinity and contractile activity of **3** and showed that it had high affinity for rat uterine membrane receptors, but a maximal response plateau was not always obtained in contraction studies using isolated rabbit aortic rings. In our hands the high affinity for AT_1 receptors (rat pituitary) was confirmed. However, in the rabbit aorta strips it had full contractile activity ($n = 5$). In fact, **3** and **4b** seem to have a similar pharmacological profile in the rabbit aorta strip test (Figure 2) which may indicate that **3** interacts with the Ang II receptor in a γ-turn conformation.

Conclusion

We have substituted amino acid residues 3−5 in Ang II with two different γ-turn mimetics giving four diastereomeric Ang II analogues. Introduction of γ-turn mimetic **9** (Scheme 1) in Ang II resulted in two diastereomeric peptidomimetics (**4a**,**b**), which bind to the AT_1 receptor with equal or almost equal affinity compared to Ang II. The finding that one of the diastereomers (**4b**) is an agonist with 300-fold reduced potency as compared to Ang II indicates that Ang II is not adopting a γ-turn around Tyr[4] when interacting with the receptor. The suggestion by Nikiforovich et al.[6] of an open turn in this region seems to us very likely. The reason for the inactivity of Ang II analogues **5a**,**b** containing γ-turn mimetic **10** may be due to a negative steric interaction with the receptor of the bicyclic moiety and/or that γ-turn mimetic **10** is not equally effective as mimetic **9** in mimicking a γ-turn.

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries on an Axel Kistner or an Electrothermal apparatus. The ^1H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 (67.8) MHz or a Varian Unity 400 spectrometer at 400 (100.58) MHz (final products). Chemical shifts are reported as δ values (ppm) downfield from Me_4Si. IR spectra were recorded on a Perkin-Elmer 298 or 1600 FT-IR instrument and are recorded in ν_{max} (cm^{-1}). Mass spectroscopy was performed on an Applied Biosystems BIOION 20 plasma desorption mass spectrometer. Samples were applied on aluminized mylar foils, coated with electrosprayed nitrocellulose, by drying from EtOH/H_2O/TFA mixtures and removal of excess liquid by nitrogen. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden. Flash column chromatography was carried out using Merck silica gel 60 (40−63 and 15−40 μm) and 60G (5−40 μm, compunds **25**−**32**). Thin-layer chromatography (TLC) was carried out using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm; E. Merck). Chromatographic spots were visualized by UV and/or spraying with an acidic, ethanolic solution of *p*-anisaldehyde or an ethanolic solution of ninhydrin followed by heating. For preparative TLC, plates precoated with silica gel 60 F_{254} (2.0 mm; E. Merck) were used. Optical rotations were obtained on a Perkin-Elmer Model 241 polarimeter. Amino acid analyses and peptide content measurements were performed by Dr. M. Sundquist on 24 h hydrolysates with an LKB 4151 alpha plus analyzer using ninhydrin detection.

Materials. SPPS resins and amino acid derivatives were bought from Fluka Chemie (Switzerland), NovaBiochem (Switzerland), Millipore, Saxon Biochemicals (FRG), or Bachem (Switzerland). THF was dried and distilled from sodium and benzophenone. Diisopropylamine was distilled and stored over NaOH. DMF was stored over 3 Å molecular sieves. All other commercial chemicals were used without further purification.

Solid-Phase Peptide Synthesis (SPPS). Most peptides were synthesized by manual SPPS using Fmoc/*tert*-butyl protection. The starting polymer was 2-chlorotrityl chloride resin derivatized with Fmoc-Phe-OH[60] or preloaded Fmoc-Phe-Wang resin. For the Fmoc amino acids the side chain protecting groups used are as follows: His(Boc), Arg(Pmc), and Asp(O^{t}Bu). Removal of the Fmoc group was achieved by reaction with 20% piperidine in DMF for 5 + 15 min. Coupling of the amino acids (3 equiv) was normally performed with TBTU or PyBOP (3 equiv) in the presence of HOBt (3 equiv) and DIEA (6 equiv) in DMF for 30−60 min. Histidine was

occasionally coupled as the pentafluorophenyl ester. At the end of each coupling cycle a resin sample (1−3 mg) was transferred to an Eppendorf tube. The Fmoc group was removed by treatment with 20% piperidine/DMF (200 μL) for 10 min. Ether (1 mL) was added, and the resin was carefully washed by decantation with additional portions of ether and allowed to dry. The peptide was released by reaction with 95% aqueous TFA (100 μL) for 15−30 min. An aliquot (10 μL) was added to 50% aqueous EtOH (50 μL) for PDMS analysis.

Synthesis of Cyclic Peptides 1−3 and 6−8. The peptides **1**−**3** and **6**−**8** were obtained from the Biomolecular Resource Facility, University of Lund, Sweden. In short, the solid-phase synthesis of these compounds was carried out on Merrifield or Pam resins using Boc/Bzl protection. The amino acids were coupled as HOBt esters or as preformed symmetrical anhydrides. The fully deprotected octapeptides **1**−**3** were obtained by cleavage with liquid HF (HF/DMS/*p*-cresol/*p*-thiocresol, 10:1:0.75:0.25; 1.5−2.5 h at 0−5 °C), whereas the linear tripeptides **6**−**8** were cleaved from the resin by reaction with methylamine (33% in EtOH; 3 d at room temperature) before removal of the thiol protecting groups with HF as above. In all cases the disulfide bridges were formed by oxidation with iodine (20% AcOH/MeOH; 5 min). The products were purified by RP-HPLC on a Kromasil C8 support using a 0.1% TFA−MeCN gradient. The purity was established by HPLC and PDMS analysis.

(*E*)-Methyl 4-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-6-oxo-3-hexenoate (13). 1-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-4-methoxy-1,4-cyclohexadiene (**12**) (30.0 g, 79.2 mmol), prepared according to literature,[28,29] was dissolved in MeOH (75 mL), ether (50 mL), and CH_2Cl_2 (20 mL), cooled to −58 °C, ozonized for 2.5 h at an O_3 flow rate of 2.5 g/h (130 mmol), quenched by addition of dimethyl sulfide (Me_2S; 10 mL), and allowed to reach room temperature overnight. The concentrated mixture was purified by gradient flash chromatography (ether/pentane, 1:2 to 1:1) to yield **13** (53 mmol, 20.8 g, 67%) as a colorless oil, which trimerized on prolonged chromatography or standing: TLC R_f 0.35 (ether/pentane, 1:2); ^{1}H-NMR ($CDCl_3$) δ 1.05 (s, 9H, *t*-Bu), 3.08 (d, J = 7.3 Hz, 2H, 2), 3.17 (d, J = 2.0 Hz, 2H, 5), 3.68 (s, 3H, OMe), 4.15 (s, 2H, CH_2O), 5.90 (t, J = 7.3 Hz, 1H, 3), 7.36−7.43 (m, 6H, Ar), 7.63−7.67 (m, 4H, Ar), 9.56 (t, J = 2.1 Hz, 1H, 6); ^{13}C-NMR ($CDCl_3$) δ 19.2 (Si*C*Me$_3$), 26.8 (*t*-Bu), 33.2 (2), 43.5 (5), 52.0 (OMe), 68.0 (CH_2O), 121.8 (3), 127.8, 129.8 (Ar), 133.1 (4), 133.5, 135.6 (Ar), 171.5 (1), 198.5 (6); IR_{CHCl_3} 2725m, 1740s, 1730s, 1110s.

Trimer: TLC R_f 0.27 (ether/pentane, 1:2); ^{1}H-NMR ($CDCl_3$) δ 1.01 (s, 27H, *t*-Bu), 2.36 (d, J = 5.6 Hz, 6H, 5), 3.18 (d, J = 7.3 Hz, 6H, 2), 4.16 (s, 6H, CH_2O), 4.36 (t, J = 5.6 Hz, 3H, 6), 5.83 (t, J = 7.3 Hz, 3H, 3), 7.37−7.44 (m, 18H, Ar), 7.65−7.71 (m, 12H, Ar); ^{13}C-NMR ($CDCl_3$) δ 19.3 (Si*C*Me$_3$), 26.8 (*t*-Bu), 32.4 (5), 33.2 (2), 53.7 (OMe), 67.7 (CH_2O), 104.2 (6), 119.1 (3), 127.7, 129.6 (Ar), 133.5 (4), 135.6, 136.8 (Ar), 172.4 (1); IR_{CHCl_3} 1730s.

(*E*)-Methyl 4-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-6-hydroxy-3-hexenoate (14). The aldehyde and its trimer **13, 13a** (8.67 g, 21.1 mmol), were dissolved in MeOH, and sodium borohydride (0.397 g, 10.5 mmol) was added in small portions. After 3 h the reaction was quenched (NH_4Cl) and the mixture filtered and concentrated to yield a white residue, which was dissolved in aqueous $NaHCO_3$ (150 mL) and extracted with ether (4 × 130 mL). The combined organic phases were dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (ether/pentane, 2:1) to give **14** (8.26 g, 95%): TLC R_f 0.45 (ether/pentane, 2:1); ^{1}H-NMR ($CDCl_3$) δ 1.07 (s, 9H, *t*-Bu), 1.86 (t, exchangeable by D_2O, 1H, OH), 2.33−2.47 (m, 2H, 5), 3.15 (d, J = 7.6 Hz, 2H, 2), 3.63 (m, 2H, 6), 3.69 (s, 3H, OMe), 4.11 (s, 2H, CH_2O), 5.74 (m, 1H, 3), 7.36−7.47 (m, 6H, Ar), 7.65−7.69 (m, 4H, Ar); ^{13}C-NMR ($CDCl_3$) δ 19.1 (Si*C*Me$_3$), 26.8 (*t*-Bu), 31.9 (5), 33.1 (2), 51.9 (OMe), 60.8 (6), 67.8 (CH_2O), 120.1 (3), 127.7, 129.66, 129.74 (Ar), 133.1 (4), 135.5 (Ar), 172.5 (1); IR_{CHCl_3} 3450bm, 1730s. Anal. ($C_{24}H_{32}O_4Si$) C, H.

(*E*)-Methyl 4-[[(*tert*-Butyldiphenylsilyl)oxy]methyl]-6-(tosyloxy)-3-hexenoate (15). The alcohol **14** (8.2 g, 19.4 mmol), triethylamine (6.03 g, 59.6 mmol, 8.3 mL), and (dimethylamino)pyridine (0.125 g, 0.99 mmol) were mixed together with CH_2Cl_2 and cooled to 0 °C. Tosyl chloride (5.68 g, 29.8 mmol) was added carefully, and the reaction mixture was then allowed to reach room temperature overnight. The mixture was poured into aqueous $NaHCO_3$ (200 mL), and the organic phase was washed with $NaHCO_3$ (1 × 100 mL) and 10% citric acid (3 × 130 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (CH_2Cl_2) to give **15** (10.14 g, 90%): TLC R_f 0.24 (CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ 1.00 (s, 9H, *t*-Bu), 2.40−2.47 (m, 5H, Ar-CH_3, 5), 3.05 (d, J = 7.3 Hz, 2H, 2), 3.68 (s, 3H, OMe), 3.98−4.03 (m, 4H, 6, CH_2O), 5.71 (t, J = 7.3 Hz, 1H, 3), 7.25−7.29 (m, 2H, Ar), 7.33−7.43 (m, 6H, Ar), 7.59−7.62 (m, 4H, Ar), 7.72 (d, J = 8.2 Hz, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 19.1 (Si*C*Me$_3$), 21.6 (Ar-*C*H$_3$), 26.7 (*t*-Bu), 27.9 (5), 33.0 (2), 51.8 (OMe), 67.3 (CH_2O), 68.4 (6), 121.0 (3), 127.7, 127.8, 129.7 (2C), 132.9 (Ar), 133.1 (4), 135.4, 136.0, 144.6 (Ar), 171.8 (1); IR_{CHCl_3} 1733s, 1360s, 1175s. Anal. ($C_{31}H_{38}O_6SSi$) C, H.

(*E*)-Methyl 6-[[1(*S*),2(*S*)-1-(*tert*-Butoxycarbonyl)-2-methylbutyl]amino]-4-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3-hexenoate (16). The isoleucine *tert*-butyl ester (3.3 g, 17.6 mmol) was dissolved in THF (15 mL), and the tosylate **15** (2.0 g, 3.53 mmol, dissolved in THF (10 mL)) was added dropwise in 30 min at room temperature. The reaction temperature was then raised to 80 °C. After 5 days of reflux the reaction was quenched with aqueous NH_4Cl (75 mL) and the mixture extracted with EtOAc (3 × 60 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (ether/pentane, 1:2) to give **16** (1.61 g, 78%): TLC R_f 0.44 (ether/pentane, 1:2); ^{1}H-NMR ($CDCl_3$) δ 0.84−0.89 (m, 6H, 2′Me, 4′), 1.06 (s, 9H, *t*-BuSi), 1.09−1.2 (m, 1H, 3′), 1.36−1.58 (m, 12H, *t*-BuO, NH, 2′, 3′), 2.22 (m, 2H, 5), 2.4 (m, 1H, 6), 2.6 (m, 1H, 6), 2.86 (d, J = 5.9 Hz, 1H, 1′), 3.15 (d, J = 7.3 Hz, 2H, 2), 3.69 (s, 3H, OMe), 4.12 (s, 2H, CH_2O), 5.76 (t, J = 7.3 Hz, 1H, 3), 7.34−7.42 (m, 6H, Ar), 7.65−7.68 (m, 4H, Ar); ^{13}C-NMR ($CDCl_3$) δ 11.5 (4′), 15.4 (2′Me), 19.2 (Si*C*Me$_3$), 25.8 (3′), 26.5, 26.8 (*t*-BuSi), 28.1 (*t*-BuO), 29.0 (5), 33.1 (2), 38.4 (2′), 46.8 (6), 51.7 (OMe), 66.4 (1′), 67.0 (CH_2O), 80.7 (O*C*Me$_3$), 117.5 (3), 127.6, 129.6, 133.6, 135.5 (Ar), 139.5 (4), 172.4 (CO), 174.3 (CO); IR_{CHCl_3} 3324w 1745s, 1730s. Anal. ($C_{34}H_{51}NO_5Si$) C, H, N.

(*E*)-Methyl 6-[[1(*S*),2(*S*)-1-(*tert*-Butoxycarbonyl)-2-methylbutyl]amino]-4-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3-hexenoate (16). Isoleucine *tert*-butyl ester hydrochloride (3.87 g, 17.3 mmol) and the aldehyde **13** (4.10 g, 10 mmol) were mixed in dry MeOH (30 mL) with NaOAc (1.43 g, 17.5 mmol) for 10 min and treated with freshly ground 3 Å molecular sieves (14 g) prior to the slow addition of $NaBH_3CN$ (1.07 g, 17 mmol). The mixture was stirred at room temperature for 2 h, filtered, and concentrated. The residue was dissolved in H_2O (100 mL), extracted with EtOAc, dried (Na_2SO_4), concentrated, and purified by flash chromatography (ether/petroleum ether, 1:1) to give **16** (3.20 g, 55%) contaminated with less than 10% of an isomer. Spectroscopic data were consistent with data described above. Anal. ($C_{34}H_{51}NO_5Si$) C, H, N.

1-[1(*S*),2(*S*)-1-(*tert*-Butoxycarbonyl)-2-methylbutyl]-5-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (17). The methyl *tert*-butyl diester **16** (33 mmol) was dissolved in dioxane (75 mL) and treated with 1 M aqueous NaOH (66 mL, 2 equiv) at room temperature for 6 h. The mixture was brought to pH 3 by 3 M aqueous HCl and extracted with EtOAc. The combined acidic extracts were dried (Na_2SO_4) and concentrated. The residual oil was filtered through silica gel using (I) ether/pentane (1:2) and (II) EtOAc. The ethyl acetate fractions yielded the desired acid on concentration sufficiently pure for immediate cyclization. The acid (10 mmol) was dissolved in dry DMF (200 mL) and cooled to 0 °C prior to the addition of DPPA (28 mmol, 7.7 g, 6 mL) and Na_2CO_3 (100 mmol, 90 g). The mixture was stirred at 0 °C for 3 days under N_2 atmosphere, then filtered (CH_2Cl_2), and concentrated below 30 °C. The residue was extracted with ether and filtered through silica gel. The concentrated filtrates were purified by flash chromatography (ether/pentane, 1:2) to give the lactam as colorless oil (5.80 g, 32% over two steps): TLC R_f 0.27 (ether/pentane, 1:2); ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3H, 4′), 0.94 (d, J = 6.5 Hz, 3H, 2′Me), 1.02−1.14 (m, 10H, *t*-BuSi, 3′), 1.35−1.46 (m, 10H, *t*-BuO, 3′), 1.92 (m, 1H, 2′), 2.22 (m, 2H, 6), 3.28 (m, 2H, 3), 3.72 (t, J = 5.8 Hz,

2H, 7), 3.97 (s, 2H, CH_2O), 4.84 (d, J = 10.5 Hz, 1H, 1′), 5.67 (bt, J = 5.9 Hz, 1H, 4), 7.32−7.44 (m, 6H, Ar), 7.62−7.66 (m, 4H, Ar); ^{13}C-NMR ($CDCl_3$) δ 10.9 (4′), 15.4 (2′Me), 19.1 (Si*C*Me_3), 25.0 (3′), 26.7 (Si*C*Me_3), 27.8 (*C*Me_3), 29.7 (6), 34.5 (2′), 35.3 (3), 41.2 (7), 60.8 (1′), 68.1 (CH_2O), 81.2 (*C*Me_3), 114.9 (4), 127.6, 129.6, 133.2, 135.4 (Ar), 138.6 (5), 170.5 (CO), 173.6 (CO); IR_{CDCl_3} 3420bm, 1717s, 1647s. Anal. ($C_{33}H_{47}NO_4Si$) C, H, N.

1-[1(*S*),2(*S*)-(*tert*-Butoxycarbonyl)-2-methylbutyl]-5-(hydroxymethyl)-2,3,6,7-tetrahydro-1*H*-azepin-2-one (18). Deprotection: 17 (1.90 g, 3.45 mmol) was dissolved in CH_2Cl_2 (15 mL) and treated with tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$; 1.18 g, 3.73 mmol) dissolved in dry THF (15 mL) at room temperature for 6 h. The solution was filtered through silica gel using (I) ether/pentane (1:1) and (II) EtOAc. Solvent removal yielded the alcohol as thin needles (765 mg, 71%): TLC R_f 0.34 (EtOAc); mp 92 °C; $[\alpha]^{23}_D$ −81.9 ± 0.5° (c = 1.031, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H, 4′), 0.95 (d, J = 6.6 Hz, 3H, 2′Me), 1.0−1.14 (m, 1H, 3′), 1.34−1.43 (m, 1H, 3′), 1.45 (s, 9H, *t*-Bu), 1.92 (m, 1H, 2′), 2.32 (m, 2H, 6), 3.30 (m, 2H, 3), 3.74 (t, J = 5.8 Hz, 2H, 7), 3.95 (s, 2H, CH_2O), 4.85 (d, J = 10.6 Hz, 1H, 1′), 5.67 (m, 1H, 4); ^{13}C-NMR ($CDCl_3$) δ 10.9 (4′), 15.5 (2′Me), 25.1 (3′), 27.8 (OC*Me*$_3$), 29.8 (6), 34.5 (2′), 35.3 (3), 41.3 (7), 60.9 (1′), 67.5 (CH_2O), 81.5 (O*C*Me$_3$), 115.9 (4), 139.7 (5), 170.5 (CO), 173.6 (CO); IR_{CHCl_3} 3616s, 3428bm, 1722s, 1636s; GC−MS (HP-1) 25.89 min 311 (0.1, M^+), 282 (1), 210 (100, $-CO_2t$-Bu), 192 (30, $-CO_2t$-Bu, $-H_2O$). Anal. ($C_{17}H_{29}NO_4$) C, H, N.

3(*R*,*S*)-3-[4-(Benzyloxy)benzyl]-1-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-5-(hydroxymethyl)-2,3,6,7-tetrahydro-1*H*-azepin-2-one (19). Benzylation: A round bottom flask (25 mL) was charged under N_2 atmosphere with dry THF (4 mL) and freshly generated LDA (3.09 mmol) and cooled to −78 °C. A solution of **16** (320 mg, 1.03 mmol) in dry THF (4 mL) was added over 12 min. The solution was rapidly warmed to −40 °C to give a stable gel and after 20 min cooled to −78 °C prior to the slow addition of 4-(benzyloxy)benzyl bromide (512 mg, 1.85 mmol) in dry THF (2 mL). The clear solution was stirred at −78 °C for 10 min and then rapidly warmed to −25 °C, kept at this temperature for 30 min, and finally warmed to 0 °C, and the reaction was quenched by saturated NH_4Cl solution (10 mL). The mixture was suspended in EtOAc (15 mL), washed with H_2O (3 × 15 mL), dried (Na_2SO_4), filtered through silica gel, and concentrated to a yellow oil. The oil was purified by flash chromatography (ether/pentane, 2:1; R_f 0.24) to obtain a 9:5 mixture of the diastereomers (322 mg, 62%), which can be separated by repeated preparative TLC using $CHCl_3$/EtOH (16:1).

Major isomer: TLC R_f 0.16 ($CHCl_3$/EtOH, 100:1); $[\alpha]^{26}_D$ −31.5 ± 0.5° (c = 0.729, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H, 4′), 0.97 (d, J = 6.6 Hz, 3H, 2′Me), 1.00−1.12 (m, 1H, 3′), 1.28−1.45 (m, 1H, 3′), 1.43 (s, 9H, *t*-Bu), 1.62 (bs, 1H, OH), 1.85 (m, 1H, 2′), 2.32 (m, 2H, 6), 2.71 (dd, J = 14.2, 9.2 Hz, 1H, 3-CH_aAr), 3.27 (dd, J = 14.2, 5.6 Hz, 1H, 3-CH_bAr), 3.74−3.93 (m, 5H, CH_2O, 7, 3), 4.88 (d, J = 10.2 Hz, 1H, 1′), 5.03 (s, 2H, $PhCH_2$), 5.44 (bs, 1H, 4), 6.90 (d, J = 8.6 Hz, 2H, $BnOC_6H_4$), 7.17 (d, J = 8.6 Hz, 2H, $BnOC_6H_4$), 7.32−7.45 (m, 5H, Bn); ^{13}C-NMR ($CDCl_3$) δ 11.2 (4′), 15.8 (2′Me), 25.5 (3′), 28.0 (OC*Me*$_3$), 30.1 (6), 33.9 (2′), 35.8 (3-CH_2Ar), 41.3 (3), 43.0 (7), 61.8 (1′), 68.1 (OCH_2Ar), 70.0 (CH_2OH), 81.5 (*C*Me_3), 114.8 (Ar), 123.6 (4), 127.5, 127.9, 128.6, 130.1, 132.3, 137.0 (Ar), 139.1 (5), 158.2 (Ar), 170.5 (CO), 174.8 (CO); IR_{CHCl_3} 3600m, 3430bm, 1723s, 1650s. Anal. ($C_{31}H_{41}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

Minor isomer: TLC R_f 0.15 ($CHCl_3$/EtOH, 100:1); $[\alpha]^{26}_D$ −41.0 ± 0.5° (c = 0.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.74 (t, J = 7.3 Hz, 3H, 4′), 0.92 (d, J = 6.6 Hz, 3H, 2′Me), 1.00−1.12 (m, 1H, 3′), 1.28−1.45 (m, 1H, 3′) 1.45 (s, 9H, *t*-Bu), 1.67 (bs, 1H, OH), 1.85 (m, 1H, 2′), 2.32 (bs, 2H, 6), 2.72 (dd, J = 14.2, 9.2 Hz, 1H, 3-CH_aAr), 3.27 (dd, J = 14.2, 5.3 Hz, 1H, 3-CH_bAr), 3.74−3.93 (m, 5H, CH_2O, 7, 3), 4.89 (d, J = 10.5 Hz, 1H, 1′), 5.03 (s, 2H, $PhCH_2$), 5.42 (bs, 1H, 4), 6.89 (d, J = 8.6 Hz, 2H, $BnOC_6H_4$), 7.17 (d, J = 8.6 Hz, 2H, $BnOC_6H_4$), 7.30−7.45 (m, 5H, Bn); ^{13}C-NMR ($CDCl_3$) δ 10.6 (4′), 15.5 (2′Me), 24.8 (3′), 27.9 (OC*Me*$_3$), 29.6 (6), 33.9 (2′), 36.3 (3-CH_2Ar), 39.9 (3), 43.0 (7), 60.5 (1′), 68.1 (OCH_2Ar), 70.0 (*C*H_2OH), 81.5 (*C*Me_3), 114.7 (Ar), 122.8 (4), 127.5, 127.9, 128.6, 130.1, 132.3, 137.1 (Ar), 138.8 (5), 157.2 (Ar), 171.0 (CO), 174.7 (CO); IR_{CHCl_3} 3600m, 3432bm, 1720s, 1650s. Anal. ($C_{31}H_{41}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

3(*R*,*S*)-3-[4-(Benzyloxy)benzyl]-1-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-5-(phthalimidomethyl)-2,3,6,7-tetrahydro-1*H*-azepin-2-one (20). Amination: The diastereomeric mixture of **19** (2.37 mmol, 1.20 g), triphenylphosphine (2.4 mmol, 0.65 g), and phthalimide (2.6 mmol, 0.39 g) was dissolved in dry THF (25 mL) and treated with diethyl azodicarboxylate (DEAD; 2.8 mmol, 0.46 mL) under N_2 atmosphere for 16 h. The mixture was concentrated to a yellow oil and triturated with ether/pentane (1:1; 50 mL). The extracts were filtered, concentrated, and purified by gradient flash chromatography (ether/pentane, 1:2, to ether) to give **20** as a inseparable set of diastereomers (1.48 g, 98%); TLC R_f 0.59 (ether/pentane, 2:1).

Major isomer: ^{1}H-NMR ($CDCl_3$) δ 0.75 (t, J = 7.4 Hz, 3H, 4′), 0.90 (d, J = 6.4 Hz, 3H, 2′Me), 0.89−1.00 (m, 1H, 3′), 1.21−1.37 (m, 1H, 3′), 1.39 (s, 9H, *t*-Bu), 1.82 (m, 1H, 2′), 2.23 (m, 2H, 6), 2.70 (dd, J = 14.2, 8.6 Hz, 1H, 3-CH_aAr), 3.24 (dd, J = 14.2, 6.4 Hz, 1H, 3-CH_bAr), 3.63−3.90 (m, 3H, 3, 7), 4.06 (m, 2H, CH_2N), 4.84 (d, J = 10.4 Hz, 1H, 1′), 4.99 (s, 2H, $PhCH_2$), 5.43 (m, 1H, 4), 6.77−6.86 (m, 2H, $BnOC_6H_4$), 7.05−7.14 (m, 2H, $BnOC_6H_4$), 7.31−7.46 (m, 5H, Bn), 7.68−7.76 (m, 2H, phthal), 7.79−7.87 (m, 2H, phthal); ^{13}C-NMR ($CDCl_3$) δ 10.9 (4′), 15.7 (2′Me), 25.4 (3′), 28.0 (OC*Me*$_3$), 31.3 (6), 33.7 (2′), 36.3 (3-CH_2), 41.0 (3), 43.2 (7), 44.1 (CH_2N), 61.6 (1′), 70.0 ($PhCH_2$), 81.4 (*C*Me_3), 114.7, 123.3 (Ar), 125.8 (4), 127.4, 127.8, 128.5, 130.1, 131.9, 132.4, 133.5, 134.0 (Ar), 137.5 (5), 157.2 (Ar), 167.8 (CO), 170.5 (CO), 174.2 (CO).

Minor isomer: ^{1}H-NMR ($CDCl_3$) δ 0.70 (t, J = 7.4 Hz, 3H, 4′), 0.88 (d, J = 6.4 Hz, 3H, 2′Me), 0.89−1.00 (m, 1H, 3′), 1.21−1.37 (m, 1H, 3′), 1.37 (s, 9H, *t*-Bu), 1.85 (m, 1H, 2′), 2.23 (m, 2H, 6), 2.69 (dd, J = 14.2, 8.1 Hz, 1H, 3-CH_aAr), 3.24 (dd, J = 14.2, 6.4 Hz, 1H, 3-CH_bAr), 3.63−3.90 (m, 3H, 3, 7), 4.06 (m, 2H, CH_2N), 4.83 (d, J = 11.0 Hz, 1H, 1′), 4.99 (s, 2H, $PhCH_2$), 5.33 (m, 1H, 4), 6.77−6.86 (m, 2H, $BnOC_6H_4$), 7.05−7.14 (m, 2H, $BnOC_6H_4$), 7.31−7.46 (m, 5H, Bn), 7.68−7.76 (m, 2H, phthal), 7.79−7.87 (m, 2H, phthal); ^{13}C-NMR ($CDCl_3$) δ 10.5 (4′), 15.4 (2′Me), 24.7 (3′), 27.8 (OC*Me*$_3$), 30.8 (6), 33.7 (2′), 35.4 (3-CH_2), 39.7 (3), 43.0 (7), 43.9 (CH_2N), 60.5 (1′), 70.0 ($PhCH_2$), 81.3 (*C*Me_3), 114.6, 123.3 (Ar), 124.3 (4), 127.4, 127.8, 128.5, 130.1, 131.9, 132.4, 133.6, 134.0 (Ar), 137.2 (5), 157.2 (Ar), 167.8 (CO), 170.9 (CO), 173.7 (CO); IR_{CHCl_3} 3530bw, 1773m, 1716s, 1651m; PDMS (MW 636.8) 637.6 ($M^+ + H^+$), 660.6 (M^+ + Na). Anal. ($C_{39}H_{44}N_2O_6$) C, H, N.

3(*R*,*S*)-3-[4-(Benzyloxy)benzyl]-1-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-5-[[(9-fluorenylmethyloxycarbonyl)amino]methyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (21). The diastereomeric phthalimide **20** (0.67 mmol, 427 mg) was dissolved in dry EtOH (6 mL) and treated with hydrazine hydrate ($H_2NN_2H \cdot H_2O$; 1.4 mmol, 68 μL) under N_2 atmosphere for 24 h. The mixture was filtered through silica gel ($CHCl_3$/EtOH, 3:1) and concentrated to give the crude amine. Partial purification of the residue by flash chromatography ($CHCl_3$/EtOH, 8:1) gave 192 mg, 57%; TLC R_f 0.51 (ether/pentane, 2:1). The diastereomeric amine, TBAF·$3H_2O$ (0.63 μmol, 20 mg), and Na_2CO_3 (1.14 mmol, 120 mg) were suspended in dry dioxane (4 mL) and treated with Fmoc-Cl (0.53 mmol, 137 mg) under N_2 atmosphere for 20 h. The mixture was filtered through silica gel (EtOAc) and concentrated to leave a yellow foam which was purified by flash chromatography on silica gel 60, 15−40 μm, using EtOAc/petroleum ether (1:3) to give 227.7 mg, 83%; TLC R_f 0.42 (EtOAc/petroleum ether, 1:3); IR_{CHCl_3} 3460m, 1780s, 1730bs, 1650s.

3(*R*,*S*)-3-[4-(Benzyloxy)benzyl]-1-[1(*S*),2(*S*)-1-carboxy-2-methylbutyl]-5-[[(9-fluorenylmethyloxycarbonyl)amino]-methyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (22a,b). The Fmoc protected diastereomeric *tert*-butyl esters **21** (227.7 mg, 312 μmol) were dissolved in CH_2Cl_2 (3 mL) and treated with a mixture of CH_2Cl_2/TFA/anisole (10/3/1; 7 mL) at 0 °C for 14 h. The mixture was filtered through silica gel ($CHCl_3$) to leave the crude product as a white foam upon concentration. The diastereomers were separated by flash chromatography on silica gel 60, 15−40 μm ($CHCl_3$/2-propanol, 11:1), to yield the

pure compounds in a ratio of 5:3 at a total yield of 95% (185 + 111 μmol, 124.2 + 74.7 mg).

Major isomer 22a: TLC R_f 0.43 ($CHCl_3$/*i*-PrOH, 11:1); $[\alpha]^{24}_D$ −21.4 ± 0.3° (c = 2.218, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.85 (m, 3H, 4′), 1.02 (d, J = 6.1 Hz, 3H, 2′Me), 0.90−1.10 (bs, 1H, 3′), 1.24−1.45 (bs, 1H, 3′), 2.01 (bs, 2H, 2′), 2.19 (bs, 1H, 6), 2.71 (m, 1H, 3-CH_aAr), 3.22 (m, 1H, 3-CH_bAr), 3.42 (bs, 1H, 3), 3.61 (bs, 2H, 7), 3.80−4.10 (m, 2H, 5-CH_2N), 4.19 (m, 1H, Fmoc), 4.41 (d, J = 6.1 Hz, 2H, Fmoc), 4.68 (d, J = 8.2 Hz, 1H, 1′), 4.94 (s, 2H, $PhCH_2$), 5.30 (s, 1H, 4), 5.65 (bs, 1H, NH), 6.84 (d, J = 7.9 Hz, 2H, $BnOC_6H_4$), 7.11 (d, J = 7.9 Hz, 2H, $BnOC_6H_4$), 7.22−7.45 (m, 9H, Fmoc, Ph), 7.59 (m, 2H, Fmoc), 7.76 (d, J = 7.4 Hz, 2H, Fmoc), 9.12 (bs, 1H, COOH); ^{13}C-NMR ($CDCl_3$) δ 11.1 (4′), 16.0 (2′Me), 25.5 (3′), 30.2 (6), 34.8 (2′), 36.2 (3-CH_2N), 43.1 (7), 43.5 (3), 47.3 (5-CH_2N), 47.7 (Fmoc), 64.3 (1′), 66.7 (Fmoc), 69.9 ($PhCH_2$), 114.8, 120.0 (Ar), 123.3 (4), 125.0, 127.1, 127.4, 127.8, 127.9, 128.6, 130.2, 131.7, 136.6 (Ar), 137.1 (5), 141.3, 143.9 (Ar), 156.5 (OCON), 157.3 (Ar), 174.3 (CO), 175.5 (CO); IR_{CHCl_3} 3610m, 3500bw, 3460s, 1780s, 1730bs, 1650s; PDMS (MW 672.8) 674.2 (M^+ + H), 695.8 (M^+ + Na), 712.6 (M^+ + K). Anal. ($C_{42}H_{44}N_2O_6$) N; C: calcd, 74.98; found, 66.2; H: calcd, 6.59; found, 5.9.

Minor isomer 22b: TLC R_f 0.37 ($CHCl_3$/*i*-PrOH, 11:1) $[\alpha]^{24}_D$ −10.9 ± 0.3° (c = 1.195, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.83 (m, 3H, 4′), 0.89−1.05 (m, 1H, 3′), 1.00 (d, J = 6.4 Hz, 3H, 2′Me), 1.19−1.39 (m, 1H, 3′), 2.08 (m, 1H, 2′), 2.35 (m, 2H, 6), 2.71 (m, 1H, 3-CH_aAr), 3.22 (m, 1H, 3-CH_bAr), 3.40−3.70 (m, 3H, 7, 3), 3.84 (m, 2H, 5-CH_2N), 4.18 (t, J = 6.0 Hz, 1H, Fmoc), 4.38 (d, J = 6.0 Hz, 2H, Fmoc), 4.80 (d, J = 8.0 Hz, 1H, 1′), 4.92 (s, 2H, $PhCH_2$), 5.30 (s, 1H, 4), 6.10 (bs, 1H, NH), 6.82 (d, J = 7.8 Hz, 2H, $BnOC_6H_4$), 7.14 (d, J = 7.8 Hz, 2H, $BnOC_6H_4$), 7.20−7.34 (m, 9H, Fmoc, Ph), 7.55−7.65 (m, 2H, Fmoc), 7.70−7.80 (m, 2H, Fmoc), 8.6 (bs, 1H, COOH); ^{13}C-NMR ($CDCl_3$) δ 10.6 (4′), 15.8 (2′Me), 25.0 (3′), 29.8 (6), 33.0 (2′), 36.2 (3-CH_2), 41.5 (3), 42.8 (7), 47.2 (5-CH_2N), 47.4 (Fmoc), 61.9 (1′), 66.8 (Fmoc), 70.0 ($PhCH_2$), 114.8 (Ar), 120.0 (Fmoc), 122.3 (4), 125.1, 127.2, 127.5, 127.8, 127.9, 128.6, 130.2, 132.0, 136.3 (Ar), 136.8 (5), 141.3 (Fmoc), 143.9 (Ar), 156.6 (OCON), 157.3 (Ar), 174.1 (CO), 175.3 (CO); IR_{CHCl_3} 3600w, 3440m, 1780s, 1720bs, 1650s. Anal. ($C_{42}H_{44}N_2O_6$) H; C: calcd, 74.98; found, 68.1. N: calcd, 4.10; found, 3.65.

Azepine-Based Ang II Mimetics 4a,b. 4a, Synthesis: The Fmoc-protected template **22a** (83 mg, 123 μmol), TBTU (39.6 mg, 123 μmol), HOBt·H_2O (16.7 mg, 123 μmol), and DIEA (43 μL, 247 μmol) were dissolved in DMF (1.8 mL) and added to preswollen His(Boc)-Pro-Phe-Wang resin (250 mg, 123 μmol) in a 5 mL disposable syringe equipped with a porous polyethylene filter. After mixing by slow rotation at room temperature for 3 h the resin was filtered off and washed with DMF (6 × 3 mL). PDMS analysis of a deprotected resin sample showed, in addition to the expected compound, a peak corresponding to the tetramethylguanylated tripeptide. The free tripeptide could not be detected. After deprotection with 20% piperidine/DMF (2 × 3 mL, 5 + 15 min) and washing with DMF (6 × 3 mL), the peptide was further elongated by reaction with, in turn, Fmoc-Arg(Pmc)-OH and Fmoc-Asp(O^{t}Bu)-OH as described above (general procedures). Fmoc deprotection, washing with DMF and CH_2Cl_2, and drying in air and in vacuo afforded the partially protected peptide resin (313 mg, 66% yield according to the weight increase).

Cleavage: I. TFMSA. Part of the resin (150 mg, 59 μmol) was reacted with 95% aqueous TFA (4 mL) for 1 h. The mixture was filtered through a small plug of glass wool in a Pasteur pipet and the resin washed with TFA (2 × 1 mL). The combined filtrates were evaporated in a stream of dry nitrogen to ca. 1 mL, and the product was precipitated by the addition of cold anhydrous ether (5 mL). The precipitate was collected by centrifugation, washed with ether (4 × 4 mL), and dried to furnish 48.5 mg (74%) of crude benzylated **4a**. Finally, the benzyl group was removed by reaction with TFMSA/TFA/DMS/*m*-cresol (1:5:3:1), 0.1 mL/g, at 0 °C for 15−30 min. The crude final product **4a** was precipitated and isolated as above and immediately submitted to gel filtration.

II. HF. A second batch of the partially protected peptide resin (187 mg) was treated with HF (5 mL) in the presence of *m*-cresol (300 μL) and DMS (200 μL) at 0 °C for 1 h. After removal of HF in vacuo, the resin was transferred to a glass filter funnel and washed several times with ether, $CHCl_3$ and finally ether. The fully deprotected peptide **4a** was then extracted into 1 M AcOH (3 × 2.5 mL) and lyophilized. PDMS showed, in addition to the expected compound, the presence of a benzylated product (small amount), the tetramethylguanylated tripeptide, and the peptide Asp-Arg-His-Pro-Phe.

Purification: The crude peptide was dissolved in 0.1 M NH_4OAc, pH 8.5, and purified by gel filtration on a Sephadex G15 column (1 × 100 cm) in the same buffer followed by preparative RP-HPLC on a Waters Radial Pak 10 μm μBondapak C18 column (2.5 × 10 cm) with a 40 min gradient of 10−40% MeCN or a 30 min gradient of 15−30% MeCN in 0.1% aqueous TFA at a flow rate of 8 mL/min. The separation was monitored at 220 and 254 nm and by PDMS. The total yield of **4a** was 11.4 mg. Amino acid analysis: Asp 0.97, Arg 0.96, His 1.03, Pro 1.01, Phe 1.02 (54% peptide). PDMS (MW 1013.4): 1014.2 (M + H^+), 1035.0 (M + Na^+), 1052.3 (M + K^+).

4b: This peptide was prepared from **22b** by the same procedure as described above for **4a**. Cleavage and debenzylation were accomplished by treatments with TFA and TFMSA, respectively (procedure I above). Additional chromatographic steps were included in the purification of **4b**. The material recovered after RP-HPLC was dissolved in 20 mM Na phosphate, 0.1 M NaCl, pH 7.0 (buffer A), and applied onto a Cu^{2+}-loaded column of Chelating Superose (1 × 2 cm) at a flow rate of 1 mL/min. After washing with the starting buffer (12 mL), the peptide was desorbed by gradient elution (buffer B: 100 mM Na phosphate, 0.1 M NaCl, pH 3.8) using a 6 min gradient of 0−80% B followed by a 42 min gradient of 80−100% B. The separation was monitored at 254 nm and by analytical RP-HPLC. The fractions containing the product were desalted and further purified by rechromatography on the C18 support. The final yield of **4b** was 2.6 mg. Amino acid analysis: Asp 1.00, Arg 0.98, Ile 0.12, His 0.99, Pro 1.01, Phe 1.02 (59% peptide). PDMS: 1014.4 (M + H^+), 1036.3 (M + Na^+).

7-Nitro-3-isochromanone (24). Trifluoroacetic anhydride (60 mL) was added to a solution of 3-isochromanone (**23**) (7.4 g, 50 mmol)[46,61,62] and ammonium nitrate (7.9 g, 100 mmol) in CH_2Cl_2 (20 mL), and the reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated, and the residue was purified by flash chromatography to give a mixture of 6-nitro and 7-nitro isomers in the ratio of 1:5 (7.8 g, 81%): TLC R_f 0.24 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 3.85 (s, 2H, 4), 5.42 (s, 2H, 1), 7.44 (d, J = 8.1 Hz, 1H, 5), 8.16 (d, J = 2.2 Hz, 1H, 8), 8.23 (dd, J = 8.1, 2.2 Hz, 1H, 6); ^{13}C-NMR ($CDCl_3$) δ 36.2 (4), 69.1 (1), 120.0, 124.0, 128.2, 133.1, 138.3, 147.2 (7), 168.6 (3); $IR_{cap.film}$ 1757, 1529, 1348. Anal. ($C_9H_7NO_4$) C, H, N.

4(*R,S*)-4-[4-(Benzyloxy)benzyl]-7-nitro-3-isochromanone (25). Compound **24** (7.1 g, 30 mmol) was dissolved in THF (250 mL), cooled to −78 °C, and treated with LDA (20 mL, 2 M, 40 mmol). The mixture was warmed to −30 °C and stirred for 30 min. 4-(Benzyloxy)benzyl bromide (11.08 g, 40 mmol) was added, and the reaction mixture was stirred at 0 °C overnight. The solution was poured into aqueous citric acid (5%, 500 mL) and extracted by CH_2Cl_2 (3 × 200 mL). The organic phase was concentrated and purified by flash chromatography to give compound **25** (2.8 g, 24%): TLC R_f 0.50 (petroleum ether/EtOAc, 2:1), R_f 0.90 (1% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) 3.23 (dd, J_{app} = 12.0, 5.2 Hz, 1H, $C_6H_4CH_{2a}$), 3.32 (dd, J_{app} = 12.0, 6.8 Hz, 1H, $C_6H_4CH_{2b}$), 4.08 (dd, J = 6.8, 5.2 Hz, 1H, 4), 4.60 (d, J = 14.7 Hz, 1H, 1′), 5.04 (s, 2H, $C_6H_5CH_2$), 5.16 (d, J = 14.7 Hz, 1H, 1″), 6.83 (m, 4H, $BnOC_6H_4$), 7.13 (d, J = 8.3 Hz, 1H, 5), 7.32−7.43 (m, 5H, C_6H_5-CH_2), 7.97 (d, J = 2.2 Hz, 1H, 8), 8.14 (dd, J = 8.3, 2.2 Hz, 1H, 6); ^{13}C-NMR ($CDCl_3$) δ 38.3 ($C_6H_4CH_2$), 47.6 (4), 68.7 (1), 70.0 ($PhCH_2$), 115.1, 119.5, 123.4, 127.5, 127.9, 128.0, 128.5, 128.7, 130.2, 132.7, 136.6, 140.9, 147.0 (7), 158.9, 170.7 (3); $IR_{cap.film}$ 1745, 1531, 1347. Anal. ($C_{23}H_{19}NO_5$) C, H, N.

Ethyl (*R,S*)-2-[2-(Hydroxymethyl)-4-nitrophenyl]-3-[4-(benzyloxy)phenyl]propanoate (26). Compound **25** (3.9 g, 10 mmol) was treated with H_2SO_4 (10 mL) in EtOH (200 mL) at reflux overnight. The solution was poured into ice/water,

neutralized with Na_2CO_3, extracted with CH_2Cl_2 (3 × 100 mL), concentrated, and purified by flash chromatography to give compound **26** (2.1 g, 48%) and starting material (1.3 g, 33%): TLC R_f 0.50 (petroleum ether/EtOAc, 2:1), R_f 0.71 (1% MeOH in CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) δ 1.16 (t, J = 7.1 Hz, 3H, CH_2CH_3), 2.99 (dd, J = 13.7, 7.7 Hz, 1H, 3a), 3.42 (dd, J = 13.7, 7.7 Hz, 1H, 3b), 4.11 (m, 2H, CH_2CH_3), 4.23 (t, J = 7.7 Hz, 1H, 2), 4.56 (d, J = 13.2 Hz, 1H, $CH_{2a}OH$), 4.68 (d, J = 13.2 Hz, 1H, $CH_{2b}OH$), 5.01 (s, 2H, $C_6H_5CH_2$), 6.83 (d, J = 8.8 Hz, 2H, $BnOC_6H_4$), 7.00 (d, J = 8.8 Hz, 2H, $BnOC_6H_4$), 7.32−7.42 (m, 5H, C_6H_5), 7.69 (d, J = 8.5 Hz, 1H, 6′), 8.15 (dd, J = 8.5, 2.6 Hz, 1H, 5′), 8.23 (d, J = 2.6 Hz, 1H, 3′); ^{13}C-NMR ($CDCl_3$) δ 13.9 (Me), 38.5 ($C_6H_4CH_2$), 48.6 (2), 61.5 (CH_3CH_2), 62.3 (CH_2OH), 69.9 (3), 114.9, 123.9, 123.3, 127.4, 127.9, 128.5, 128.6, 129.9, 130.3, 136.8, 140.6, 144.2, 147.0, 157.5, 172.3; $C_{25}H_{25}NO_6$, MW 435.4811; HRMS calcd 435.168 172 8, found 435.1707.

Ethyl (*R,S*)-2-[2-(Bromomethyl)-4-nitrophenyl]-3-[4-(benzyloxy)phenyl]propanoate (27). The compound **26** (870 mg, 2 mmol) was treated with CBr_4 (1.33 g, 4 mmol) and triphenylphosphine (1.05 g, 4 mmol) in THF at room temperature for 3 h. After concentration, the residue was purified by flash chromatography to give compound **27** (580 mg, 58%): TLC R_f 0.82 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 1.14 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.00 (dd, J = 13.7, 6.6 Hz, 1H, 3a), 3.42 (dd, J = 13.7, 8.8 Hz, 1H, 3b), 4.11 (m, 2H, CH_2-CH_3), 4.22 (t, J_{app} = 6.4 Hz, 1H, 2), 4.28 (d, J_{app} = 10.7 Hz, 1H, $CH_{2a}Br$), 4.55 (d, J = 10.9 Hz, 1H, $CH_{2b}Br$), 5.01 (s, 2H, $C_6H_5CH_2O$), 6.87 (d, J = 8.8 Hz, 2H, $C_6H_4CH_2$), 7.09 (d, J = 8.8 Hz, 2H, $C_6H_4CH_2$), 7.33−7.43 (m, 5H, C_6H_5), 7.75 (dd, J = 7.3, 1.5 Hz, 1H, 6′), 8.15−8.21 (m, 2H, 3′, 5′); ^{13}C-NMR ($CDCl_3$) δ 14.0 (Me), 29.4 (CH_2Br), 38.6 (3), 49.0 (2), 61.5 (CH_3CH_2), 70.0 ($C_6H_5CH_2$), 115.0, 123.9, 125.2, 127.4, 127.9, 128.6, 129.5, 130.0, 130.3, 136.9, 137.6, 145.2, 146.8, 157.7, 171.9; IR_{CHCl_3} 1730, 1524, 1348; $C_{25}H_{24}NO_5Br$, MW 498.38; HRMS calcd ($C_{25}H_{24}NO_5Br^{79}$) 497.0838, found 497.0840; calcd ($C_{25}H_{24}NO_5Br^{81}$) 499.0817, found 499.0815.

Ethyl 2(*R,S*)-3-[4-(Benzyloxy)phenyl]-2-[2-[[[(1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]amino]methyl]-4-nitrophenyl]propanoate (28). The solution of compound **27** (747 mg, 1.5 mmol), triethylamine (303 mg, 3 mmol), and isoleucine *tert*-butyl ester (842 mg, 4.5 mmol) in THF (50 mL) was heated to 45 °C and stirred for 2 h. After concentration, the residue was purified by flash chromatography to give a mixture of diastereomers **28** (825 mg, 91%): TLC R_f 0.88 (petroleum ether/EtOAc, 2:1).

4(*R/S*)-4-[4-(Benzyloxy)benzyl]-2-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-7-nitro-1,4-dihydro-3(2*H*)-isoquinolinone (29a,b). The compound **28** (800 mg, 1.56 mmol) was treated with acetic acid (2 drops) in toluene at reflux overnight. After concentration, the residue was separated by flash chromatography to give compounds **29a** (340 mg, 47%) and **29b** (330 mg, 45%). **29a**: TLC R_f 0.60 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 0.78 (t, J = 7.3 Hz, 3H, Me-Ile), 0.88−1.00 (m, 1H, CH_{2a}-Ile), 1.01 (d, J = 6.6 Hz, 3H, Me-Ile), 1.15−1.23 (m, 1H, CH_{2b}-Ile), 1.52 (s, 9H, *t*-Bu), 2.08 (m, 1H, CH-Ile), 2.98 (dd, J = 13.5, 8.8 Hz, 1H, CH_aBn), 3.22 (dd, J = 13.5, 5.1 Hz, 1H, CH_bBn), 3.91 (dd, J = 8.8, 5.1 Hz, 1H, 4), 4.33 (m, 2H, 1), 5.02 (s, 2H, $C_6H_4CH_2$), 5.03 (d, J = 10.7 Hz, 1H, αH-Ile), 6.78 (m, 4H, Bn), 6.86 (d, J = 8.3 Hz, 1H, 5), 7.30−7.43 (m, 5H, BnO), 7.98 (dd, J = 8.3, 2.3 Hz, 1H, 6), 8.03 (d, J = 2.2 Hz, 1H, 8); ^{13}C-NMR ($CDCl_3$) δ 10.5 (Me-Ile), 15.6 (Me-Ile), 24.9 (CH_2-Ile), 28.1 (*t*-Bu), 33.0 (CH-Ile), 37.9 (CH_2Bn), 45.5 (4), 50.7 (CH-Ile), 60.6 (1), 69.9 ($PhCH_2$), 81.9 (*t*-Bu), 114.8, 120.3, 122.3, 127.4, 127.9, 128.5, 128.9, 128.9, 130.2, 133.6, 136.8, 142.8, 146.6, 157.6, 169.8 (CO), 170.6 (CO); $IR_{cap.film}$ 1728, 1655, 1526m, 1346. Anal. ($C_{33}H_{38}N_2O_6 \cdot {}^1/_4H_2O$) C, H, N.

29b: TLC R_f 0.54 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H, Me-Ile), 1.01 (d, J = 6.7 Hz, 3H, Me-Ile), 1.10−1.20 (m, 1H, CH_{2a}-Ile), 1.37 (s, m, 9H, *t*-Bu), 1.37−1.42 (m, 1H, CH_{2b}-Ile), 2.00 (m, 1H, CH-Ile), 2.99 (dd, J = 13.9, 9.2 Hz, 1H, CH_aBn), 3.23 (dd, J = 13.9, 4.8 Hz, 1H, CH_b-Bn), 3.92 (dd, J = 9.2, 4.8 Hz, 1H, 4), 4.11 (d, J = 16.1 Hz, 1H, 1′), 4.77 (d, J = 16.1 Hz, 1H, 1″), 5.02 (s, 2H, $C_6H_4CH_2$), 5.04 (d, J = 10 Hz, 1H, αH-Ile), 6.76 (m, 4H, Bn), 6.86 (d, J = 8.9 Hz, 1H, 5), 7.35−7.45 (m, 5H, BnO), 7.99 (dd, J = 8.9, 2.2 Hz, 1H, 6), 8.04 (d, J = 2.2 Hz, 1H, 8); ^{13}C-NMR ($CDCl_3$) δ 11.0 (Me-Ile), 15.6 (Me-Ile), 25.9 (CH_2-Ile), 27.9 (*t*-Bu), 34.5 (CH-Ile), 38.2 (CH_2Bn), 46.0 (4), 50.7 (CH-Ile), 60.4 (1), 69.2 ($PhCH_2$), 81.9 (*t*-BuO), 114.8, 120.4, 122.2, 127.4, 128.0, 128.6, 128.9, 130.0, 130.3, 133.6, 136.8, 142.9, 146.7, 157.7, 170.0 (CO), 170.6 (CO); $IR_{cap.film}$ 1730, 1655, 1523, 1345. Anal. ($C_{33}H_{38}N_2O_6$) C, H, N.

4(*R/S*)-7-Amino-2-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (30a). The compound **29a** (560 mg, 1 mmol) was reduced by treatment with ammonium formate (630 mg, 10 mmol), formic acid (460 mg, 10 mmol), and 10% Pd/C (100 mg) in CH_2Cl_2 (50 mL) at room temperature for 1 h. The mixture was filtered, concentrated, and purified by flash chromatography to give compound **30a** (382 mg, 87%): TLC R_f 0.10 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 0.76 (t, J = 7.3 Hz, 3H, Me-Ile), 0.90−0.94 (m, 1H, CH_{2a}-Ile), 0.98 (d, J = 6.4 Hz, 3H, Me-Ile), 1.15−1.22 (m, 1H, CH_{2b}-Ile), 1.50 (s, 9H, *t*-Bu), 2.06 (m, 1H, CH-Ile), 2.79 (dd, J = 13.2, 8.5 Hz, 1H, CH_aBn), 3.04 (dd, J = 13.2, 5.3 Hz, 1H, CH_bBn), 3.70 (dd, J = 8.3, 5.3 Hz, 1H, 4), 4.09 (d, J = 15.8 Hz, 1H, 1′), 4.21 (d, J = 15.8 Hz, 1H, 1″), 5.02 (d, J = 10.7 Hz, 1H, αH-Ile), 6.42−6.50 (m, 3H, 5, 6, 8), 6.64 (d, J = 8.5 Hz, 2H, Bn), 6.73 (d, J = 8.5 Hz, 2H, Bn); ^{13}C-NMR ($CDCl_3$) δ 10.6 (Me-Ile), 15.7 (Me-Ile), 24.6 (CH_2-Ile), 28.1 (*t*-Bu), 32.9 (CH-Ile), 38.5 (CH_2-Bn), 45.8 (4), 50.1 (CH-Ile), 60.8 (1), 81.8 (*t*-Bu), 111.6, 114.6, 115.2, 125.2, 128.8, 129.0, 130.5, 132.5, 144.7, 155.1, 170.0 (CO), 173.3 (CO); IR_{CHCl_3} 1726, 1651. Anal. ($C_{26}H_{34}N_2O_4$) C, H, N.

4(*R/S*)-7-Amino-2-[1(*S*),2(*S*)-1-(*tert*-butoxycarbonyl)-2-methylbutyl]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (30b). The compound **29b** (535 mg, 0.95 mmol) gave **30b** by using the conditions described for compound **30a**: yield 345 mg, 83%; TLC R_f 0.10 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 0.90 (t, J = 7.3 Hz, 3H, Me-Ile), 0.99 (d, J = 6.6 Hz, 3H, Me-Ile), 1.03−1.12 (m, 1H, CH_{2a}-Ile), 1.26−1.36 (m, 1H, CH_{2b}-Ile), 1.34 (s, 9H, *t*-Bu), 1.97 (m, 1H, CH-Ile), 2.86 (dd, J = 13.2, 8.5 Hz, 1H, CH_aBn), 3.07 (dd, J = 13.2, 4.5 Hz, 1H, CH_bBn), 3.74 (dd, J = 8.3, 4.5 Hz, 1H, 4), 4.05 (d, J = 15.5 Hz, 1H, 1′), 4.48 (d, J = 15.6 Hz, 1H, 1″), 4.98 (d, J = 10 Hz, 1H, αH-Ile), 6.40−6.50 (m, 3H, 5, 6, 8), 6.64 (d, J = 8.5 Hz, 2H, Bn), 6.70 (d, J = 8.5 Hz, 2H, Bn); ^{13}C-NMR ($CDCl_3$) δ 10.9 (Me-Ile), 15.7 (Me-Ile), 25.6 (CH_2-Ile), 27.9 (*t*-Bu), 34.0 (CH-Ile), 38.8 (CH_2Bn), 46.3 (4), 59.7 (CH-Ile), 60.6 (1), 81.6 (*t*-Bu), 111.5, 114.6, 115.1, 125.1, 128.9, 129.0, 130.5, 132.5, 144.7, 155.2, 170.2 (CO), 173.0 (CO); IR_{CHCl_3} 1724, 1648. Anal. ($C_{26}H_{34}N_2O_4$) C, H, N.

4(*R/S*)-2-[1(*S*),2(*S*)-1-(*tert*-Butoxycarbonyl)-2-methylbutyl]-7-[(9-fluorenylmethyloxycarbonyl)amino]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (31a). The compound **30a** (220 mg, 0.5 mmol) was treated with Fmoc-Cl (143 mg, 0.55 mmol) in pyridine at room temperature overnight. The reaction mixture was concentrated and purified by flash chromatography to give compound **31a** (282 mg, 86%): TLC R_f 0.21 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ 0.70 (t, J = 7.3 Hz, 3H, Me-Ile), 0.83−0.91 (m, 1H, CH_{2a}-Ile), 0.95 (d, J = 6.6 Hz, 3H, Me-Ile), 1.11 (m, 1H, CH_{2b}-Ile), 1.46 (s, 9H, *t*-Bu), 2.00 (m, 1H, CH-Ile), 2.77 (dd, J = 13.2, 8.8 Hz, 1H, CH_aBn), 3.05 (dd, J = 13.2, 4.9 Hz, 1H, CH_bBn), 3.71 (dd, J = 8.8, 4.9 Hz, 1H, 4), 4.15−4.24 (m, 3H, 1, CH-Fmoc), 4.50 (d, J = 6.0 Hz, 2H, CH_2-Fmoc), 4.99 (d, J = 10.5 Hz, 1H, αH-Ile), 6.50 (d, J = 8.1 Hz, 1H, 5), 6.64 (m, 4H, Bn), 6.81−6.92 (m, 2H, 6, 8), 7.23−7.40 (m, 4H, Fmoc), 7.57 (d, 2H, Fmoc), 7.75 (d, 2H, Fmoc); ^{13}C-NMR ($CDCl_3$) δ 10.5 (Me-Ile), 15.7 (Me-Ile), 24.7 (CH_2-Ile), 28.1 (*t*-Bu), 32.8 (CH-Ile), 38.3 (CH_2-Bn), 45.9 (CH-Fmoc), 47.0 (4), 53.4 (CH-Ile), 60.9 (1), 66.8 (CH_2-Fmoc), 81.8 (*t*-BuO), 115.2, 117.6, 120.0, 124.8, 127.1, 127.8, 128.5, 128.8, 130.1, 130.4, 132.5, 136.3, 141.3, 143.6, 153.5 (CO-Fmoc), 155.1, 169.7 (CO), 172.8 (CO); $IR_{cap.film}$ 3311, 1727, 1629. Anal. ($C_{41}H_{44}N_2O_6 \cdot {}^1/_4H_2O$) C, H, N.

4(*R/S*)-2-[1(*S*),2(*S*)-1-(*tert*-Butoxycarbonyl)-2-methylbutyl]-7-[(9-fluorenylmethyloxycarbonyl)amino]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (31b). The compound **30b** (220 mg, 0.5 mmol) gave **31b** by using the conditions described for compound **31a**: yield 289 mg, 88%; TLC R_f 0.21 (petroleum ether/EtOAc, 2:1); ^{1}H-NMR ($CDCl_3$) δ

0.91 (t, J = 7.3 Hz, 3H, Me-Ile), 1.00 (d, J = 6.6 Hz, 3H, Me-Ile), 1.06−1.15 (m, 1H, CH_{2a}-Ile), 1.24−1.32 (m, 1H, CH_{2b}-Ile), 1.34 (s, 9H, *t*-Bu), 1.97 (m, 1H, CH-Ile), 2.88 (dd, J = 13.2, 8.8 Hz, 1H, CH_aBn), 3.11 (dd, J = 13.2, 4.3 Hz, 1H, CH_bBn), 3.79 (dd, J = 8.4, 4.3 Hz, 1H, 4), 4.10 (d, J = 15.4 Hz, 1H, 1′), 4.25 (t, J = 6.5 Hz, 1H, CH-Fmoc), 4.52 (d, 2H, CH_2-Fmoc), 4.56 (d, 1H, 1″), 5.01 (d, J = 9.8 Hz, 1H, αH-Ile), 6.55 (d, J = 8.3 Hz, 1H, 5), 6.67 (m, 4H, Bn), 6.93 (bd, 1H, 6), 7.04 (s, 1H, 8), 7.30 (t, 2H, Fmoc), 7.41 (t, 2H, Fmoc), 7.61 (d, 2H, Fmoc), 7.78 (d, 2H, Fmoc); ^{13}C-NMR ($CDCl_3$) δ 10.9 (Me-Ile), 15.8 (Me-Ile), 25.7 (CH_2-Ile), 27.8 (*t*-Bu), 34.1 (CH-Ile), 38.6 (CH_2Bn), 46.5 (CH-Fmoc), 47.0 (4), 53.4 (CH-Ile), 60.8 (1), 66.9 (CH_2-Fmoc), 81.7 (*t*-BuO), 115.1, 117.6, 120.0, 124.9, 127.1, 127.7, 128.4, 128.7, 130.0, 130.5, 132.4, 136.3, 141.6, 143.6, 153.5 (CO-Fmoc), 155.2, 169.9 (CO), 172.5 (CO); $IR_{cap.film}$ 3317bm, 1727, 1624. Anal. ($C_{41}H_{44}N_2O_6 \cdot {}^1/_4H_2O$) C, H, N.

4(*R/S*)-2-[1(*S*),2(*S*)-1-Carboxy-2-methylbutyl]-7-[(9-fluorenylmethyloxycarbonyl)amino]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (32a). The compound **31a** (220 mg, 0.33 mmol) was treated with TFA in CH_2Cl_2 at room temperature for 1 h. The reaction mixture was concentrated and purified by flash chromatography to give compound **32a** (178 mg, 89%): TLC R_f 0.66 (EtOH/EtOAc, 1:2); ^{1}H-NMR ($CDCl_3$ + CD_3OD) δ 0.83 (t, J = 7.1 Hz, 3H, Me-Ile), 0.95−1.05 (m, 1H, CH_{2a}-Ile), 0.99 (d, J = 6.6 Hz, 3H, Me-Ile), 1.26 (m, 1H, CH_{2b}-Ile), 2.15 (m, 1H, CH-Ile), 3.04 (m, 2H, CH_2-Bn), 3.80 (dd, J = 7.6, 4.8 Hz, 1H, 4), 3.96 (d, J = 15.8 Hz, 1H, 1′), 4.28 (t, J = 6.3 Hz, 1H, CH-Fmoc), 4.39 (d, 1H, 1″), 4.62 (m, 2H, CH_2-Fmoc), 4.89 (d, J = 10.9 Hz, 1H, αH-Ile), 6.52 (s, 1H, 8), 6.60 (m, 5H, 6, Bn), 6.63 (d, J = 7.0 Hz, 1H, 5), 7.28 (t, 2H, Fmoc), 7.38 (t, 2H, Fmoc), 7.57 (d, 2H, Fmoc), 7.75 (d, 2H, Fmoc); ^{13}C-NMR ($CDCl_3$) δ 10.4 (Me-Ile), 15.8 (Me-Ile), 24.5 (CH_2-Ile), 32.3 (CH-Ile), 40.2 (CH_2Bn), 47.0 (CH-Fmoc), 48.6 (4), 53.4 (CH-Ile), 61.7 (1), 67.0 (CH_2-Fmoc), 114.9, 115.2, 118.2, 120.1, 124.9, 127.8, 128.2, 129.4, 130.6, 132.4, 136.5, 141.3 and 143.6, 155.5 (CO-Fmoc), 173.6 (CO), 174.4 (CO); $IR_{cap.film}$ 3319, 1718, 1613. Anal. ($C_{37}H_{36}N_2O_6 \cdot {}^1/_4CF_3COOH$) C, H, N.

4(*R/S*)-2-[1(*S*),2(*S*)-1-Carboxy-2-methylbutyl]-7-[(9-fluorenylmethyloxycarbonyl)amino]-4-(4-hydroxybenzyl)-1,4-dihydro-3(2*H*)-isoquinolinone (32b). The compound **31b** (220 mg, 0.33 mmol) gave **32b** by using the conditions described for compound **32a**: yield 168 mg, 84%; TLC R_f 0.66 (EtOH/EtOAc, 1:2); ^{1}H-NMR ($CDCl_3$ + CD_3OD) δ 0.83 (t, J = 7.1 Hz, 3H, Me-Ile), 0.95−1.02 (m, 1H, CH_{2a}-Ile), 0.99 (d, J = 6.4 Hz, 3H, Me-Ile), 1.27 (m, 1H, CH_{2b}-Ile), 2.16 (m, 1H, CH-Ile), 3.01 (m, 2H, CH_2-Bn), 3.84 (m, 2H, 1′, 4), 4.24 (m, 2H, 1″, CH-Fmoc), 4.54 (m, 2H, CH_2-Fmoc), 4.99 (d, J = 10.9 Hz, 1H, αH-Ile), 6.53−6.62 (m, 6H, 6, 8, Bn), 6.63 (d, J = 7.0 Hz, 1H, 5), 7.28 (t, 2H, Fmoc), 7.39 (t, 2H, Fmoc), 7.57 (d, 2H, Fmoc), 7.76 (d, 2H, Fmoc); ^{13}C-NMR ($CDCl_3$) δ 10.6 (Me-Ile), 15.7 (Me-Ile), 25.4 (CH_2-Ile), 33.6 (CH-Ile), 39.5 (CH_2Bn), 47.0 (CH-Fmoc), 49.0 (4), 53.4 (CH-Ile, 1), 67.1 (CH_2-Fmoc), 115.2, 120.0, 124.8, 127.8, 128.2, 129.0, 130.6, 131.5, 136.5, 141.3, 143.5, 154.9 (CO-Fmoc), 174.2 (CO), 174.4 (CO); IR_{CHCl_3} 3430, 3302bm, 1728, 1634, 1614. Anal. ($C_{37}H_{36}N_2O_6 \cdot {}^1/_4CF_3COOH$) C, H, N.

Isoquinolinone-Based Ang II Mimetics 5a,b. 5a, Synthesis: The Fmoc-protected template **32a** (29.6 mg, 49 μmol), PyBOP (51.0 mg, 98 μmol), $HOBt \cdot H_2O$ (33.1 mg, 245 μmol), and DIEA (40 μL, 234 μmol) dissolved in DMF (500 μL) were reacted with His(Boc)-Pro-Phe-2-chlorotrityl resin (121 mg, 47.2 μmol) in a 2 mL syringe vessel as described above for the synthesis of **4a.** The coupling was allowed to proceed for 20 h, at which point PDMS analysis of a deprotected resin sample indicated that the reaction had gone to completion. The resin, after washing with DMF (6 × 1 mL), was deprotected with 20% piperidine/DMF (2 × 1 mL, 10 + 20 min) and washed with DMF (6 × 1 mL), CH_2Cl_2 (3 × 1 mL), and ether (3 × 1 mL). After drying in vacuo 129 mg of the partially protected peptide resin were recovered. One-half of this material (64.4 mg, 23.6 μmol) was submitted to coupling with Fmoc-Arg(Pmc)-OH (5 equiv) using PyBOP (5 equiv), $HOBt \cdot H_2O$ (10 equiv), and 4-methylmorpholine (NMM; 15 equiv) for activation. The reaction which was carried out in DMF (500 μL) was allowed to proceed for 19 h. PDMS analysis showed that the coupling was still incomplete. Therefore, the arginine derivative (4 equiv) was recoupled with PyBroP (4 equiv) and DIEA (8 equiv) in DMF (500 μL) for 1 h. After washing with DMF (6 × 1 mL), deprotection (3 × 1 mL, 5 + 5 + 20 min), and washing with DMF (6 × 1 mL), Fmoc-Asp(O^{t}Bu)-OH was introduced by the same double-coupling procedure to yield 65.9 mg of the partially protected peptide resin. MS analysis now indicated complete reaction.

Cleavage: The resin (65.9 mg, 18.3 μmol) was reacted in the syringe with TFA/H_2O/triethylsilane (90:5:5; 1 mL) for 1.5 h, filtered, and rinsed with TFA (3 × 500 μL). The combined filtrates were evaporated in a stream of dry nitrogen to ca. 500 μL, and the product was precipitated by addition of cold ether (12 mL). The precipitate was collected by centrifugation, washed with ether (3 × 4 mL), and dried to yield 19.7 mg of crude product.

Purification: The crude peptide was dissolved in 0.1% aqueous TFA (6 mL) and purified by RP-HPLC on a 10 μm Vydac C18 column (2.2 × 25 cm) using a 60 min gradient of 0−60% MeCN in 0.1% TFA at a flow rate of 4 mL/min. The separation was monitored at 230 nm and by PDMS. Some of the fractions were submitted to rechromatography using the same conditions. The fractions containing the pure product were combined and lyophilized to give 6.2 mg (33% total yield) of **5a**. Amino acid analysis: Asp 1.04, Arg 1.01, His 0.98, Pro 0.96, Phe 1.01 (69% peptide). PDMS (MW 1034.7): 1035.9 (M + H^+), 1057.5 (M + Na^+).

5b: This peptide was prepared from **32b** by the same sequence of reactions. The final yield of **5b** was 3.7 mg (22%). Amino acid analysis: Asp 1.04, Arg 0.91, His 1.02, Pro 0.98, Phe 1.05 (66% peptide). PDMS: 1035.5 (M + H^+), 1057.6 (M + Na^+).

Rat Pituitary AT_1 Receptor Binding Assay. Pituitary membranes containing Ang II AT_1 receptors were prepared from tissues collected from male Sprague−Dawley rats (Alab AB, Sollentuna, Sweden) in a weight range of 200−220 g. The animals were adapted to the environment as previously described.[63] They were decapitated without any prior drug treatments, and the pituitaries were removed and kept on dry ice. Subsequently all glands were transferred to a vessel and homogenized by ultrasonication at 0 °C, in 25 mM Tris-HCl buffer (pH 7.4) containing 10% (w/v) sucrose (50 mL of buffer/g of tissue). The homogenate was centrifuged at 1000*g* for 15 min, and the pellet was collected and suspended in 25 mM Tris-HCl (pH 7.4). All procedures were carried out at 5 °C or on ice, and the suspensions were immediately used or stored at −80 °C.

In the binding assay the incubation mixture contained [^{125}I]-Ang II (10^5 cpm), pituitary membranes, 100 μM bacitracin, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EGTA, and 0.2% BSA buffered at pH 7.4 with 50 mM Tris-HCl in a final volume of 0.5 mL. A presumable interference by binding to AT_2 receptors was blocked by the addition of 1 μM CGP-42211. Binding analysis was also performed in the absence of BSA. Samples were incubated at 25 °C for 1 h, and the reaction was terminated by the addition of 0.5 mL of cold 50 mM Tris-HCl buffer (pH 7.4). The precipitates were collected by centrifugation (10000*g* and 5 °C for 10 min) and counted in a γ-counter. Compounds to be tested were dissolved in the same buffer as above.

Calculation and Binding Constants. After correction for nonspecific binding, the bound radioactivity in the presence of a given concentration of the test compound was compared to that of a control to determine the percent inhibition. The concentration of the test compound required to inhibit the specific binding of [^{125}I]Ang II by 50% (IC_{50}) was calculated using the EBDA-LIGAND computer program (Biosoft Cambridge, U.K.). In the case of binding to pituitary AT_1 sites, the reported IC_{50} values represent an average of at least two determinations from separate assays.

Vascular Contractility Studies. Male New Zealand white rabbits (Sollentuna, Sweden) in a weight range of 2.5−3.5 kg were anesthetized by intravenous injection of pentobarbitone (Mebumal vet, 30 mg/kg of body weight). The thoracic aorta was dissected and placed into prewarmed (38.5 °C) and oxygenated Kreb's bicarbonate buffer. Excessive fat and connective tissue were removed, and the aorta was cut into rings of 2 mm. The segments obtained were mounted in 3 mL organic baths containing Krebs' buffer (120 mM NaCl,

4.75 mM KCl, 2.54 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.19 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11 mM D-(+)-glucose). The buffer was kept at 38.5 °C and pH 7.4 and aerated with 95% O_2 and 5% CO_2. Isometric contraction was recorded using Radnoti force transducers. Initial resting tension was set to 2 g, and the strips were equilibrated for about 90 min. During this time the strips were stimulated once by the addition of Ang II into the baths to give a final concentration of 300 nM. After the maximal contractile response to this peptide was reached, the strips were rinsed and allowed to relax to base-line tension. After equilibration a control cumulative concentration−contractile response curve for Ang II (3×10^{-10}−10^{-7} M) was recorded. Thereafter the aorta strips were repeatedly washed and allowed to return to base-line tension. Subsequent studies were divided in two groups of experiments: one for studies of agonistic properties and the other for studies of antagonistic properties of the test compounds.

In the agonist experiments the contractile response of the test compound was recorded at concentrations of 3×10^{-10}−10^{-5} M to produce a cumulative concentration−response curve in analogy with that of Ang II.

In the antagonist experiments the aorta strips were preincubated (20 min) separately at three fixed concentrations (0.1, 1.0, and 10 μM) of the test compound before a second cumulative concentration−contractile response curve for Ang II was recorded. The results of the agonist/antagonist experiments are expressed as a percentage of maximum control contractile force obtained from the first cumulative concentration−response curve for Ang II.

^{1}H-NMR Spectroscopy of 6−8. Spectra were recorded at 400 MHz with 12−27 mM solutions in DMSO-d_6 at 25−35 °C using a Varian Unity spectrometer. Chemical shifts are indirectly referenced to the residual solvent signal at 2.49 ppm. Coupling constants were extracted from lD proton spectra. Signal assignments were made from DQ-COSY,[64] TOCSY,[65] and ROESY[65] or NOESY[66,67] spectra, which all were run in phase-sensitive mode using the method of States et al.[66] The spectral width was 3200−3700 Hz. Between 16 and 48 transients for 2150f_1 increments with a relaxation delay of 1.5 s following the acquisition time were acquired. Mixing times were set to 60 ms for the TOCSY experiments, and a series of mixing times varying from 0.1 to 0.6 s for each sample was used in the ROESY and NOESY experiments. A time-shared spin−lock field > 4000 Hz was used in the ROESY experiments. The data matrices were zero-filled to 2 × 2K before Fourier transformation.

Conformational Energy Calculations. The calculations of **6**−**8** were performed using the Amber* all atom force field and the all atom charge set as implemented in the program Macromodel version 4.5.[17] The general born solvent accessible surface area (GB/SA) method for water developed by Still[16] was used in all calculations unless otherwise stated. The number of torsion angles allowed to vary simultaneously during each Monte Carlo step ranged from 1 to $n - 1$, where n equals the total number of rotatable bonds ($n = 12$ in **1** and $n = 10$ in **2** and **3**). All torsion angles except the amide bonds, which were set in the trans configuration, were defined as rotatable. Conformational searches was conducted by use of the systematic unbound multiple minimum search (SUMM) method[68] in the batchmin program (command SPMC); 10 000 step runs were performed, and those conformations within 50 kJ/mol of the global minimum were kept. The ring closure bond was defined as the bond between the sulfur atoms. Torsional memory and geometrical preoptimization were used. Truncated Newton conjugated gradient (TNCG) minimization with a maximum of 500 iterations was used in the conformational search with the default derivate convergence set at a value of 0.05 (kJ/mol)/Å. In the subsequent minimization to fully converged structures a maximum of 5000 steps of TNCG minimization was followed by a maximum of 5000 steps of full matrix Newton Raphson (FMNR) minimization with the convergence criteria being 0.001 (kJ/mol)/Å in both runs.

The calculations of model compounds **33** and **34** were performed using the MM2* force as implemented in the program Macromodel version 4.5.[17] Conformational searches was conducted by use of the SUMM method[68] in the batchmin program (command SPMC); 1000 step runs were performed, and those conformations within 50 kJ/mol of the global minimum were kept. Torsional memory and geometrical preoptimization were used. Truncated Newton conjugated gradient (TNCG) minimization with a maximum of 2000 iterations was used in the conformational search with the default derivate convergence set at a value of 0.001 (kJ/mol)/Å.

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Supporting Information Available: Experimental details describing the pharmacological investigation of antagonism of Ang II-induced pressor response in spontaneously hypertensive rats and a table of ^{1}H-NMR chemical shifts for tripeptides **6**−**8** (2 pages). Ordering information is given on any current masthead page.

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(11) To further evaluate compounds **2** and **3**, we studied their antagonistic activity in two pharmacological in vivo models (for experimental details, see Supporting Information). The first model investigated the antihypertensive response in conscious spontaneously hypertensive rats (SHR), using DuP 753 (losartan) as a reference compound. Compound **2** displayed weak antihypertensive effects compared to DuP 753. Compound **3**, however, showed a pronounced hypotensive effect even at very low concentrations (0.001 μmol/kg). Higher concentrations than 0.1 μmol/kg compound **3** could not be given due to adverse cardiac effects. These effects did not seem to be related to parasympatic activity as similar results were obtained in rats pretreated with atropine. In the second model, SHR were constantly infused with Ang I. No dose dependent hypotensive effect was seen for compound **2** or **3**, even at a dose of 10 μmol/kg compound **2** and 1 μmol/kg compound **3**. DuP 753 investigated in the latter in vivo model showed a dose dependent hypotensive effect on the mean arterial blood pressure. The antagonistic/hypotensive effect that compound **3** displays in one of the in vivo

models could be argued as being an effect on the renin angiotensin system as compound **3** did not show any dose dependent hypotensive effect during Ang I infusion. This would rather support the agonistic profile seen in the rabbit aorta strips model.

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