Vinyl Sulfide Cyclized Analogues of Angiotensin II with High Affinity and Full Agonist Activity at the AT_1 Receptor

Petra Johannesson,[†] Gunnar Lindeberg,[†] Anja Johansson,[†] Gregory V. Nikiforovich,[‡] Adolf Gogoll,[§] Barbro Synnergren,[||] Madeleine Le Grèves,[||] Fred Nyberg,[||] Anders Karlén,[†] and Anders Hallberg*[,†]

Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden, Department of Biochemistry and Molecular Biophysics, Washington University, P.O. Box 8036, St. Louis, Missouri 63110, Department of Organic Chemistry, Uppsala University, Box 531, SE-751 21 Uppsala, Sweden, and Department of Pharmaceutical Biosciences, Biological Research on Drug Dependence, Uppsala University, Box 591, SE-751 24 Uppsala, Sweden

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Vinyl sulfide cyclized analogues of the octapeptide angiotensin II that are structurally related to the cyclic disulfide agonist c[$Hcy^{3,5}$]Ang II have been prepared. The synthesis relies on the reaction of the mercapto group of a cysteine residue in position 3 with the formyl group of allysine incorporated in position 5 of angiotensin II. A mixture of the cis and the trans isomers was formed, and these were separated and isolated by RP-HPLC. Thus, the three-atom CH_2−S−S element of the AT_1 receptor agonist c[$Hcy^{3,5}$]Ang II has been displaced by a bioisosteric three-atom S−CH═CH element. A comparative conformational analysis of the 13-membered ring systems of c[$Hcy^{3,5}$]Ang II and the 13-membered cyclic vinyl sulfides with cis and trans configuration, respectively, suggested that all three systems adopted very similar low-energy conformations. This similarity was also reflected in the bioactivity. Both of the compounds that contained the ring systems encompassing the cis or trans vinyl sulfide elements between positions 3 and 5 exhibited K_i values less than 2 nM and exerted full agonism at the AT_1 receptor. In contrast, vinyl sulfide cyclization involving the amino acid residues 5 and 7 rendered inactive compounds. The cyclic vinyl sulfides that have agonist activity were both shown to possess low-energy conformers compatible with the previously proposed 3D model for the bioactive conformation of Ang II.

Introduction

Knowledge of the bioactive conformations of biologically active peptides is invaluable for the understanding of receptor activation and for the stepwise conversion of target peptides into less peptidic analogues. Because of the inherent flexibility of linear peptides in solution and since preferred solution conformations do not necessarily correspond to those adopted when activating the receptor, the direct determination of bioactive conformations is still a formidable endeavor. Thus, in the wait for 3D structural data of peptide/G-protein coupled receptor complexes to become generally available, alternative strategies must be explored. Constrained peptide analogues may provide indirect information about the topological requirements within the peptide−receptor complex. The principle of reducing flexibility and thereby limiting the unfavorable entropy loss upon binding has been widely used.[1] Cyclization is a powerful tool for imposing conformational constraints, and a variety of methods have been employed for the preparation of cyclic peptides.[1−3] Cyclizations by disulfide and amide bond formation are most common, but more elaborate processes, e.g., ring-closing metathesis,[4−10] and the use of thioether,[11−15] dithioether,[16−21] ureido,[22] and saturated aliphatic[23] bridges has also been successfully applied. Importantly, for obtaining receptor activation, it is critical that the cyclization procedure employed enforces orientation of the important side chain elements into the correct regions of the receptor protein. Access to a variety of cyclization methods that allow for conformational fine-tuning should therefore be highly desirable.

Several cyclic analogues of the hypertensive octapeptide angiotensin II (Ang II, **1**, Chart 1) have been prepared.[20,24−33] Among these, the monocyclic disulfides c[$Cys^{3,5}$]Ang II, c[$Cys^{3}Hcy^{5}$]Ang II, c[$Hcy^{3}Cys^{5}$]Ang II, and c[$Hcy^{3,5}$]Ang II (**2**) (Chart 1), with ring sizes from 11 to 13 atoms, all showed high affinity for the AT_1 receptor. While the 11- and 12-membered ring analogues exerted less than 2% of the activity of Ang II, the 13-membered c[$Hcy^{3,5}$]Ang II (**2**) was found to be a full agonist, only 2 times less potent than Ang II itself (pD_2 = 8.48 versus 8.81 for Ang II).[25]

We wanted access to alternative monocyclization methods that would deliver active Ang II analogues encompassing ring systems with slightly different but overall similar conformational properties compared to the disulfides but that are devoid of the redox-sensitive disulfide bridge. Such constrained and active analogues would serve as valuable tools in the ongoing[32−40] search for the bioactive conformation of Ang II. We therefore prepared the 13-membered ring analogues **13** and **14** (Scheme 2) of Ang II, encompassing cis and trans vinyl sulfide bridges between residues 3 and 5. The cyclization was also performed between residues 5 and 7 to give

* To whom correspondence should be addressed. Phone: +46 18 471 4284. Fax: +46 18 471 4976. E-mail: Anders.Hallberg@bmc.uu.se.
[†] Department of Medicinal Chemistry, Uppsala University.
[‡] Washington University.
[§] Department of Organic Chemistry, Uppsala University.
[||] Department of Pharmaceutical Biosciences, Uppsala University.

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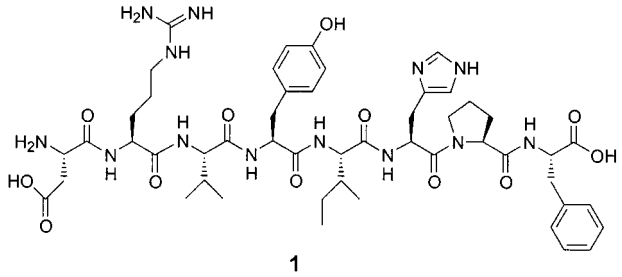
Chart 1



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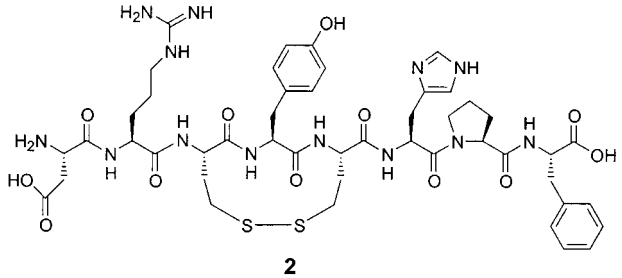

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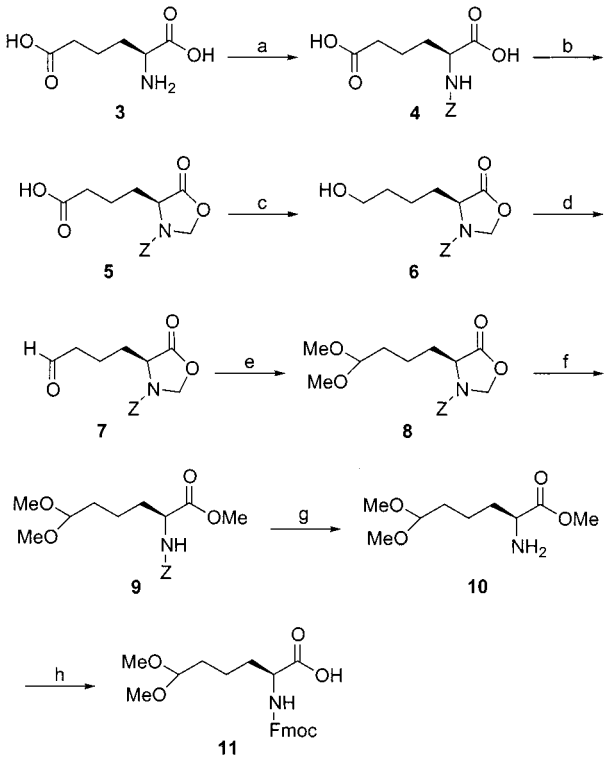
the Ang II analogues **16** and **17** (Scheme 3). The cyclized vinyl sulfides are attractive targets because they can also serve as precursors for further modifications. They were prepared by a novel cyclization reaction using a protected derivative of the *ω*-formyl-α-amino acid allysine[41] as building block.

Results

1. Synthesis. The vinyl sulfide synthesis relies on the reaction of a thiol with the formyl group of allysine. The synthetic route to the dimethylacetal-protected allysine derivative **11**, which is used as a building block for peptide synthesis, is outlined in Scheme 1. This synthesis is analogous to the one we previously reported[31] for the lower homologue, derived from L-glutamic acid. The commercially available L-2-aminoadipic acid (L-Aad) (**3**) was employed as the starting material. The synthesis requires diprotection of the nitrogen to avoid the spontaneous cyclization of the nitrogen onto the aldehyde function.[42−44] Therefore, the nitrogen was protected both with a benzyloxycarbonyl (Z) group and with an oxazolidinone. The latter group was used for the simultaneous protection of the nitrogen as well as of the α-carboxyl group.[45] The resulting compound **5** was then ready for the transformation of the side chain carboxyl function into a formyl group. The conversion of carboxylic acid **5** to aldehyde **7** was performed via reduction with borane dimethyl sulfide to the alcohol **6** and subsequent oxidation with PCC. The resulting aldehyde **7** was protected as the dimethyl acetal **8**. Compound **8** was converted to the free amine **10** through cleavage of the oxazolidinone with sodium methoxide in methanol to give the methyl ester **9**, followed by removal of the benzyloxycarbonyl group by catalytic hydrogenation. The methyl ester of compound **10** was hydrolyzed with potassium hydroxide, and the zwitterion formed was directly treated with Fmoc-Cl and aqueous sodium carbonate in dioxane to give the Fmoc-protected allysine derivative **11**.

The building block **11** was incorporated by solid-phase peptide synthesis (SPPS) into position 5 of the precursor peptide **12**, which also contains a trityl-protected cysteine residue in position 3 (Scheme 2). Upon acidic

Scheme 1[a]



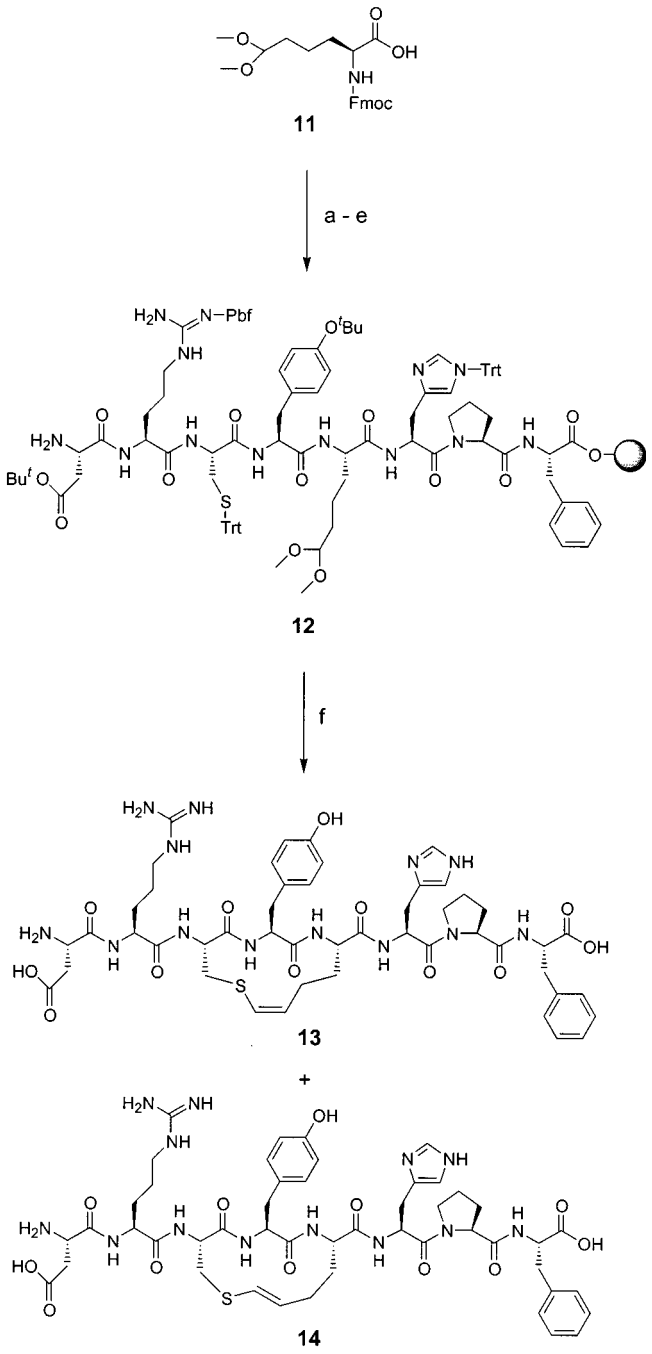
[a] Reagents: (a) Z-Cl, Na_2CO_3, H_2O/dioxane; (b) paraformaldehyde, TsOH, benzene, 67%, over two steps from **3**; (c) $Me_2S{\cdot}BH_3$, THF; (d) PCC, $NaHCO_3$, CH_2Cl_2; (e) TsOH, MeOH, 61%, over three steps from **5**; (f) NaOMe, MeOH; (g) H_2, Pd/C, EtOH, 62%, over two steps from **8**; (h) (i) KOH(aq), MeOH, (ii) Fmoc-Cl, Na_2CO_3, H_2O/dioxane, 70%.

deprotection and cleavage from the resin, cyclization was achieved and the Ang II analogues **13** and **14**, which encompass a vinyl sulfide bridge between positions 3 and 5, were obtained. The cis (**13**) and trans (**14**) isomers were formed in an approximate ratio of 1:2, and the total yield after purification by RP-HPLC was 10%.

The precursor peptide **15**, which incorporates the masked allysine in position 5 and a cysteine now in position 7, was also prepared in order to furnish the 5−7 vinyl sulfide bridged analogues **16** and **17** (Scheme 3). We were not able to separate the cis (**16**) and trans (**17**) isomers by RP-HPLC, but the ratio cis/trans was determined from NMR to be approximately 3:2 and the total isolated yield was 8%.

2. Structural Characterization. Proton NMR signals of the Ang II analogues **13** and **14** were assigned from results from primitive exclusive correlation spectroscopy (PE-COSY),[46] total correlation spectroscopy (TOCSY),[47] and rotating-frame Overhauser enhancement spectroscopy (ROESY)[48] as described previously.[30,49] Connectivity between CH_2 and S−C*H*=CH in the CH_2−S−CH=CH− segment was established by nuclear Overhauser effect (NOE) from H_ϵ of allysine to H_β of cysteine. The cis geometry of the vinyl sulfide double bond (Ang II analogue **13**) was established from the coupling constant $3J_{H\delta-H\epsilon} = 9.2$ Hz, and the trans geometry (Ang II analogue **14**) was established from the coupling constant $3J_{H\delta-H\epsilon} = 15$ Hz. PE-COSY, TOCSY, and ROESY spectra were also recorded for the mixture of Ang II analogues **16** and **17**. Comparison with the spectra from compounds **13** and **14** together with the

Scheme 2[a]

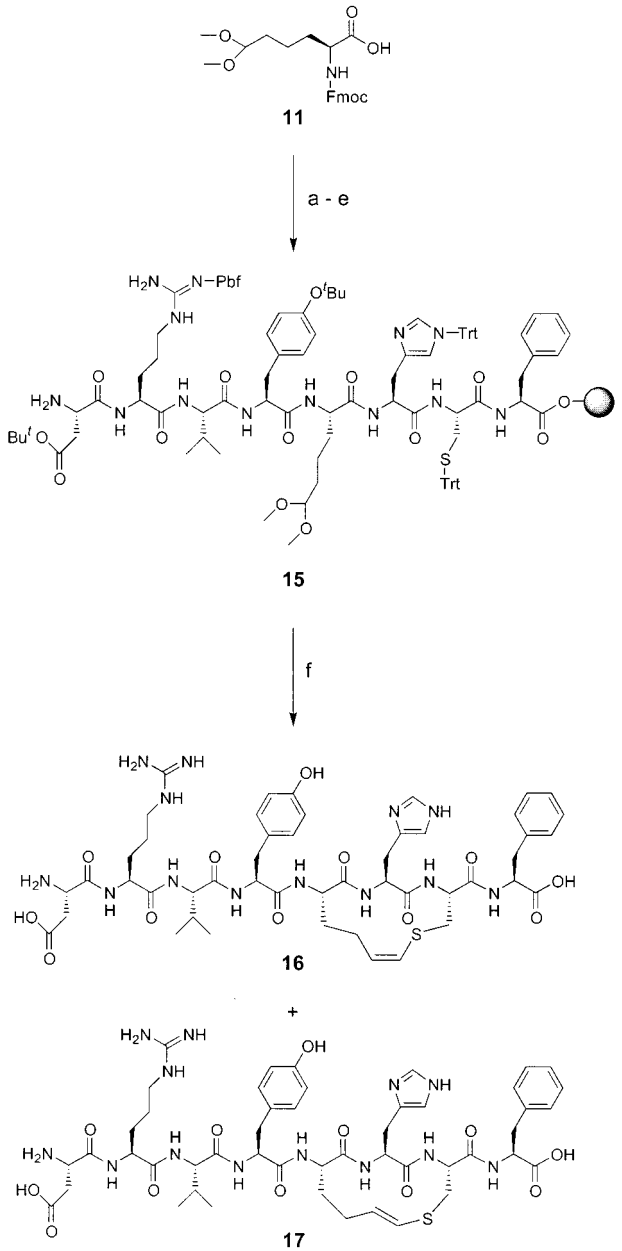


[a] Reagents: (a) (i) His(Trt)-Pro-Phe-Wang resin, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-methylmorpholine (NMM), DMF, (ii) piperidine, DMF; (b) (i) Fmoc-Tyr(ᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (c) (i) Fmoc-Cys(Trt), HBTU, NMM, DMF, (ii) piperidine, DMF; (d) (i) Fmoc-Arg(Pbf), HBTU, NMM, DMF, (ii) piperidine, DMF; (e) (i) Fmoc-Asp(OᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (f) 95% aqueous TFA.

assignment of certain key signals allowed the identification of vinyl sulfides **16** and **17**.

3. Conformational Characterization. 3.a. Theoretical Calculations on Model Tripeptides. The aim of this part of the study was to characterize the new vinyl sulfide ring systems of Ang II analogues **13** and **14** and to compare them to the ring system of c[Hcy^{3,5}]-Ang II (**2**) as well as to a linear peptide such as Ang II. The conformational analysis was performed on the blocked tripeptide model compounds **13m** and **14m** of

Scheme 3[a]



[a] Reagents: (a) (i) His(Trt)-Cys(Trt)-Phe-Wang resin, HBTU, NMM, DMF, (ii) piperidine, DMF; (b) (i) Fmoc-Tyr(ᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (c) (i) Fmoc-Val, HBTU, NMM, DMF, (ii) piperidine, DMF; (d) (i) Fmoc-Arg(Pbf), HBTU, NMM, DMF, (ii) piperidine, DMF; (e) (i) Fmoc-Asp(OᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (f) 95% aqueous TFA.

octapeptides **13** and **14**, respectively (Chart 2). Ac-Ala-Ala-Ala-NMe (**1m**) and Ac-c[Hcy-Ala-Hcy]-NMe (**2m**), which were used as model compounds for Ang II and for the 13-membered ring analogue c[Hcy^{3,5}]Ang II (**2**), were included for comparison. The conformational properties of **1m** and **2m** have recently been described.[20] The computational method that was used was the same as in the present study. We used the Amber force field and the GB/SA water solvation model[50] within Macromodel (version 6.5),[51] and all conformations within 5 kcal/mol of the lowest energy minimum were studied. In addition to these conformations, we also analyzed the conformations between 5 and 10 kcal/mol in order to evaluate whether new conformational families would appear. The number of conformations within 5 (10) kcal/mol of the

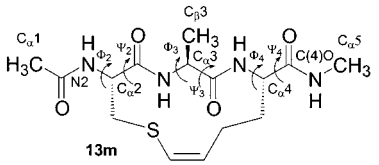
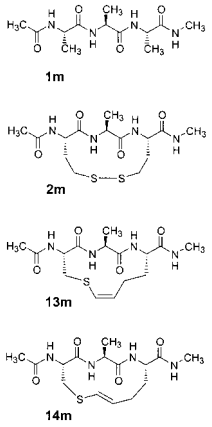


Figure 1. Parameters used to characterize the model compounds, here exemplified for model compound **13m**.

Chart 2



global energy minimum found for model compounds **13m**, **14m**, **1m**, and **2m**, were 36 (139), 22 (109), 20 (148), and 50 (303), respectively. To study whether the cis or trans vinyl sulfide changed the conformational preference of the peptide backbone within the cycle, the Ψ_2, Φ_3, Ψ_3, and Φ_4 torsion angles (Figure 1) were compared. We also investigated the overall effect of the monocyclizations by analyzing the values of the virtual torsion angles $X_1 = (N2-C_\alpha2-C_\alpha3-C_\beta3)$, $X_2 = (C_\beta3-C_\alpha3-C_\alpha4-C(4)O)$, and $X_3 = (N2-C_\alpha2-C_\alpha4-C(4)O)$ (see Figure 1 for notation of the atoms). X_1, X_2, and X_3 describe the relative directions of the incoming backbone, the side chain attached to $C_\alpha3$, and the outgoing backbone with respect to each other.

The results are shown in Figure 2, where the torsion angles of all conformations within 5 kcal/mol of the lowest energy minimum are plotted as red triangles and the conformations between 5 and 10 kcal/mol as blue circles. The Ψ_3, Φ_3 and Ψ_2, Φ_4 plots within 5 and 10 kcal/mol of the lowest energy minimum are very similar for **13m** and **14m.** This indicates that the cis and trans vinyl sulfide groups induce similar backbone conformational properties. When the same plots are compared to those of the 13-membered disulfide **2m**, there is also an overall resemblance. When **13m** and **14m** are compared to the linear **1m**, the picture is also the same except that conformers possessing dihedral angle values around $\Phi3 = 60°$ and $\Phi4 = 60°$ seem to be energetically less favorable for the vinyl sulfides and to some extent also for the disulfide **2m**. It thus appears that for the 13-membered cycles in this study, the backbone torsion angles within the ring are similar.

The X_1-X_3 plots within 5 and 10 kcal/mol of the lowest energy minimum are also similar for **13m** and **14m**. These plots also resemble those of the disulfide analogue **2m**. However, when the X_1, X_2, and X_3 torsion angles of the cyclic analogues are compared to those of the linear **1m**, it becomes evident that the linear **1m** can adopt many additional conformers not available to the cyclic **13m** and **14m** because of the added ring constraints of the cyclic compounds. Almost all conformations of the cyclic analogues have X_1 values between 0° and 180° and X_2 values between 0° and −180°. This is most probably related to the stereochemistry of the C_α-carbon in residues 3−5.

The cis and trans vinyl sulfide cyclized **13m** and **14m** adopt fewer low-energy conformations compared to the disulfide **2m**. However, as deduced from the conformational analysis, the overall conformational preferences of the tripeptides are comparable. The topographical similarity between the vinyl sulfides and the disulfide is also illustrated in Figure 3, where the lowest energy conformations of **13m**, **14m**, and **2m** are superimposed.

3.b. Modeling of Octapeptides. The conformational analysis of the agonistic octapeptides **13** and **14** will be described elsewhere.[52] It has been performed using the previously described buildup strategy[35] and the ECEPP/2 force field.[53,54] Some of the low-energy conformers that were found for the octapeptides **13** and **14** display a good overlap with one of the possible bioactive conformations of Ang II proposed by Nikiforovich and Marshall earlier.[35] This particular bioactive conformation of Ang II has been supported recently by results obtained with a series of potent cyclic Ang II agonists.[52] The conformers of compounds **13** (green) and **14** (magenta) with the best fit to this bioactive conformation of Ang II[35] (white) are shown in Figure 4. As is evident from Figure 4, the general spatial positions of the pharmacophore side chains of Tyr[4], His[6], and Phe[8] as well as the C-terminal carboxyl group overlap very well indeed. The main difference can be found in the $C_\alpha-C_\beta$ bond vector of the Tyr[4] residue. (Note that the buildup procedure employed[52] does not specify low-energy conformations within the accuracy required to distinguish side chain rotamers; however, the particular conformations of compounds **13** and **14** depicted in Figure 4 are of low energy.)

4. Pharmacology. 4.a. In Vitro Binding Affinity. Compounds **13** and **14** and compounds **16** and **17** were evaluated in a radioligand binding assay based on displacement of [^{125}I]Ang II from AT_1 receptors in rat liver membranes[55] (Table 1). Ang II, c[Hcy^{3,5}]Ang II, and the non-peptide AT_1 antagonist DuP 753 (Losartan) were used as reference substances. Analogues **13** and **14** were both found to bind with high affinity, 1.7 nM, to the AT_1 receptor. Compounds **16** and **17**, which were tested as a mixture, did not show any affinity to the receptor.

4.b. Functional Data. Ang II analogues **13** and **14** were evaluated for possible agonistic properties in a vascular contractility study using rabbit aorta (Table 1). Both analogues **13** and **14** behaved as full agonists (Figure 5) and were about 20 and 10 times less active, respectively, than Ang II itself. For both of the compounds the concentration−response curves were potentiated with a maximum effect more than 15% higher than Ang II. The agonistic properties of **13** and **14** were completely blocked upon addition of the non-peptide AT_1 antagonist DuP 753. Compounds **16** and **17** were not tested in the functional assay because they lacked affinity for the AT_1 receptor.

Discussion

ω-Formyl-α-amino acids have frequently been used for the construction of bicyclic thiazabicycloalkane

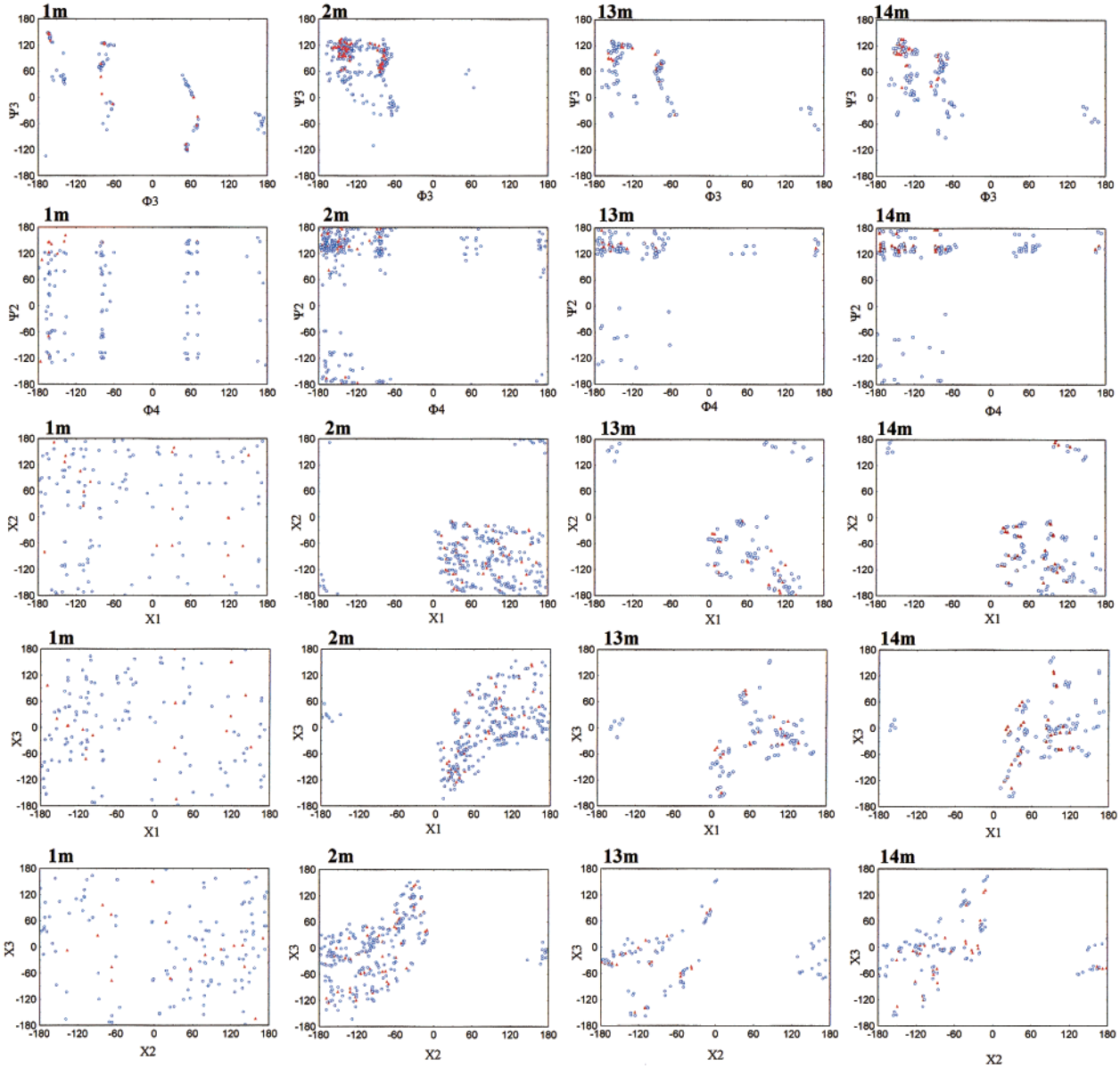


Figure 2. Scatter plots of torsion angles for all conformations below 5 kcal/mol (red triangles) and between 5 and 10 kcal/mol (blue circles) for model compounds **1m**, **2m**, **13m**, and **14m**.

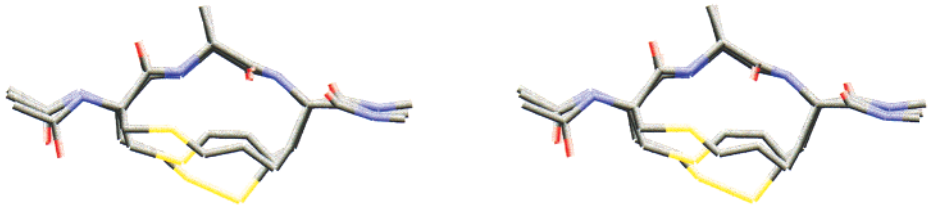


Figure 3. Stereoimage of the rmsd best fit of the lowest energy conformations of **13m** and **14m** to **2m**. N2, $C_\alpha 2$, $C_\alpha 3$, $C_\beta 3$, $C_\alpha 4$, and C(4)O were included in the fitting procedure. The rmsd values were 0.15 Å for **13m** and 0.17 Å for **14m**.

dipeptide units.[56−60] The thiazabicycloalkanes are most often synthesized through reaction of the formyl function of the *ω*-formyl-α-amino acid with a neighboring nitrogen and sulfur atom, followed by intramolecular N-acylation to give bicyclization and to provide a thiazolidine in the final step. We previously reported a spontaneous bicyclization, which delivered tripeptide mimetic thiazabicycloalkanes upon deprotection of octapeptides encompassing masked *ω*-formyl-α-amino acids.[30,31] The regioselectivity of this cyclization could be directed toward either the C-terminal or the N-terminal end of the peptide by simply altering the chain length of the *ω*-formyl-α-amino acid from two to three carbons, as illustrated in Scheme 4. We attributed the driving force for this regioselectivity to the ready formation of five-membered rings in favor of other ring sizes. Robl et al.[57] have used the *ω*-formyl-α-amino acid allysine coupled to homocysteine to accomplish a similar bicyclization via a six-membered *N*-acyliminium ion. They used this to prepare 7,6-fused thiazabicycloalkane dipeptide units. In contrast, when precursor peptides **12** and **15** are deprotected, cyclization to nitrogen to

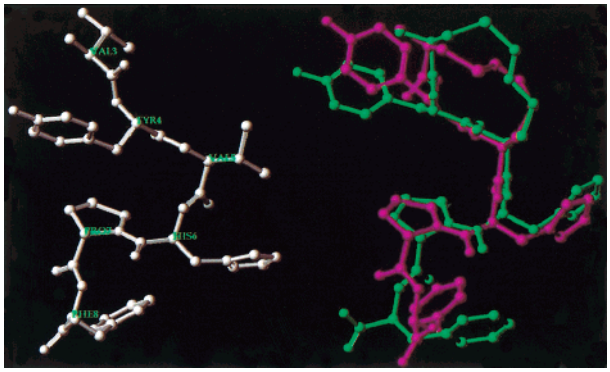


Figure 4. Low-energy conformers (relative energy less than 10 kcal/mol) of compounds **13** (green) and **14** (magenta), with the best fit to the suggested bioactive conformation number II of Ang II[35] (white). Geometrical similarity was assessed by overlapping the C_α and C_β atoms for the Val[3]-Phe[8] fragment of Ang II. The rmsd values were 1.1 and 1.0 Å for **13** and **14**, respectively. Only fragments 3−8 are depicted. All hydrogens are omitted for clarity.

Table 1. In Vitro Rat Liver AT_1 Receptor Binding Affinities and Agonistic Activities in Vascular Contractility Studies on Rabbit Aorta

compound	AT_1 receptor binding affinities K_i (nM) ± SEM	agonistic activities EC_{50} (nM) ± SEM
Ang II	0.31 ± 0.08	1.40 ± 0.28
c[Hcy[3,5]]Ang II	0.23 ± 0.14	
DuP 753	25 ± 4.7	
13	1.7 ± 0.26	26.0 ± 6.79
14	1.7 ± 0.32	16.1 ± 3.52
16	no affinity[a]	
17	no affinity[a]	

[a] Compounds **16** and **17** were tested for binding as a mixture (approximate ratio 3:2). This mixture did not show any affinity below 1 μM.

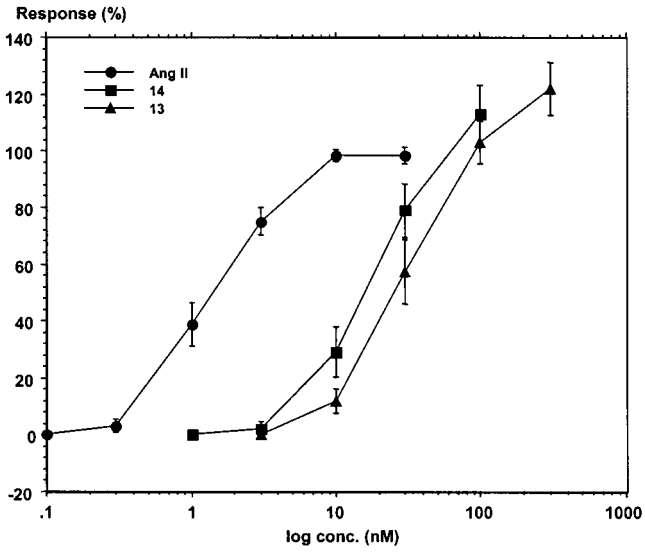
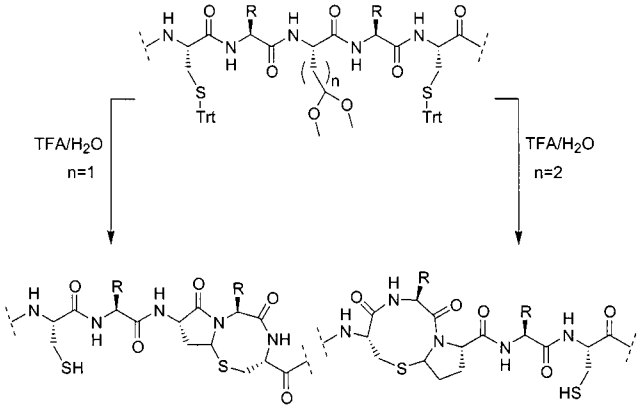


Figure 5. Cumulative concentration−response curves for the contractile effects of Ang II (●), **13** (▲), and **14** (■) in isolated rabbit aorta strips. Values represent the mean ± SEM (n = 11 for Ang II; n = 4 for **13** and **14**).

generate a six-membered ring does not seem to occur, but instead, the formyl function cyclizes to the sulfur atom only. We speculate that the reaction occurs via the formation of hemithioacetal-like compounds that undergo elimination of water to form both the monocyclic cis and trans vinyl sulfide compounds. Crescenza et al.[61] have reported the formation of vinyl sulfides through elimination−addition of enol triflates that occurs via an intermediate allene in their synthesis of monocyclic seven-membered dipeptide units. Besides their work, the formation of vinyl sulfides as a method for the cyclization of peptides has, to the best of our knowledge, not been used previously.

Scheme 4[a]



[a] Previously reported bicyclization procedure.[30,31] The regioselectivity can be directed either toward the C-terminal or N-terminal end of the peptide by altering the chain length of the incorporated masked ω-formyl-α-amino acid from two to three carbons.

We envisioned that the 13-membered vinyl sulfide ring systems would exhibit overall similar properties but with slightly different conformational properties compared to the corresponding disulfide-based ring systems. The vinyl sulfides should therefore exhibit the proper requirements and the potential to be exploited for conformational fine-tuning of structure activity relationships (SARs) of cyclic peptide analogues. From a pharmacokinetic viewpoint it would also be of value to assess whether compounds containing the vinyl sulfide ring systems display higher metabolic stability than the disulfides. Encouraged by the high affinity and agonistic properties reported for the 13-membered Ang II analogue c[Hcy[3,5]]Ang II (**2**),[25] we therefore prepared Ang II analogues **13** and **14** with the cis and trans vinyl sulfide bridges between residues 3 and 5. Indeed, the pharmacological evaluation showed both of these analogues to display high affinities and full agonist properties at the AT_1 receptor. As an additional example of the cyclization method, Ang II analogues **16** and **17**, cyclized between residues 5 and 7, were prepared. The 5−7 region of Ang II has not been as extensively studied as the 3−5 region. With the exception of the bicyclic c[Sar[1], Hcy[5], Mpc[7]]Ang II, which was shown to be a weak partial agonist with 10 times lower affinity than Ang II,[29] cyclization between residues 5 and 7 has only rendered inactive compounds.[29−31] In line with this, the 5−7 cyclized vinyl sulfides **16** and **17** showed no affinities for the rat AT_1 receptor.

We were initially surprised that the conformational differences between the cis and trans vinyl sulfide ring systems were so small, as is shown in the conformational analysis on the tripeptide model compounds. These similarities are, however, further supported by the modeling of octapeptides **13** and **14** as well as by their similar pharmacological profiles. The very similar binding affinities of the cis and trans vinyl sulfide

cyclized octapeptides **13** and **14** indicate that there are no specific interactions between the ring-closed loops and the receptor.

Initially, we also expected that the vinyl sulfides would restrict the conformational freedom to a further extent than is suggested by the conformational analysis. The expected overall topographical similarities of the cis and trans vinyl sulfide ring systems to the corresponding 13-membered disulfide ring system in c[Hcy^{3,5}]-Ang II were, however, confirmed. Hence, it appears that vinyl sulfides can be considered as methylene disulfide bioisosteres, at least in 13-membered cyclized peptide systems. To determine whether vinyl sulfides provide proper surrogates for methylene disulfides in other ring systems, further conformational analyses are required.

Both the agonistic octapeptides **13** and **14** can present the previously proposed bioactive conformation of Ang II,[35] as is shown in Figure 4. Note that the absolute orientation of the aromatic rings of the pharmacophore groups that is displayed in this figure is not confirmed by experimental data. One of the possible ways to address this issue is by the use of analogues with the conformationally restricted side chains, as the β-methyl-Phe[62] and β-methyl-Tyr. The two new Ang II agonists **13** and **14** have also served as valuable tools in computational modeling experiments. This has resulted in a refined proposed model of the bioactive conformation of Ang II.[52]

Conclusion

In summary a new 1−3 cyclization method that delivers cis and trans vinyl sulfides and that provides a complement to the common disulfide cyclizations has been developed. The 13-membered ring systems that are formed adopt low-energy conformations very similar to those from the 1−3 disulfide cyclizations with homocysteine residues, as deduced from conformational analysis. Incorporation of the 13-membered vinyl sulfide ring systems into Ang II produced agonists that were almost as potent as c[Hcy^{3,5}]Ang II. Thus, the vinyl sulfide cyclization should serve as a valuable tool in the fine-tuning of models of bioactive conformations. Although only two examples of 1−3 vinyl sulfide cyclizations are given herein, we believe that the method should be applicable to the cyclization of other short target peptides. The fact that the double bond of vinyl sulfides can in general be functionalized provides a special advantage of the cyclization procedure.

Experimental Section

Chemistry. General Comments. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 at 270 (67.8) MHz or on a JEOL JNM-EX400 or a Varian Unity 400 spectrometer at 400 (100.6) MHz. Spectra were recorded at ambient temperature unless otherwise noted. Chemical shifts are reported as δ values (ppm) referenced to Me_4Si. IR spectra were recorded on a Perkin-Elmer model 1605 FT-IR instrument and are reported as ν_{max} (cm^{-1}). Optical rotations were measured at ambient temperature on a Perkin-Elmer model 241 polarimeter. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden. Flash column chromatography was done using Riedel-de Haën silica gel S (32−63μm). Mass spectroscopy was carried out on an Applied Biosystems (Uppsala, Sweden) BIOION 20 plasma desorption mass spectrometer. Amino acid analyses and peptide content determinations were performed at the Department of Biochemistry, Biomedical Centre, Uppsala, Sweden, on 24 h hydrolyzates with an LKB 4151 alpha plus analyzer, using ninhydrin detection.

Solid-Phase Peptide Synthesis (SPPS). The peptides were synthesized on an 80 μmol scale with a Symphony instrument (Protein Technologies, Inc., Tucson, AZ) using Fmoc/*tert*-butyl protection. The starting polymer was Fmoc-Phe-Wang resin (0.51 mmol/g), and the side chain protecting groups were as follows: Asp(O^{t}Bu), Arg(Pbf), Cys(Trt), Tyr(^{t}Bu), and His(Trt). The Fmoc group was removed by 20% piperidine/DMF using a two-step treatment, 5 + 10 min. Coupling of the amino acids (125 μmol) was done in DMF (2.5 mL) using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (125 μmol) in the presence of *N*-methylmorpholine (NMM) (0.5 mmol). Double couplings (2 × 30 min) were used except for the introduction of compound **11** (single coupling, 1 h). At the end of each coupling cycle, the remaining amino groups were capped by addition of 20% acetic anhydride/DMF (1.25 mL) to the coupling mixture and allowing the reaction to proceed for 5 min. After completion of the synthesis, the Fmoc group was removed and the partially protected peptide resin was washed with several portions of DMF and CH_2Cl_2 and dried in a stream of nitrogen and in vacuo. Yields for the purified Ang II analogues were corrected for peptide content.

L-4-(3-(Benzyloxycarbonyl)-5-oxo-1,3-oxazolidin-4-yl)-butanoic Acid (5).[63−65] Commercially available L-2-aminoadipic acid (L-Aad) (**3**) (7.00 g, 43.4 mmol) was dissolved in a mixture of 10% aqueous Na_2CO_3 (200 mL) and dioxane (200 mL) and cooled to 0 °C. Benzylchloroformate (Z-Cl) (95%) (7.2 mL, 48 mmol) was added dropwise over 10 min, and 10% aqueous Na_2CO_3 was added until pH 9 was attained. The reaction mixture was stirred at 0 °C for 1 h and thereafter was washed with diethyl ether (3 × 400 mL). Diethyl ether (400 mL) was added to the water phase, which was then acidified with 1 M aqueous HCl until pH 2 was attained. The phases were separated, and the water phase was further extracted with diethyl ether (3 × 400 mL). The combined organic layers (4 × 400 mL) were dried ($MgSO_4$) and evaporated to give crude Z−L-Aad (**4**)[66,67] as white crystals (12.1 g, 40.9 mmol). Crude Z−L-Aad (**4**) (6.00 g, 20.3 mmol) was dissolved in benzene (200 mL), and paraformaldehyde (95%) (1.22 g, 38.6 mmol) and *p*-toluenesulfonic acid (monohydrate, 98.5%) (0.230 g, 1.19 mmol) were added. The reaction mixture was refluxed with azeotropic removal of water, using a Dean−Stark apparatus, for 18 h. Most of the benzene was evaporated, and the residue was dissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography (gradient system, CH_2Cl_2 to 2% MeOH in CH_2Cl_2) to give product **5** (4.45 g, 67% over two steps, from compound **3**), as an oil: $[\alpha]_D$ +84.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.56−2.18 (m, 4H, CH_2CH_2), 2.29−2.47 (m, 2H, CH_2), 4.30−4.40 (m, 1H, CH), 5.13−5.27 (m, 3H, $OCH_{2a}N$ and OCH_2Ar), 5.55 (br s, 1H, $OCH_{2b}N$), 7.34−7.41 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$) δ 19.4, 29.9, 33.1 ($CH_2CH_2CH_2$), 54.5 (CH), 68.0 (OCH_2Ar), 77.9 (OCH_2N), 128.2, 128.5, 128.6 (CH Ar), 135.1 (ipso Ar), 152.9 (CO Z), 172.0, 178.5 (C-1, C-5); IR (neat) 3200, 1802, 1724. Anal. ($C_{15}H_{17}NO_6$) C, H, N.

L-3-(Benzyloxycarbonyl)-4-(4-hydroxybutyl)-1,3-oxazolidin-5-one (6).[63] Compound **5** (4.11 g, 13.4 mmol) was dissolved in dry THF (160 mL) under N_2 atmosphere and was cooled to 0 °C. Borane−methyl sulfide complex (2.0 M in THF, 7.0 mL, 14 mmol) was added dropwise over 15 min, whereafter the reaction mixture was slowly allowed to reach room temperature and was then stirred for 18 h. Concentration afforded crude **6** (4.31 g) as a white foam that could be used in the next step without further purification. An analytical sample was prepared by purification by flash column chromatography (gradient system, CH_2Cl_2 to 1% MeOH in CH_2Cl_2): $[\alpha]_D$ +86.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.23−1.75 (m, 5H, CH_2CH_2 and OH), 1.82−2.17 (m, 2H, CH_2), 3.61 (br s, 2H, CH_2OH), 4.25−4.40 (m, 1H, CH), 5.12−5.28 (m, 3H, $OCH_{2a}N$ and OCH_2Ar), 5.51 (br s, 1H, $OCH_{2b}N$), 7.34−7.41 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$) δ 20.4, 30.2, 31.8 ($CH_2CH_2CH_2$), 54.7 (CH),

61.9 (CH_2OH), 67.7 (OCH_2Ar), 77.8 (OCH_2N), 128.1, 128.4, 128.5 (CH Ar), 135.2 (ipso Ar), 152.7 (CO Z), 172.3 (C-5); IR (neat) 3448, 1798, 1715 cm^{-1}. Anal. ($C_{15}H_{19}NO_5$) C, H, N.

L-4-(3-(Benzyloxycarbonyl)-5-oxo-1,3-oxazolidin-4-yl)-butanal (7).[65] To a solution of alcohol **6** (860 mg, 2.93 mmol) in CH_2Cl_2, Celite (1.80 g), PCC (1.84 g, 8.37 mmol), and solid $NaHCO_3$ (0.29 g, 3.45 mmol) were added. The reaction mixture was stirred at room temperature for 5 h and thereafter filtered through a plug of SiO_2, using EtOAc/petroleum diethyl ether 1:1 as eluent. Concentration afforded crude aldehyde **7** (660 mg, 2.27 mmol) as a colorless oil that could be used in the next step without further purification. An analytical sample was prepared by purification by flash column chromatography (gradient system, CH_2Cl_2 to 2% MeOH in CH_2Cl_2): $[\alpha]_D$ +94.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.54−2.17 (m, 4H, CH_2CH_2), 2.38−2.59 (m, 2H, CH_2), 4.30−4.40 (m, 1H, CH), 5.13−5.30 (m, 3H, $OCH_{2a}N$ and OCH_2Ar), 5.55 (br s, 1H, $OCH_{2b}N$), 7.34−7.42 (m, 5H, Ar), 9.72 (br s, 1H, CHO); ^{13}C NMR ($CDCl_3$) δ 16.7, 29.7, 42.8 ($CH_2CH_2CH_2$), 54.4 (CH), 67.8 (OCH_2Ar), 77.8 (OCH_2N), 128.1, 128.4, 128.5 (CH Ar), 135.1 (ipso Ar), 152.7 (CO Z), 171.9 (C-5), 201.0 (CHO); IR (neat) 2728, 1799, 1715 cm^{-1}. Anal. ($C_{15}H_{17}NO_5$) C, H, N.

L-3-(Benzyloxycarbonyl)-4-(4,4-dimethoxybutyl)-1,3-oxazolidin-5-one (8). Aldehyde **7** (460 mg, 1.58 mmol) and *p*-toluenesulfonic acid (monohydrate, 98.5%) (15 mg, 78 μmol) were dissolved in MeOH (30 mL). After the mixture was stirred at room temperature for 2.5 h, most of the solvent was evaporated and the residue was partitioned between EtOAc (50 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic phase was washed with brine (20 mL), dried ($MgSO_4$), and concentrated. Purification by flash column chromatography (gradient system, CH_2Cl_2 to 2% MeOH in CH_2Cl_2) gave the product **8** as a colorless oil (383 mg, 1.14 mmol, 61% over three steps, from compound **5**): $[\alpha]_D$ +81.4° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.22−1.70 (m, 4H, CH_2CH_2), 1.80−2.13 (m, 2H, CH_2), 3.29 (s, 6H, $CH(OCH_3)_2$), 4.24−4.38 (m, 2H, CH and $CH(OCH_3)_2$), 5.13−5.27 (m, 3H, $OCH_{2a}N$ and OCH_2Ar), 5.54 (br s, 1H, $OCH_{2b}N$), 7.32−7.40 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$) δ 19.3, 30.3, 31.9 ($CH_2CH_2CH_2$), 52.6, 52.8 ($CH(OCH_3)_2$), 54.7 (CH), 67.8 (OCH_2Ar), 77.8 (OCH_2N), 103.9 ($CH(OCH_3)_2$), 128.2, 128.5, 128.6 (CH Ar), 135.2 (ipso Ar), 152.8 (CO Z), 172.1 (C-5); IR (neat) 1802, 1716 cm^{-1}. Anal. ($C_{17}H_{23}NO_6$) C, H, N.

L-2-(Benzyloxycarbonylamino)-6,6-dimethoxyhexanoic Acid Methyl Ester (9). Dimethylacetal **8** (4.70 g, 13.9 mmol) was dissolved in dry MeOH (300 mL) under N_2 atmosphere and cooled to −12 °C. Sodium methoxide (95%) (790 mg, 13.9 mmol) was suspended in MeOH (300 mL) and added dropwise over 1 h, whereafter the reaction mixture was slowly allowed to reach room temperature. After the mixture was stirred for 6 h, 10% aqueous citric acid was added until pH 7 was attained and then half of the solvent was evaporated. The residue was poured into EtOAc (400 mL) and washed with 20% aqueous NaCl (2 × 250 mL). The water phases were further extracted with EtOAc (2 × 250 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give **9** as a colorless oil (4.23 g, 12.5 mmol). Compound **9** was used in the next step without further purification. An analytical sample was prepared through purification by flash column chromatography (gradient system, CH_2Cl_2 to 1.5% MeOH in CH_2Cl_2): $[\alpha]_D$ +7.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.25−1.45 (m, 2H, CH_2), 1.50−1.92 (m, 4H, CH_2 and CH_2), 3.29 (s, 6H, $CH(OCH_3)_2$), 3.73 (s, 3H, $COOCH_3$), 4.32 (dd, J = 5.7, 5.7 Hz, 1H, H-6), 4.33−4.43 (m, 1H, H-2), 5.10 (s, 2H, OCH_2Ar), 5.39 (br d, J = 8.1 Hz, 1H, NH), 7.28−7.40 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$) δ 20.2, 31.7, 32.1 ($CH_2CH_2CH_2$), 52.1 ($COOCH_3$), 52.5, 52.6 ($CH(OCH_3)_2$), 54.2 (C-2), 66.7 (OCH_2Ar), 103.9 (C-6), 127.9, 128.0, 128.3 (CH Ar), 136.1, (ipso Ar), 155.7 (CO Z), 172.7 (C-1); IR (neat) 3375, 1732 cm^{-1}. Anal. ($C_{17}H_{25}NO_6$) C, H, N.

L-2-Amino-6,6-dimethoxyhexanoic Acid Methyl Ester (10).[57,68−72] Compound **9** (3.32 g, 9.78 mmol) and 10% Pd/C (550 mg, 517 μmol) were mixed in absolute EtOH (170 mL) and stirred under H_2 (1 atm) at room temperature for 2.5 h. The mixture was filtered through Celite and concentrated. The residue was purified by flash column chromatography (gradient system, CH_2Cl_2 to 3.5% MeOH in CH_2Cl_2) to give the product **10** (1.38 g, 62% over two steps, from compound **8**) as a colorless oil: $[\alpha]_D$ +16.4° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.36−1.82 (m, 8H, $CH_2CH_2CH_2$ and NH_2), 3.30 (s, 6H, $CH(OCH_3)_2$), 3.44 (dd, J = 5.4, 7.3 Hz, 1H, H-2), 3.72 (s, 3H, $COOCH_3$), 4.35 (dd, J = 5.4, 5.4 Hz, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 20.6, 32.0, 34.5 ($CH_2CH_2CH_2$), 51.8 ($COOCH_3$), 52.6 (2C, $CH(OCH_3)_2$), 54.2 (C-2), 104.1 (C-6), 176.3 (C-1); IR (neat) 3375, 1732 cm^{-1}. Anal. ($C_9H_{19}NO_4$) C, H, N.

L-2-((9-Fluorenylmethoxycarbonyl)amino)-6,6-dimethoxyhexanoic Acid (11). To compound **10** (100 mg, 487 μmol) dissolved in MeOH (6 mL) was added 1 M aqueous KOH (490 μL, 490 μmol), and the mixture was stirred at room temperature for 15 h. Then 10% aqueous citric acid was added until pH 6−7 was attained, and the reaction mixture was concentrated to give a solid residue. This residue was dissolved in a mixture of 10% aqueous Na_2CO_3 (10 mL) and dioxane (5 mL) and cooled to 0 °C. Fmoc-Cl (97%) (190 mg, 712 μmol) dissolved in dioxane (5 mL) was added dropwise, whereafter the reaction mixture was allowed to reach room temperature. The pH was kept around 10−11. Stirring at room temperature was continued for 48 h. Then 10% aqueous citric acid was added until pH 8 was attained, and the reaction mixture was washed with diethyl ether (4 × 50 mL). Diethyl ether (150 mL) was added to the water phase, which was then acidified to pH 3 with 10% aqueous citric acid, under vigorous stirring. The phases were separated, and the water phase was further extracted with diethyl ether (2 × 150 mL). The combined organic layers (3 × 150 mL) were washed with water (2 × 200 mL), dried ($MgSO_4$), and concentrated to give the building block **11** (141 mg, 70%) as a white foam: $[\alpha]_D$ +8.7° (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.35−2.01 (m, 6H, $CH_2CH_2CH_2$), 3.32 (s, 6H, $CH(OCH_3)_2$), 4.23 (dd, J = 6.9, 6.9 Hz, 1H, CH Fmoc), 4.35−4.53 (m, 4H, H-2, H-6 and CH_2 Fmoc), 5.42 (br d, J = 8.1 Hz, 1H, NH), 7.32 (m, 2H, Ar Fmoc), 7.41 (m, 2H, Ar Fmoc), 7.58−7.63 (m, 2H, Ar Fmoc), 7.77 (m, 2H, Ar Fmoc); ^{13}C NMR ($CDCl_3$) δ 20.3, 31.8, 31.9 ($CH_2CH_2CH_2$), 47.0 (CH Fmoc), 52.6, 52.7 ($CH(OCH_3)_2$), 53.5 (C-2), 66.9 (CH_2 Fmoc), 104.1 (C-6), 119.9, 125.0, 127.0, 127.6 (CH Ar Fmoc), 141.2, 143.6, 143.7 (ipso Fmoc), 156.1 (CO Z), 176.0 (C-1); IR (neat) 3320, 1715. Anal. ($C_{23}H_{27}NO_6 \cdot 1.5H_2O$) C, H, N.

Ang II Analogues 13 and 14. Automated SPPS according to the general procedure, starting with 159 mg (81.1 μmol) Fmoc-Phe-Wang resin, produced 285 mg of the partially protected peptide polymer (weight increase corresponding to 97% yield). Part of the resin (256 mg, 72.0 μmol) was cleaved and deprotected with 95% aqueous TFA (4 mL) for 1.5 h. The resin was filtered off and washed with 5% triethylsilane/TFA (3 × 400 μL). An additional portion of triethylsilane (50 μL) was added in order to decolorize the filtrate. After 20 min the peptide was precipitated with cold diethyl ether (40 mL), collected by centrifugation, washed with diethyl ether (4 × 15 mL), and dried to yield 81.0 mg. The crude product was divided into five aliquots, each dissolved in 0.1% aqueous TFA containing 7% MeCN (4.3 mL), and chromatographed on a Vydac 10 μm C18 column (1 cm × 25 cm) using a 60 min gradient of 15−45% MeCN in 0.1% aqueous TFA at a flow rate of 3 mL/min. The separation was monitored at 230 nm and by plasma desorption mass spectrometry (PDMS). The fractions corresponding to the two major peaks both contained material of the expected mass. **13**: 2.6 mg (3.4%); PDMS (MW 1046.1) 1047.9 ($M + H^+$); amino acid analysis Asp, 1.02; Arg, 0.96; Tyr, 0.97; His, 1.01; Pro, 1.02; Phe, 1.01; ^1H NMR (DMSO-d_6, 30 °C, 400 MHz) δ 1.46 (m, 2H, Hγ'/Hγ-Arg), 1.49 (m, 1H, Hβ'-Arg), 1.58 (m, 1H, Hβ-Arg), 1.66 (m, 1H, Hβ'-allysine), 1.77 (m, 1H, Hβ-allysine), 1.79 (m, 1H, Hβ'-Pro), 1.81 (m, 2H, Hγ'/Hγ-Pro), 1.89 (m, 1H, Hγ'-allysine), 2.04 (m, 1H, Hβ-Pro), 2.21 (m, 1H, Hγ-allysine), 2.64 (m, 1H, Hβ'-Tyr), 2.66 (m, 1H, Hβ'-Asp), 2.82 (m, 1H, Hβ-Asp), 2.86 (m, 1H, Hβ'-His), 2.88 (m, 1H, Hβ-Tyr), 2.90 (m, 2H, Hβ/Hβ'-Cys), 2.94 (m, 1H, Hβ'-Phe), 3.03 (m, 1H, Hβ-His), 3.04 (m, 1H, Hβ-Phe), 3.09 (m, 2H, Hδ/Hδ'-Arg), 3.45 (m, 1H, Hδ'-Pro), 3.56 (m, 1H, Hδ-Pro), 4.11 (m, 1H, Hα-Asp), 4.25 (m, 1H, Hα-allysine), 4.33 (m, 1H, Hα-

Arg), 4.38 (m, 1H, Hα-Pro), 4.42 (m, 1H, Hα-Phe), 4.51 (m, 1H, Hα-Cys), 4.51 (m, 1H, Hα-Tyr), 4.76 (m, 1H, Hα-His), 5.46 (m, 1H, Hδ-allysine), 5.89 (d, J = 9.2 Hz, 1H, Hε-allysine), 6.61 (m, 2H, meta-Tyr), 6.96 (m, 2H, ortho-Tyr), 7.17−7.27 (m, 5H, Phe), 7.32 (m, 1H, H4-His), 7.63 (d, J = 7.3 Hz, 1H, NH-allysine), 7.64 (m, 1H, NHε-Arg), 7.97 (d, J = 7.7 Hz, 1H, NH-His), 8.02 (d, J = 7.3 Hz, 1H, NH-Cys), 8.28 (d, J = 7.6 Hz, 1H, NH-Phe), 8.52 (d, J = 8.9 Hz, 1H, NH-Tyr), 8.58 (d, J = 7.8 Hz, 1H, NH-Arg), 8.85 (m, 1H, H2-His). **14**: 4.9 mg (6.5%); PDMS 1047.2 ($M + H^+$); amino acid analysis Asp, 1.04; Arg, 1.01; Tyr, 0.98; His, 1.00; Pro, 0.98; Phe, 0.99; ^{1}H NMR (DMSO-d_6, 30 °C, 400 MHz) δ 1.47 (m, 3H, Hβ′-Arg, Hγ′/Hγ-Arg), 1.58 (m, 2H, Hβ/Hβ′-allysine), 1.59 (m, 1H, Hβ-Arg), 1.78 (m, 3H, Hβ′-Pro, Hγ′/Hγ-Pro), 2.02 (m, 1H, Hβ-Pro), 2.08 (m, 2H, Hγ′/Hγ-allysine), 2.64 (m, 1H, Hβ′-Tyr), 2.66 (m, 1H, Hβ′-Asp), 2.82 (m, 1H, Hβ-Asp), 2.84 (m, 1H, Hβ′-Cys), 2.86 (m, 1H, Hβ-Tyr), 2.88 (m, 1H, Hβ′-His), 2.90 (m, 1H, Hβ-Cys), 2.93 (m, 1H, Hβ′-Phe), 3.03 (m, 1H, Hβ-Phe), 3.04 (m, 1H, Hβ-His), 3.07 (m, 2H, Hδ/Hδ′-Arg), 3.48 (m, 1H, Hδ′-Pro), 3.57 (m, 1H, Hδ-Pro), 4.11 (m, 1H, Hα-Asp), 4.24 (m, 1H, Hα-allysine), 4.33 (m, 1H, Hα-Arg), 4.39 (m, 1H, Hα-Pro), 4.43 (m, 1H, Hα-Phe), 4.44 (m, 1H, Hα-Cys), 4.48 (m, 1H, Hα-Tyr), 4.75 (m, 1H, Hα-His), 5.41 (ddd, J = 7.3, 7.3, 15.0 Hz, 1H, Hδ-allysine), 5.75 (d, J = 15.0 Hz, 1H, Hε-allysine), 6.60 (m, 2H, meta-Tyr), 6.97 (m, 2H, ortho-Tyr), 7.17−7.27 (m, 5H, Phe), 7.33 (m, 1H, H4-His), 7.64 (m, 1H, NHε-Arg), 7.64 (d, J = 7.8 Hz, 1H, NH-allysine), 7.93 (d, J = 7.4 Hz, 1H, NH-Cys), 8.16 (d, J = 7.7 Hz, 1H, NH-His), 8.27 (d, J = 7.7 Hz, 1H, NH-Phe), 8.58 (d, J = 7.7 Hz, 1H, NH-Arg), 8.66 (d, J = 8.6 Hz, 1H, NH-Tyr), 8.87 (m, 1H, H2-His).

Ang II Analogues 16 and 17. The partially protected peptide resin was recovered in 98% yield according to the measured increase in mass. Part of the resin (281 mg, 79.1 μmol) was cleaved and deprotected as described for **13** and **14** to yield 88.3 mg of the crude product. The peptide was divided into five aliquots and purified as above. The peaks corresponding to products **16** and **17** were not resolved, and their fractions were therefore collected together. (**16** + **17**): 7.0 mg (8.4%); PDMS (MW 1048.0) 1049.8 ($M + H^+$); amino acid analysis Asp, 1.03; Arg, 0.99; Val, 0.98; Tyr, 0.97; His, 1.02; Phe, 1.00. ^{1}H NMR of the mixture of **16** + **17** indicated the formation of vinyl sulfides by analogy with **13** + **14**, by observation of the following signals. ^{1}H NMR (DMSO-d_6, 30 °C, 400 MHz) δ 5.34 (ddd, J = 7.1, 7.1, 15.1 Hz, 1H, Hδ-allysine, trans isomer), 5.55 (m, 1H, Hδ-allysine, cis isomer), 5.97 (d, J = 15.1 Hz, 1H, Hε-allysine, trans isomer), 5.97 (d, J = 9.2 Hz, 1H, Hε-allysine, cis isomer); cis/trans ratio = 63:37.

Rat Liver Membrane AT_1-Receptor Binding Assay. Rat liver membranes were prepared according to the method of Dudley et al.[55] Binding of [^{125}I]Ang II to membranes was conducted in a final volume of 0.5 mL of 50 mM Tris-HCl (pH 7.4), supplemented with 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.025% bacitracin, and 0.2% BSA and containing rat liver homogenate corresponding to 5 mg of the original tissue weight, [^{125}I]Ang II (0.036 nM), and variable concentrations of the test substance. Samples were incubated at 25 °C for 1 h, and binding was terminated by filtration through Whatman GF/B glass-fiber filter sheets, using a Brandel cell harvester. The filters were washed with 4 × 2 mL of Tris-HCl (pH 7.4) and transferred to tubes. The radioactivity was measured in a γ counter. Nonspecific binding was determined in the presence of 1 μM Ang II. All experiments were performed in triplicate except for Ang II, which was performed in quadruplicate. K_i values were calculated using the Cheng−Prusoff equation (K_d = 1.1 ± 0.08 nM, [L] = 0.036 nM).

Functional Assay, Vascular Contractility Studies on Rabbit Aorta. Male New Zealand white rabbits weighing 2.5−3.5 kg were killed by a blow to the head. The thoracic aorta was excised immediately and placed in 38 °C oxygenated Kreb's bicarbonate buffer of the following composition: NaCl 120 mM, KCl 4.75 mM, $CaCl_2$ 2.54 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.19 mM, $NaHCO_3$ 25 mM, and D-(+)-glucose 11 mM. The aorta was rinsed of blood, and the connective tissue was removed before the aorta was cut into 3 mm segments and mounted in water-jacketed organ baths (3 mL) containing Kreb's bicarbonate buffer, maintained at 38 °C and oxygenated with a gas mixture of 93.5% O_2 and 6.5% CO_2. One end of the aorta strips was anchored to a stationary support; the other end was connected to an isometric force transducer (Radnoti Glass Technology, Inc.), and the isometric contractions were recorded on an ink-writing recorder. The aorta strips were stretched stepwise to a resting tension of 2 g, which was maintained throughout the experiment, and were then allowed to equilibrate for 60 min. After equilibration a control cumulative concentration−contractile response curve for Ang II (3×10^{-10} to 10^{-7} M) was recorded. Thereafter, the aorta strips were repeatedly washed and allowed to return to baseline tension. When stabilized, the contractile response of the test compounds was recorded at concentrations of 3×10^{-10} to 10^{-6} M to produce a cumulative concentration−response curve in analogy with that of Ang II. The results of the experiments are expressed as a percentage of maximum control contractile force obtained from the first cumulative concentration−response curve for Ang II.

Conformational Energy Calculations of Model Tripeptides. The calculations of **13m** and **14m** were performed using the Amber* all atom force field as implemented in the program Macromodel 6.5.[51] The general Born solvent-accessible surface area (GB/SA) method for water developed by Still[50] was used in all calculations. 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 = 8 for **13m** and **14m**). Amide bonds were fixed in the trans configuration. Conformational searches were conducted by use of the systematic unbound multiple minimum search (SUMM) method[73] in the batchmin program (command SPMC); 20 000-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 C_β and C_γ atoms of the side chain of allysine. Torsional memory and geometrical preoptimization were used. PR conjugate gradient (PRCG) minimization with a maximum of 5000 iterations was used in the conformational search with the derivative convergence set to 0.05 (kJ/mol)/Å. In the subsequent minimization to fully converged structures, a maximum of 5000 steps of PRCG minimization was followed by a maximum of 5000 steps of TNCG minimization with the convergence criteria set to 0.001 (kJ/mol)/Å in both runs.

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References

(1) Hruby, V. J. Conformational Restrictions of Biologically Active Peptides via Amino Acid Side Chain Groups. *Life Sci.* **1982**, *31*, 189−199.
(2) Toniolo, C. Conformationally Restricted Peptides through Short-Range Cyclizations. *Int. J. Pept. Protein Res.* **1990**, *35*, 287−300.
(3) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. The Synthesis of Cyclic Peptides. *J. Chem. Soc., Perkin Trans. 1* **2001**, 471−484.
(4) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. Application of Ring-Closing Metathesis to the Synthesis of Rigidified Amino Acids and Peptides. *J. Am. Chem. Soc.* **1996**, *118*, 9606−9614 and references therein.
(5) Ripka, A. S.; Bohacek, R. S.; Rich, D. H. Synthesis of Novel Cyclic Protease Inhibitors Using Grubbs Olefin Metathesis. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 357−360.
(6) Piscopio, A. D.; Miller, J. F.; Koch, K. Ring Closing Metathesis in Organic Synthesis: Evolution of a High Speed, Solid Phase Method for the Preparation of β-Turn Mimetics. *Tetrahedron* **1999**, *55*, 8189−8198.
(7) Fink, B. E.; Kym, P. R.; Katzenellenbogen, J. A. Design, Synthesis, and Conformational Analysis of a Proposed Type I β-Turn Mimic. *J. Am. Chem. Soc.* **1998**, *120*, 4334−4344.
(8) Reichwein, J. F.; Versluis, C.; Liskamp, R. M. J. Synthesis of Cyclic Peptides by Ring-Closing Metathesis. *J. Org. Chem.* **2000**, *65*, 6187−6195 and references therein.
(9) Prabhakaran, E. N.; Rajesh, V.; Dubey, S.; Iqbal, J. Synthesis of Cyclic Peptides as Mimics for the Constrained Conformation of Structural Analogues of HIV Protease Inhibitors. *Tetrahedron Lett.* **2001**, *42*, 339−342.

(10) Creighton, C. J.; Reitz, A. B. Synthesis of an Eight-Membered Cyclic Pseudo-Dipeptide Using Ring Closing Metathesis. *Org. Lett.* **2001**, *3*, 893−895.
(11) Rudinger, J.; Jošt, K. A Biologically Active Analogue of Oxytocin Not Containing a Disulfide Group. *Experientia* **1964**, *20*, 570−571.
(12) Yu, L.; Lai, Y.; Wade, J. V.; Coutts, S. M. A Simple and Efficient Method for the Syntheses of Thioether Cyclic Peptides. *Tetrahedron Lett.* **1998**, *39*, 6633−6636 and references therein.
(13) Feng, Y.; Pattarawarapan, M.; Wang, Z.; Burgess, K. Solid-Phase S_N2 Macrocyclization Reactions To Form β-Turn Mimics. *Org. Lett.* **1999**, *1*, 121−124.
(14) Li, H.; Jiang, X.; Howell, S. B.; Goodman, M. Synthesis, Conformational Analysis and Bioactivity of Lan-7, a Lanthionine Analog of TT-232. *J. Pept. Sci.* **2000**, *6*, 26−35.
(15) Li, H.; Jiang, X.; Goodman, M. Synthesis, Conformational Analysis and Biological Activities of Lanthionine Analogs of a Cell Adhesion Modulator. *J. Pept. Sci.* **2001**, *7*, 82−91.
(16) Mosberg, H. I.; Omnaas, J. R. Dithioether-Containing Cyclic Peptides. *J. Am. Chem. Soc.* **1985**, *107*, 2986−2987.
(17) Szewczuk, Z.; Rebholz, K. L.; Rich, D. H. Synthesis and Biological Activity of New Conformationally Restricted Analogues of Pepstatin. *Int. J. Pept. Protein Res.* **1992**, *40*, 233−242.
(18) Ueki, M.; Ikeo, T.; Iwadate, M.; Asakura, T.; Williamson, M. P.; Slaninová, J. Solid Phase Synthesis and Biological Activities of [Arg^8]-Vasopressin Methylenedithioether. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1767−1772.
(19) Ueki, M.; Ikeo, T.; Hokari, K.; Nakamura, K.; Saeki, A.; Komatsu, H. A New Efficient Method for S−CH_2−S Bond Formation and Its Application to a Djenkolic Acid-Containing Cyclic Enkephalin Analog. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 829−838 and references therein.
(20) Lindman, S.; Lindeberg, G.; Gogoll, A.; Nyberg, F.; Karlén, A.; Hallberg, A. Synthesis, Receptor Binding Affinities and Conformational Properties of Cyclic Methylenedithioether Analogues of Angiotensin II. *Bioorg. Med. Chem.* **2001**, *9*, 763−772.
(21) Walker, M. A.; Johnson, T. General Method for the Synthesis of Cyclic Peptidomimetic Compounds. *Tetrahedron Lett.* **2001**, *42*, 5801−5804.
(22) Pawlak, D.; Oleszczuk, M.; Wójcik, J.; Pachulska, M.; Chung, N. N.; Schiller, P. W.; Izdebski, J. Highly Potent Side-Chain to Side-Chain Cyclized Enkephalin Analogues Containing a Carbonyl Bridge: Synthesis, Biology and Conformation. *J. Pept. Sci.* **2001**, *7*, 128−140.
(23) Alexander McNamara, L. M.; Andrews, M. J. I.; Mitzel, F.; Siligardi, G.; Tabor, A. B. Peptides Constrained by an Aliphatic Linkage between Two C^α Sites: Design, Synthesis, and Unexpected Conformational Properties of an i,(i + 4)-Linked Peptide. *J. Org. Chem.* **2001**, *66*, 4585−4594.
(24) Sugg, E. E.; Dolan, C. A.; Patchett, A. A.; Chang, R. S. L.; Faust, K. A.; Lotti, V. J. Cyclic Disulfide Analogs of [Sar^1,Ile^8]-Angiotensin II. *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 11th* **1990**, 305−306.
(25) Spear, K. L.; Brown, M. S.; Reinhard, E. J.; McMahon, E. G.; Olins, G. M.; Palomo, M. A.; Patton, D. R. Conformational Restriction of Angiotensin II: Cyclic Analogs Having High Potency. *J. Med. Chem.* **1990**, *33*, 1935−1940.
(26) Plucinska, K.; Kataoka, T.; Yodo, M.; Cody, W. L.; He, J. X.; Humblet, C.; Lu, G. H.; Lunney, E.; Major, T. C.; Panek, R. L.; Schelkun, P.; Skeean, R.; Marshall, G. R. Multiple Binding Modes for the Receptor-Bound Conformations of Cyclic AII Agonists. *J. Med. Chem.* **1993**, *36*, 1902−1913.
(27) Matsoukas, J. M.; Hondrelis, J.; Agelis, G.; Barlos, K.; Gatos, D.; Ganter, R.; Moore, D.; Moore, G. J. Novel Synthesis of Cyclic Amide-Linked Analogues of Angiotensins II and III. *J. Med. Chem.* **1994**, *37*, 2958−2969.
(28) Vlahakos, D.; Matsoukas, J. M.; Ancans, J.; Moore, G. J.; Iliodromitis, E. K.; Marathias, K. P.; Kremastinos, D. T. Biological Activity of the Novel Cyclic Angiotensin II Analog [Sar^1,Lys^3-Glu^5]ANG II. *Lett. Pept. Sci.* **1996**, *3*, 191−194.
(29) Zhang, W.-J.; Nikiforovich, G. V.; Pérodin, J.; Richard, D. E.; Escher, E.; Marshall, G. R. Novel Cyclic Analogs of Angiotensin II with Cyclization between Positions 5 and 7: Conformational and Biological Implications. *J. Med. Chem.* **1996**, *39*, 2738−2744.
(30) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Karlén, A.; Hallberg, A. Bicyclic Tripeptide Mimetics with Reverse Turn Inducing Properties. *J. Med. Chem.* **1999**, *42*, 601−608.
(31) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Synnergren, B.; Nyberg, F.; Karlén, A.; Hallberg, A. Angiotensin II Analogues Encompassing 5,9- and 5,10-Fused Thiazabicycloalkane Tripeptide Mimetics. *J. Med. Chem.* **1999**, *42*, 4524−4537.
(32) Matsoukas, J. M.; Polevaya, L.; Ancans, J.; Mavromoustakos, T.; Kolocouris, A.; Roumelioti, P.; Vlahakos, D. V.; Yamdagni, R.; Wu, Q.; Moore, G. J. The Design and Synthesis of a Potent Angiotensin II Cyclic Analogue Confirms the Ring Cluster Receptor Conformation of the Hormone Angiotensin II. *Bioorg. Med. Chem.* **2000**, *8*, 1−10.
(33) Polevaya, L.; Mavromoustakos, T.; Zoumboulakis, P.; Grdadolnik, S. G.; Roumelioti, P.; Giatas, N.; Mutule, I.; Keivish, T.; Vlahakos, D. V.; Iliodromitis, E. K.; Kremastinos, D. T.; Matsoukas, J. Synthesis and Study of a Cyclic Angiotensin II Antagonist Analogue Reveals the Role of $\pi^*-\pi^*$ Interactions in the C-Terminal Aromatic Residue for Agonist Activity and Its Structure Resemblance with AT_1 Non-peptide Antagonists. *Bioorg. Med. Chem.* **2001**, *9*, 1639−1647.
(34) Samanen, J. M.; Peishoff, C. E.; Keenan, R. M.; Weinstock, J. Refinement of a Molecular Model of Angiotensin II (AII) Employed in the Discovery of Potent Nonpeptide Antagonists. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 909−914.
(35) Nikiforovich, G. V.; Marshall, G. R. Three-Dimensional Recognition Requirements for Angiotensin Agonists: A Novel Solution for an Old Problem. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 222−228.
(36) Nikiforovich, G. V.; Kao, J. L.-F.; Plucinska, K.; Zhang, W. J.; Marshall, G. R. Conformational Analysis of Two Cyclic Analogs of Angiotensin: Implications for the Biologically Active Conformation. *Biochemistry* **1994**, *33*, 3591−3598.
(37) Joseph, M.-P.; Maigret, B.; Scheraga, H. A. Proposals for the Angiotensin II Receptor-Bound Conformation by Comparative Computer Modeling of AII and Cyclic Analogs. *Int. J. Pept. Protein Res.* **1995**, *46*, 514−526.
(38) Balodis, J.; Golbraikh, A. Conformational Analysis of Cyclic Angiotensin II Analogs. *Lett. Pept. Sci.* **1996**, *3*, 195−199.
(39) Carpenter, K. A.; Wilkes, B. C.; Schiller, P. W. The Octapeptide Angiotensin II Adopts a Well-Defined Structure in a Phospholipid Environment. *Eur. J. Biochem.* **1998**, *251*, 448−453.
(40) Boucard, A. A.; Wilkes, B. C.; Laporte, S. A.; Escher, E.; Guillemette, G.; Leduc, R. Photolabeling Identifies Position 172 of the Human AT_1 Receptor as a Ligand Contact Point: Receptor-Bound Angiotensin II Adopts an Extended Structure. *Biochemistry* **2000**, *39*, 9662−9670.
(41) Eyre, D. R.; Paz, M. A.; Gallop, P. M. Cross-Linking in Collagen and Elastin. *Annu. Rev. Biochem.* **1984**, *53*, 717−748.
(42) Dölz, R.; Heidemann, E. Allysine Peptides and Derivatives. *Int. J. Pept. Protein Res.* **1988**, *32*, 307−320.
(43) Dölz, R.; Heidemann, E. Reactivity of the Allysine Aldehyde Group. *Connect. Tissue Res.* **1989**, *18*, 255−268.
(44) Ojima, I.; Tzamarioudaki, M.; Eguchi, M. New and Efficient Route to Pipecolic Acid Derivatives by Means of Rh-Catalyzed Intramolecular Cyclohydrocarbonylation. *J. Org. Chem.* **1995**, *60*, 7078−7079.
(45) Ben-Ishai, D. Reaction of Acylamino Acids with Paraformaldehyde. *J. Am. Chem. Soc.* **1957**, *79*, 5736−5738.
(46) Mueller, L. P.E.COSY, a Simple Alternative to E.COSY. *J. Magn. Reson.* **1987**, *72*, 191−196.
(47) Braunschweiler, L.; Ernst, R. R. Coherence Transfer by Isotropic Mixing: Application to Proton Correlation Spectroscopy. *J. Magn. Reson.* **1983**, *53*, 521−528.
(48) Bothner-By, A. A.; Stephens, R. L.; Lee, J.-m.; Warren, C. D.; Jeanloz, R. W. Structure Determination of a Tetrasaccharide: Transient Nuclear Overhauser Effects in the Rotating Frame. *J. Am. Chem. Soc.* **1984**, *106*, 811−813.
(49) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley & Sons: New York, 1986.
(50) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.
(51) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel−An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.
(52) Nikiforovich, G. V.; Karlén, A.; Hallberg, A. 3D Model for "Receptor-Bound" Conformation at the AT-1 Receptors of Angiotensin II Analogs Cyclized in Position 3 and 5. Manuscript in preparation.
(53) Nemethy, G.; Pottle, M. S.; Scheraga, H. A. Energy Parameters in Polypeptides. 9. Updating of Geometrical Parameters, Nonbonded Interactions, and Hydrogen Bond Interactions for the Naturally Occurring Amino Acids. *J. Phys. Chem.* **1983**, *87*, 1883−1887.
(54) Dunfield, L. G.; Burgess, A. W.; Scheraga, H. A. Energy Parameters in Polypeptides. 8. Empirical Potential Energy Algorithm for the Conformational Analysis of Large Molecules. *J. Phys. Chem.* **1978**, *82*, 2609−2616.
(55) Dudley, D. T.; Panek, R. L.; Major, T. C.; Lu, G. H.; Bruns, R. F.; Klinkefus, B. A.; Hodges, J. C.; Weishaar, R. E. Subclasses of Angiotensin II Binding Sites and Their Functional Significance. *Mol. Pharmacol.* **1990**, *38*, 370−377.
(56) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. Design and Synthesis of Conformationally Constrained Amino Acids as Versatile Scaffolds and Peptide Mimetics. *Tetrahedron* **1997**, *53*, 12789−12854 and references therein.

(57) Robl, J. A.; Sun, C.-Q.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Asaad, M. M.; Cheung, H. S.; Abboa-Offei, B. E.; Smith, P. L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A.; Trippodo, N. C. Dual Metalloprotease Inhibitors: Mercaptoacetyl-Based Fused Heterocyclic Dipeptide Mimetics as Inhibitors of Angiotensin-Converting Enzyme and Neutral Endopeptidase. *J. Med. Chem.* **1997**, *40*, 1570−1577.

(58) Siddiqui, M. A.; Préville, P.; Tarazi, M.; Warder, S. E.; Eby, P.; Gorseth, E.; Puumala, K.; DiMaio, J. Synthesis of Constrained Bicyclic Dipeptide Mimetics. *Tetrahedron Lett.* **1997**, *38*, 8807−8810.

(59) Witter, D. J.; Famiglietti, S. J.; Cambier, J. C.; Castelhano, A. L. Design and Synthesis of SH3 Domain Binding Ligands: Modifications of the Consensus Sequence XPpXP. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3137−3142.

(60) Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, Synthesis, and Dopamine Receptor Modulating Activity of Spiro Bicyclic Peptidomimetics of L-Prolyl-L-leucyl-glycinamide. *J. Med. Chem.* **1999**, *42*, 628−637.

(61) Crescenza, A.; Botta, M.; Corelli, F.; Santini, A.; Tafi, A. Cyclic Dipeptides. 3. Synthesis of Methyl (*R*)-6-[(*tert*-Butoxycarbonyl)-amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate and Its Hexahydro Analogues: Elaboration of a Novel Dual ACE/NEP Inhibitor. *J. Org. Chem.* **1999**, *64*, 3019−3025.

(62) Shenderovich, M. D.; Kövér, K. E.; Nikiforovich, G. V.; Jiao, D.; Hruby, V. J. Conformational Analysis of β-Methyl-*para*-nitrophenylalanine Stereoisomers of *cyclo*[D-Pen^2, D-Pen^5]Enkephalin by NMR Spectroscopy and Conformational Energy Calculations. *Biopolymers* **1996**, *38*, 141−156.

(63) Shiosaki, K.; Nadzan, A. M.; Kopecka, H.; Shue, Y.-K.; Holladay, M. W.; Lin, C. W.; Nellans, H. N. Patent WO 9119733, 1991.

(64) Nishikawa, M. Patent JP 04193871, 1992.

(65) Nishimura, K.; Fujimura, K.-i.; Matsumoto, J.; Kobayashi, T. Patent WO 0006594, 2000.

(66) Claesen, M.; Vlietinck, A.; Vanderhaeghe, H. Preparation of the Enantiomers of 2-Carbobenzyloxyaminoadipic Acid and Their 1-Benzylesters. *Bull. Soc. Chim. Belg.* **1968**, *77*, 587−596.

(67) Szirtes, T.; Kisfaludy, L.; Pálosi, É.; Szporny, L. Synthesis of Thyrotropin-Releasing Hormone Analogs. 2. Tripeptides Structurally Greatly Different from TRH with High Central Nervous System Activity. *J. Med. Chem.* **1986**, *29*, 1654−1658.

(68) Robl, J. A.; Kronenthal, D. R.; Goderey, J. D., Jr. Patent EP 629627, 1994.

(69) Robl, J. A. U.S. Patent 5508272, 1996.

(70) Godfrey, J. D., Jr.; Kronenthal, D. R.; Schwinden, M. D.; Srivastava, S. K.; Ramig, K.; Venit, J. J.; Jass, P. A.; Racha, S.; Dillon, J. L., Jr.; Soundararajan, N.; Powers, G. L.; Kotnis, A. S. Patent WO 0003981, 2000.

(71) Donovan, M. J.; Goldberg, S.; Hanson, R. L.; Jass, P. A.; Li, W.-S.; Patel, R. N.; Ramig, K.; Szarka, L. J.; Venit, J. J. Patent WO 0004179, 2000.

(72) Moniot, J. L.; Srivastava, S. K.; Winter, W. J.; Venit, J. J.; Swaminathan, S.; Ramig, K.; Jass, P. A.; Schwinden, M. D.; Dillon, J. L., Jr.; Racha, S.; Simpson, J.; Chen, C.-k.; Pack, S. K. U.S. Patent 6162913, 2000.

(73) Goodman, J. M.; Still, W. C. An Unbounded Systematic Search of Conformational Space. *J. Comput. Chem.* **1991**, *12*, 1110−1117.

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