Bicyclic Tripeptide Mimetics with Reverse Turn Inducing Properties

Petra Johannesson,[†] Gunnar Lindeberg,[‡] Weimin Tong,[†] Adolf Gogoll,[§] Anders Karlén,[†] and Anders Hallberg*[,†]

Department of Organic Pharmaceutical Chemistry, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden, Department of Medical Biochemistry and Microbiology, Uppsala University, Box 575, SE-751 23 Uppsala, Sweden, and Department of Organic Chemistry, Uppsala University, Box 531, SE-751 21 Uppsala, Sweden

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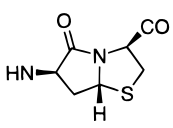
Analogues of the hypertensive octapeptide angiotensin II, comprising novel constrained 5,8-bicyclic and 5,9-bicyclic tripeptide units adopting nonclassical β-turn geometries, as deduced from theoretical conformational analysis, have been synthesized. Spontanous bicyclization upon acid-catalyzed deprotection of a model peptide, encompassing a protected ω-formyl α-amino acid in position 5 and cysteine residues in positions 3 and 7, revealed a strong preference for bicyclization toward the C-terminus. The bicyclic thiazolidine related angiotensin II analogues synthesized exhibited no affinity for the angiotensin II AT_1 receptor.

Introduction

Reverse turns are common secondary structures in proteins. The prevalence of β-turns and γ-turns, which have often been implicated as recognition elements,[1,2] has led to an intense search for templates that mimic these structures.[3−7] Mimetics where the stabilizing hydrogen bonds in β- and γ-turns have been substituted with covalent bonds[8−12] as well as mimetics enhancing the reverse turn propensity of a peptide[13−17] have been developed. Several cyclic and bicyclic dipeptide analogues intended to stabilize the peptide in a reverse turn have been reported.[3−7] Among these, the 5,5-bicyclic dipeptidomimetic **1** was designed as a type II β-turn mimetic[18] and the 6,5-bicyclic dipeptidomimetic BTD **2** as a type II′ β-turn mimetic (Chart 1). BTD has been extensively studied and used for diverse biological applications.[14,19−22] BTD and the related 4,5-bicyclic, 5,5-bicyclic, 7,5-bicyclic, and 7,6-bicyclic core structures are most frequently synthesized by reaction sequences where ring closure by intramolecular N-acylation constitutes the last step.[23−25] While numerous investigations have been devoted to constrained bicyclic dipeptides,[24] studies of the corresponding bicyclic tripeptides are rare. Access to reliable methods that, on solid phase, allow for construction of well-defined constrained tripeptide units and that constitute a complement to the commonly used disulfide-based cyclization procedures is highly desirable. Such general methods should provide important research tools for medicinal chemists in the search for bioactive conformations and in the efforts to design non-peptide ligands acting at various peptide receptors.

We herein report analogues of the hypertensive octapeptide angiotensin II (Ang II: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe)[26] incorporating the novel constrained 5,8-bicyclic and 5,9-bicyclic tripeptide units **3** and **4**, respectively (Chart 2). We also report that these tripeptidic core structures have β-turn forming properties as deduced from theoretical conformational analysis studies.

Chart 1



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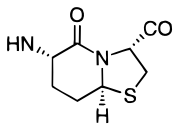

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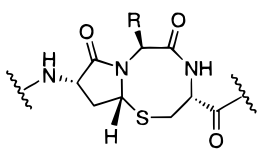
Chart 2



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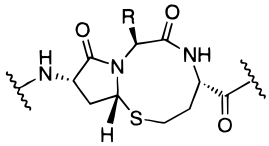

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Results

Synthesis. The building block L-2-(9-fluorenylmethyloxycarbonyl)amino-4,4-dimethoxybutanoic acid **10** was synthesized by the reaction sequence outlined in Scheme 1. Boc-Asp-O^{t}Bu **5** was transformed into the aldehyde **8**[27−29] following a procedure similar to that described by Ramsamy et al.,[27] where we used pyridinium chlorochromate (PCC) in the presence of sodium hydrogen carbonate and Celite for the oxidation. The aldehyde function was thereafter protected as the dimethyl acetal to give **9**. The Boc and *tert*-butyl groups were subsequently cleaved with HCl in methanol. The removal of these groups was accompanied with formation of a considerable amount of undesired α-methyl ester, and subsequent treatment with potassium hydroxide was needed for liberation of the zwitterion. This zwitterion was not isolated but was treated directly with Fmoc-Cl and sodium carbonate in water/dioxane[30] to give the building block **10**. Alternatively, the aldehyde **8** could be transformed directly into the Fmoc derivative **10** in approximately the same yield as for the two-step procedure via compound **9**. The synthetic route via compound **9** was preferred since we found it more reliable. Attempts to selectively cleave the Boc and *tert*-butyl protecting groups with trimethylsilyl iodide were

[†] Department of Organic Pharmaceutical Chemistry.
[‡] Department of Medical Biochemistry and Microbiology.
[§] Department of Organic Chemistry.

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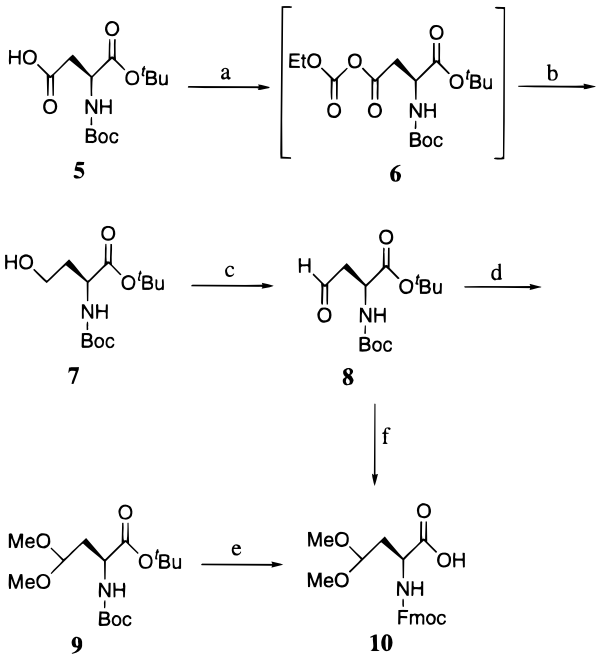
Scheme 1[a]



[a] Reagents: (a) EtOCOCl, *N*-methylmorpholine (NMM), THF; (b) $NaBH_4$, H_2O, THF; (c) PCC, $NaHCO_3$, Celite, CH_2Cl_2, 32% from **5**; (d) TsOH, MeOH, 79%; (e) (i) HCl, MeOH, (ii) KOH, MeOH, (iii) Fmoc-Cl, Na_2CO_3, H_2O/dioxane, 52%; (f) (i) HCl, MeOH, (ii) KOH, MeOH, (iii) Fmoc-Cl, Na_2CO_3, H_2O/dioxane, 42%.

unsuccessful[31] due to concomitant deprotection of the aldehyde group. The Fmoc protecting group was introduced at the end of the reaction sequence, since it was found not to be compatible with the PCC oxidation. Compound **10** was purified through extensive extractions. It was not stable to flash chromatography on silica and decomposed slowly when stored at room temperature.

The Fmoc-protected amino acid **10** was subjected to solid-phase peptide synthesis (SPPS) for the preparation of compounds **11**−**14** (Scheme 2). Acidic cleavage with trifluoroacetic acid (TFA) produced the bicyclized pseudopeptides as the predominant components in the reaction mixture. According to LC−MS analyses none of the major side products (>5% yields, as estimated from peak areas) exhibited the same molecular weights as those of the target peptides, suggesting that the synthesis of **10** and the bicyclizations proceed with high stereoselectivity and with negligible racemization/epimerization.[18,32] After purification by RP-HPLC, the pseudopeptides were isolated in 15−40% yields.

To determine the preferred direction of cyclization, the dimercapto precursor **15** was prepared on solid phase and subsequently bicyclized to yield the monomercapto derivative **16** with very high regioselectivity (Scheme 3).

Structural Determination. The structural assignment of **11**−**14** and **16** was conducted with P.E.COSY,[33] TOCSY,[34] and NOESY[35,36] or ROESY[37] NMR experiments. Selected carbon−proton connectivity information involving the cyclic part of the molecule was also obtained from HMQC[38] and HSBC[39] NMR experiments. ^{1}H NMR chemical shifts for compounds **11**−**14** and **16** are given in Table 1. The stereochemistry of the bicyclic segments was determined from NOE information as

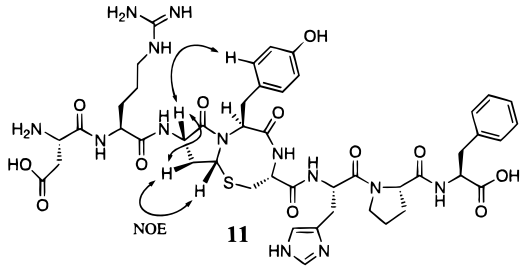
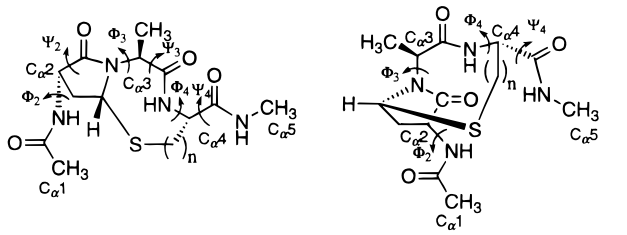


Figure 1. NOE information from Ang II analogue **11**.



β-turn form A: $C_\alpha1$-$C_\alpha4 \leq 7$ Å — **17** (n=1), **18** (n=2)

β-turn form B: $C_\alpha2$-$C_\alpha5 \leq 7$ Å — **17** (n=1), **18** (n=2)

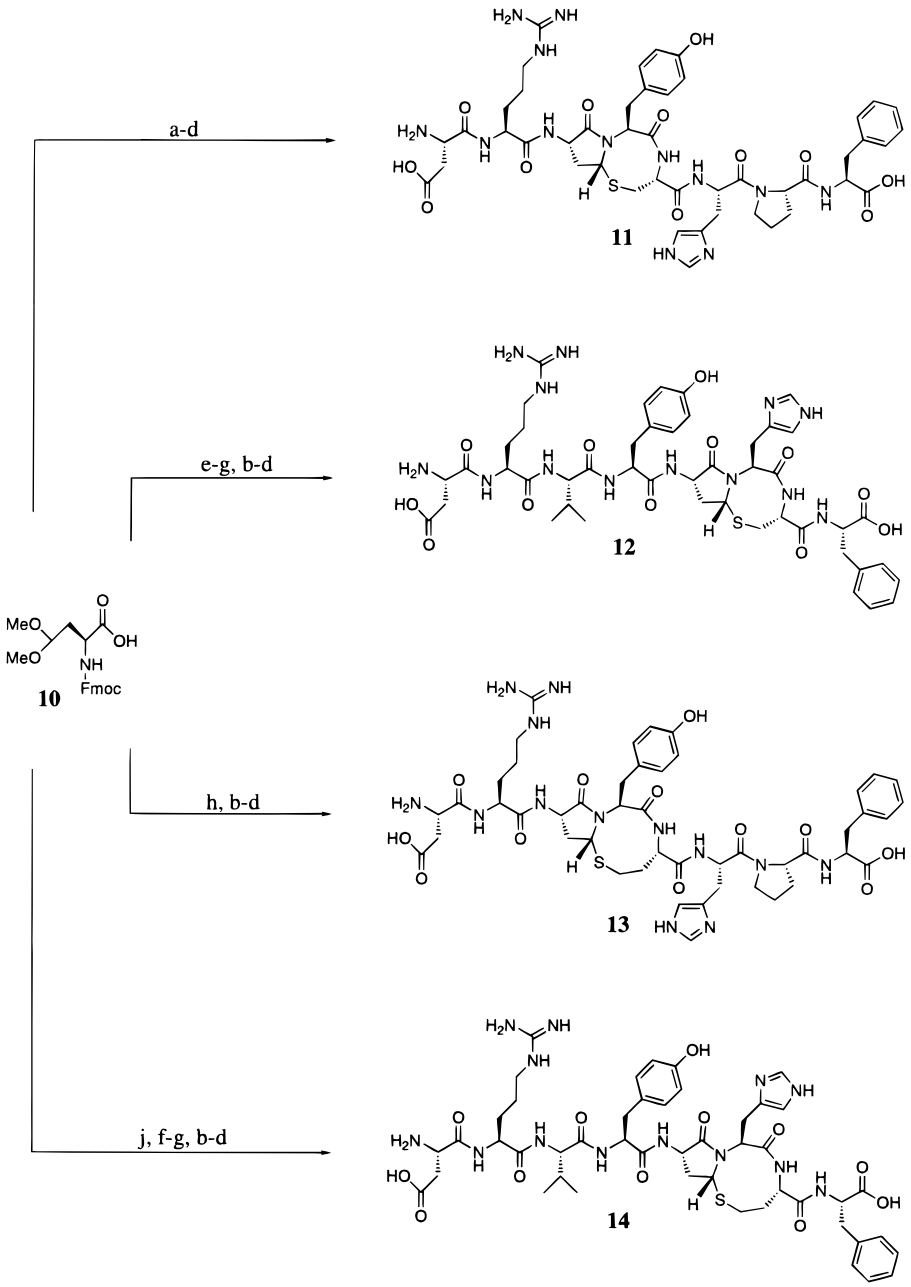
Figure 2. Possible β-turn locations in model compounds **17** and **18**.

illustrated by **11** in Figure 1. Thus, protons in the residues involved (2-amino-4-oxobutanoic acid (Aob), Tyr, Cys) were assigned using coupling information and interresidue NOEs. An NOE between the *ortho*-Tyr protons and H_α of Aob established their positions on the same face of the bicyclic ring system. H_α-Aob has an NOE to only one of the two H_β-Aob protons, which in turn has an NOE to H_γ-Aob. These three protons must therefore reside on the same face of the bicyclic ring system. This is confirmed by observation of an NOE between H_γ-Aob and H_α-Aob.

Conformational Characterization. (a) Theoretical Calculations on Model Compounds. The aim of this part of the investigation was to evaluate if the bicyclic peptide core structures have β-turn inducing properties. This was accomplished by performing a Monte Carlo conformational search using the Amber* force field and the GB/SA water solvation model[40] as implemented in the Macromodel program (version 5.5).[41] Compounds **11** and **12** derived from cysteine were modeled as the blocked bicyclic tripeptide **17**, and the homocysteine (Hcy) compounds **13** and **14** were modeled as the blocked bicyclic tripeptide **18** (Figure 2). A β-turn is most often identified as any tetrapeptide sequence occurring in a nonhelical region in which the distance from C_α of the first residue to C_α of the fourth residue is less than or equal to 7 Å.[42] A β-turn can be further subdivided into the classical, well-defined turn types (I, I′, II, II′, VIa, and VIb) based on the Φ and Ψ backbone torsion angles of residues 2 and 3 in the turn.[43] In model compounds **17** and **18**, two locations of the β-turn are possible. In the structure designated as form A, residues 1−4 adopt the β-turn, while in form B, residues 2−5 adopt the β-turn (Figure 2).

Results from the theoretical conformational analysis showed that compound **17**, used as a model of the core structure of analogues **11** and **12**, assumes four conformations within 5 kcal/mol of the lowest energy conformation identified (Table 2). Analysis of the distances

Scheme 2[a]



[a] Reagents: (a) (i) Tyr(ᵗBu)-Cys(Trt)-His(Trt)-Pro-Phe-Wang resin, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), NMM, DMF, (ii) piperidine, DMF; (b) (i) Fmoc-Arg(Pmc), HBTU, NMM, DMF, (ii) piperidine, DMF; (c) (i) Fmoc-Asp(OᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (d) TFA/H_2O; (e) (i) His(Trt)-Cys(Trt)-Phe-Wang resin, HBTU, NMM, DMF, (ii) piperidine, DMF; (f) (i) Fmoc-Tyr(ᵗBu), HBTU, NMM, DMF, (ii) piperidine, DMF; (g) (i) Fmoc-Val, HBTU, NMM, DMF, (ii) piperidine, DMF; (h) (i) Tyr(ᵗBu)-Hcy(Trt)-His(Trt)-Pro-Phe-Wang resin, HBTU, NMM, DMF, (ii) piperidine, DMF; (j) (i) His(Trt)-Hcy(Trt)-Phe-Wang resin, HBTU, NMM, DMF, (ii) piperidine, DMF. Hcy is used as the abbreviation for homocysteine.

between the C_α atoms showed that all these conformations assume a β-turn of form A. In two of the conformations (2 and 3, Table 2) both turns are present, i.e., the A and B form.[44] In Figure 3a, the similar geometries of the low-energy conformations of **17** are shown. To determine if the β-turn conformations belong to any of the classical β-turn types, we searched the conformations and allowed a $\pm 30°$ deviation from the ideal Φ and Ψ values. However, none of the β-turns identified fulfilled all four torsion angle criteria of a classical β-turn.

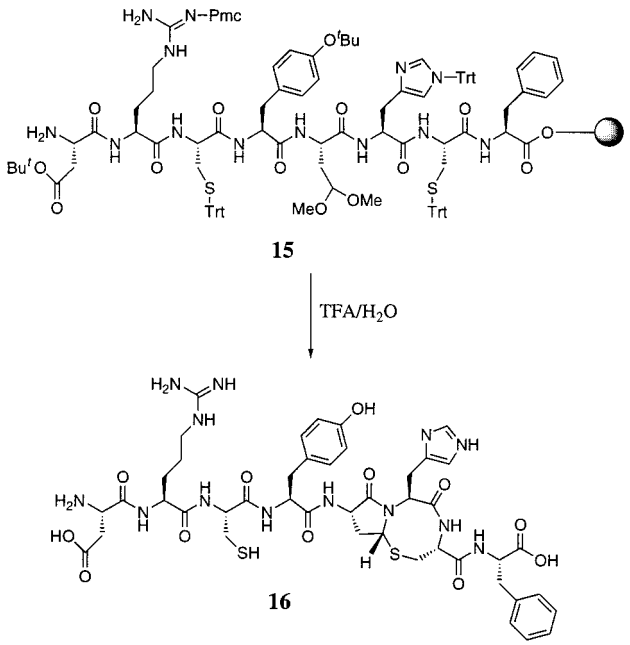
The conformational analysis study of compound **18**, used as a model of the core structure of analogues **13** and **14**, resulted in 10 conformations within 5 kcal/mol of the lowest energy conformation. Out of the 10 conformations, four adopt the form A β-turn. Two of the 10 conformations adopt a double turn[44] involving both form A and form B (conformations 3 and 8, Table 2). The similar geometries of the 10 conformations that were identified within 5 kcal/mol of the lowest energy conformation are shown in Figure 3b. None of the β-turn conformations identified could be classified into the classical β-turn types.

A comparison of the Φ and Ψ backbone torsion angles of model compounds **17** and **18** indicates that these compounds induce the same type of reverse turn geom-

Table 1. ^{1}H NMR Chemical Shifts for Ang II Analogues **11**−**14** and **16** in DMSO-d_6

compd (temp)	residue	NH	H_α	H_β	$H_{\beta'}$	other
11 (45 °C)	Asp		4.13	2.87	2.69	
	Arg	8.65	4.18	1.71	1.55	7.64 (NHϵ), 3.08 (Hδ, Hδ'), 1.55 (Hγ, Hγ')
	Aob	8.19	4.05	2.59	1.94	4.90 (Hγ)
	Tyr		4.89	3.21	2.72	7.00 (2H, ortho), 6.61 (2H, meta)
	Cys	5.95	4.43	3.05	2.85	
	His	8.31	4.81	3.05	2.93	8.86 (H2), 7.37 (H4)
	Pro		4.40	2.04	1.83	3.57−3.51 (Hδ, Hδ'), 1.83 (Hγ, Hγ')
	Phe	8.21	4.45	3.01	2.92	7.28−7.18 (5H, Ar)
12 (25 °C)	Asp		4.10	2.79	2.64	
	Arg	8.56	4.35	1.63	1.49	7.65 (NHϵ), 3.07 (Hδ, Hδ'), 1.49 (Hγ, Hγ')
	Val	7.78	4.15	1.92		0.77 (3H, Me), 0.75 (3H, Me)
	Tyr	8.11	4.38	2.85	2.67	6.98 (2H, ortho), 6.60 (2H, meta)
	Aob	8.03	4.20	2.43	1.82	4.92 (Hγ)
	His		5.03	3.38	2.98	8.86 (H2), 7.40 (H4)
	Cys	6.21	4.38	2.95	2.95	
	Phe	8.23	4.44	3.08	2.95	7.28−7.18 (5H, Ar)
13 (45 °C)	Asp		4.12	2.83	2.78	
	Arg	8.61	4.25	1.70	1.55	7.69 (NHϵ), 3.10 (Hδ, Hδ'), 1.55 (Hγ, Hγ')
	Aob	8.27	3.97	2.59	1.86	4.79 (Hγ)
	Tyr		4.88	3.18	2.70	6.99 (2H, ortho), 6.61 (2H, meta)
	Hcy	6.88	4.49	2.70	2.58	1.98 (Hγ), 1.80 (Hγ')
	His	8.14	4.80	3.07	2.92	8.85 (H2), 7.33 (H4)
	Pro		4.41	2.03	1.85	3.57 (Hδ), 3.49 (Hδ'), 1.85 (Hγ), 1.80 (Hγ')
	Phe	8.21	4.46	3.05	2.95	7.26−7.20 (5H, Ar)
14 (35 °C)	Asp		4.12	2.80	2.64	
	Arg	8.56	4.36	1.64	1.50	7.64 (NHϵ), 3.08 (Hδ, Hδ'), 1.50 (Hγ, Hγ')
	Val	7.76	4.15	1.93		0.77 (3H, Me), 0.75 (3H, Me)
	Tyr	8.02	4.43	2.86	2.70	7.01 (2H, ortho), 6.62 (2H, meta)
	Aob	8.19	4.18	2.54	1.81	4.84 (Hγ)
	His		4.99	3.29	2.98	8.89 (H2), 7.43 (H4)
	Hcy	7.03	4.51	2.01	1.81	2.67 (Hγ), 2.54 (Hγ')
	Phe	8.22	4.46	3.09	2.90	7.26−7.20 (5H, Ar)
16 (35 °C)	Asp		4.13	2.79	2.66	
	Arg	8.66	4.31	1.71	1.54	7.76 (NHϵ), 3.07 (Hδ, Hδ'), 1.50 (Hγ, Hγ')
	Cys^3	8.30	4.52	3.05	2.84	
	Tyr	8.03	4.38	2.72	2.67	6.98 (2H, ortho), 6.50 (2H, meta)
	Aob	8.28	4.20	2.49	1.89	4.93 (Hγ)
	His		5.03	3.37	2.96	8.62 (H2), 7.30 (H4)
	Cys^7	6.20	4.38	3.00	2.93	
	Phe	8.24	4.46	3.07	2.94	7.23 (5H, Ar)

Scheme 3



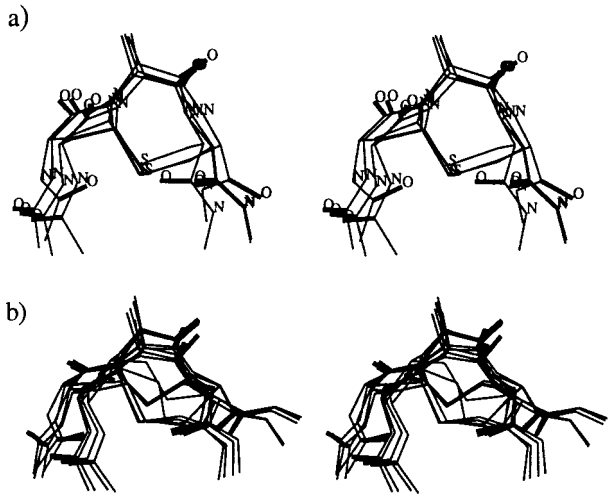


Figure 3. (a) Stereo image of the rms best fit of the four identified conformations within 5 kcal/mol of the lowest energy minimum of **17**. Heteroatoms are labeled for clarity. (b) Stereo image of the rms best fit of the 10 identified conformations within 5 kcal/mol of the lowest energy minimum of **18**. In both a and b, all heavy atoms were included in the fitting procedure.

etries (Table 2). This is also exemplified in Figure 4 in which the lowest energy β-turn conformation of **17** (conformation 1) and that of **18** (conformation 3) are superimposed. Interestingly, from Figure 4 it can also be seen that the tripeptidic core structures seem to have the potential to induce β-hairpin-like structures.[45]

According to the theoretical calculations, the bicyclic ring system does not favor the adoption of any of the classical turn types; however, it does restrict the con-

Table 2. Comparison of C_α Distances (Å) and Backbone Torsion Angles (deg) in Low-Energy Conformations (<5 kcal/mol) of **17** and **18**

compd	conf	$C_\alpha1-C_\alpha4$	$C_\alpha2-C_\alpha5$	Φ_2	Ψ_2	Φ_3	Ψ_3	Φ_4	Ψ_4	ΔE (kcal/mol)
17	1	6.9	7.8	176.9	−108.3	−148.2	62.6	169.7	160.1	0.0
	2	5.6	6.1	177.9	−104.7	−107.5	34.9	160.0	158.4	1.7
	3	7.0	6.1	176.4	−108.3	−152.5	73.3	161.6	−42.3	4.0
	4	6.6	8.0	15.7	−111.2	−155.2	65.7	170.5	164.2	4.1
18	1	8.2	10.0	177.3	−112.2	−161.8	98.2	−148.6	135.5	0.0
	2	8.2	9.3	177.2	−114.5	−169.1	89.0	−177.5	152.8	0.1
	3	5.5	6.5	179.7	−119.1	−89.3	39.5	−179.9	150.2	0.6
	4	8.0	10.1	27.4	−119.5	−168.0	100.7	−149.5	135.8	3.7
	5	8.0	9.4	23.5	−119.4	−174.3	90.3	−178.1	153.4	4.0
	6	6.3	7.2	176.9	−107.3	−107.9	28.5	−179.8	160.9	4.1
	7	6.6	7.4	177.0	−108.0	−113.4	31.9	−176.9	156.9	4.1
	8	5.8	6.5	178.3	−105.0	−99.1	24.8	173.7	167.0	4.1
	9	8.0	9.5	176.6	−132.8	−136.5	62.8	−129.7	130.1	4.5
	10	7.1	9.2	177.3	−122.4	−122.8	58.2	−127.4	129.5	4.5

Table 3. NMR Temperature Coefficients of NH Chemical Shifts for Compounds **11**−**14** and **16** in DMSO-d_6[a]

compd	residue 2	residue 3	residue 4	residue 5	residue 6	residue 7	residue 8
11	3.7	1.3	*b*	1.0	4.0	*c*	4.0
12	2.0	3.7	5.7	0.0	*b*	0.7	4.3
13	4.0	2.7	*b*	2.7	3.3	*c*	4.3
14	2.8	2.8	4.0	2.4	*b*	2.4	4.8
16[d]	3.3	2.4	4.0	1.3	*b*	0.7	3.1

[a] $\Delta\delta/\Delta T$ (ppb/K). [b] No NH (part of the bicyclic ring system). [c] No NH (proline). [d] Values are for the dimer.

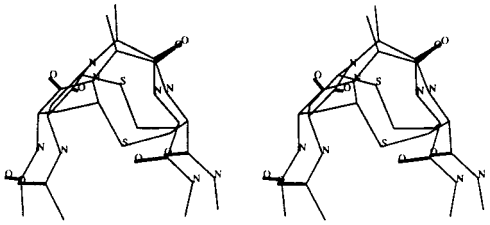


Figure 4. Stereo image of the rms best fit of the lowest energy β-turn conformation of **17** (conformation 1) and **18** (conformation 3). All heavy atoms were included in the fitting procedure.

formational space of the peptides and induces nonclassical β-turns of either form A or both forms A and B. Although the conformational analysis was performed on model compounds, considering the constrained nature of the bicyclic scaffolds, it is reasonable to assume that the octapeptides also populate these conformations in the corresponding turn regions.

(b) NMR Spectroscopy of Compounds 11−14 and 16. NMR temperature coefficients of the NH chemical shifts of compounds **11**−**14** and **16** are given in Table 3. In all compounds the NH protons of the residues closest to the five-membered ring have the lowest temperature coefficients. This is more pronounced in the compounds containing cysteine (**11**, **12**, and **16**) than in those containing homocysteine (**13** and **14**). These low temperature coefficients indicate that these protons are either intramolecularly hydrogen bonded or unexposed to solvent.[46] A hydrogen bond involving the NH proton on the N-terminal side of the five-membered ring was also seen in conformation 2 of model compound **17** and in conformations 3 and 8 of model compound **18**.

In all compounds the amide proton in the larger ring of the bicyclic system has an unusually low chemical shift (see Table 1). This may be due to a distortion of the amide bond out of planarity or to the localization of the NH proton in the shielding region of a carbonyl bond.

In Vitro Binding Affinity. Compounds **11**−**14** were evaluated in a radioligand binding assay based on displacement of ^{125}I-Ang II from AT_1 receptors in rat liver membranes.[47,48] A second binding assay, using Chinese hamster ovary (CHO) cells stably expressing the rat AT_1 receptor,[49,50] was also applied. All compounds lacked affinity for the AT_1 receptor. No IC_{50} values below 10 μM were found. Ang II displayed an affinity with a K_i value of 0.53 nM in the rat liver assay and an IC_{50} value of 1.5 nM on the CHO cells.

Discussion

Our synthetic strategy for preparation of ring skeletons **3** and **4** relies on a connection of side chains in the final step, as opposed to amide bond formation as is most commonly employed in the synthesis of the dipeptidomimetic thiazolidine counterparts (e.g., **1** and **2**).[24] The initial experiments were designed to assess the propensity of the formyl function to cyclize toward the N-terminal or C-terminal end of the peptide. Thus, an angiotensin II analogue (**15**) with cysteine residues replacing both Val^3 and Pro^7 and the masked 2-amino-4-oxobutanoic acid (Aob) replacing Ile^5 was prepared. We anticipated that cyclization toward the N-terminal end might occur via involvement of the Tyr^4 NH to furnish a 6,7-fused system. However, bicyclization in the N-terminal direction was not encountered, and only the C-terminal directed cyclization, with formation of the five-membered lactam ring as a plausible driving force affording the 5,8-fused scaffold in compound **16**, was observed (Scheme 3).

Previous monocyclizations and bicyclizations in the Tyr^4 and His^6 region of Ang II have provided bioactive angiotensin II analogues.[11,48,51−55] Therefore, we found it appropriate to incorporate the building block **10** both in position 3 and in position 5 to furnish the target peptides **11**−**14**. The bicyclizations from the mono mercapto compounds proceeded smoothly, and only the diastereoisomers with the ring-junction hydrogen on the opposite side of the incoming and outgoing backbone of the bicyclic tripeptidic ring system were encountered. Thus, the stereochemistry at the ring junction differs from that of BTD (**2**) (cf. Charts 1 and 2).

The pharmacological evaluation showed that analogues **11**−**14** lacked affinity to the AT_1 receptor and apparently the angiotensin II analogues cannot adopt a conformation that enables binding to the AT_1 receptor.[56−60] According to the theoretical conformational analysis, the model compounds **17** and **18** adopt β-turn geometries that could not be classified into the well-defined turn types. Most of the β-turn mimetics available today mimic one of the classical β-turns. However, about 40% of the β-turns found in proteins cannot be classified into any of these β-turn types.[44] Therefore it is reasonable to assume that even smaller peptides might adopt nonclassical β-turn conformations when binding to their receptors. This implies that there should be an incentive for developing turn mimetics for nonclassical reverse turns as well.

Conclusion

Constrained 5,8-fused and 5,9-fused thiazolidine related bicyclic tripeptide units with β-turn inducing properties can be prepared with high regio- and stereoselectivity after deprotection of a masked aldehyde in presynthesized angiotensin II analogues. Although only a few examples are given in the present report, we anticipate that the synthetic strategy is sufficiently flexible to accommodate amino acids other than Tyr and His in the turn region and that the methodology, suitable for combinatorial chemistry approaches, will be appreciated also in elaborations of other target peptides.

Experimental Section

Chemistry. General Comments. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 (67.8) MHz and on a JEOL JNM-EX400 or on a Varian Unity 400 spectrometer at 400 (100.5) 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 performed using Merck silica gel 60 (40−63 μm) or Riedel-de Haën silica gel S (32−63 μm). Thin-layer chromatography (TLC) was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck, or 0.25 mm, Macherey-Nagel). Chromatographic spots were visualized by UV and/or spraying with an ethanolic solution of ninhydrin (2%) followed by heating. 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 by Dr. M. Sundquist, Department of Biochemistry, Biomedical Centre, Uppsala, Sweden, on 24 h hydrolyzates with an LKB 4151 alpha plus analyzer, using ninhydrin detection. SPPS resins and amino acid derivatives were obtained from Bachem (Bubendorf, Switzerland), Calbiochem-Novabiochem (Läufelfingen, Switzerland), or Alexis Corporation (Läufelfingen, Switzerland). DMF (peptide synthesis grade) was obtained from PerSeptive Biosystems (Hamburg, Germany) and was used without further purification. HBTU was purchased from Richelieu Biotechnologies (St-Hyacinthe, Qc, Canada). The peptides were synthesized on a 60−80 μmol scale with a Symphony instrument (Protein Technologies Inc., Tucson, AZ) using Fmoc/*tert*-butyl protection. The starting polymer was Fmoc-Phe-Wang resin (0.6 mmol/g), and for the Fmoc amino acids the side chain protecting groups were as follows: Asp(O^{t}Bu), Arg(Pmc), Tyr(^{t}Bu), His(Trt), Cys(Trt), and Hcy(Trt). Removal of the Fmoc group was achieved by reaction with 20% piperidine in DMF for 5 + 10 min. Coupling of the amino acids (125 μmol) was done in DMF (2.5 mL) using HBTU (125 μmol) in the presence of NMM (0.5 mmol). Single couplings (60 min) were used for Fmoc-Hcy(Trt) and for compound **10**; double couplings (2 × 30 min) were used for the other amino acids. After the introduction of each amino acid, remaining amino groups were capped by addition of 20% acetic anhydride in 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-2-*tert*-Butoxycarbonylamino-4,4-dimethoxybutanoic Acid *tert*-Butyl Ester (9). L-2-*tert*-Butoxycarbonylamino-4-oxobutanoic acid *tert*-butyl ester **8**[27−29] (1.06 g, 3.88 mmol) and *p*-toluenesulfonic acid (monohydrate, 88.5%) (82 mg, 0.38 mmol) were dissolved in MeOH (50 mL). After being stirred at room temperature for 7 h, the reaction mixture was partitioned between ether (150 mL) and 20% aqueous $NaHCO_3$ (60 mL), and the water phase was further extracted with ether (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (gradient system: CH_2Cl_2 to 1.5% MeOH in CH_2Cl_2) to give **9** (0.98 g, 79%) as a colorless oil. TLC R_f = 0.45 (CH_2Cl_2/MeOH 97:3). $[\alpha]_D = -25.4°$ (c = 1.0, 99% EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (s, 9H, C(CH_3)$_3$), 1.45 (s, 9H, C(CH_3)$_3$), 1.96 (ddd, J = 5.4, 6.6, 14.2 Hz, 1H, 3), 2.08 (ddd app dt, J = 5.4, 14.2 Hz, 1H, 3), 3.31 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 4.25 (br m, 1H, 2), 4.44 (dd app t, J = 5.6 Hz, 1H, 4), 5.28 (br d, J = 7.2 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 27.9, 28.3 (C($\underline{C}H_3$)$_3$), 35.2 (3), 51.1 (2), 53.3, 53.4 (OCH_3), 79.4, 81.7 ($\underline{C}$(CH$_3$)$_3$), 102.2 (4), 155.2 (CO Boc), 171.2 (1). IR (neat): 3351, 1718. Anal. ($C_{15}H_{29}NO_6$) C, H, N.

L-2-(9-Fluorenylmethyloxycarbonyl)amino-4,4-dimethoxybutanoic Acid (10). Method A. Compound **9** (0.89 g, 2.79 mmol) was dissolved in a 4.5 M solution of HCl in MeOH (20 mL) and stirred at room temperature for 20 h. A 10 M aqueous KOH solution was added in small portions until the pH of the reaction mixture reached 12 (9 mL). After being stirred at room temperature for 1 h, the mixture was neutralized to pH 7 with 10% aqueous citric acid and concentrated to give a solid residue. The solid material was dissolved in a mixture of 10% aqueous Na_2CO_3 (50 mL) and dioxane (25 mL) and cooled to 0 °C. Fmoc-Cl (1.08 g, 4.17 mmol) was dissolved in dioxane (25 mL) and added dropwise, whereafter the reaction was allowed to reach room temperature. The pH was kept around 11. After the mixture was stirred at room temperature for 48 h, 10% aqueous citric acid was added to pH 7−8, and the reaction mixture was washed with ether until TLC showed the water phase to be free from impurities having R_f values higher than those of the product (4 × 90 mL). Ether (400 mL) was added to the water phase, which was then acidified to pH 3 with aqueous citric acid. The phases were separated, and the water phase was further extracted with ether (2 × 200 mL). The combined organic layers were washed with water (2 × 150 mL), dried ($MgSO_4$), and concentrated to afford pure product **10** as a white foam (0.56 g, 52%). TLC R_f = 0.3 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D = -15.8°$ (c = 1.0, 99% EtOH). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.97−2.07 (m, 1H, 3), 2.15−2.23 (m, 1H, 3), 3.31 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 4.26 (t, J = 7.1 Hz, 1H, CH Fmoc), 4.32−4.39 (m, 3H, CH_2 Fmoc and 2), 4.56 (dd, J = 4.2, 7.2 Hz, 1H, 4), 6.81 (br d, J = 8.1 Hz, 1H, NH), 7.34 (t, J = 7.5 Hz, 2H, CH Ar Fmoc), 7.42 (t, J = 7.5 Hz, 2H, CH Ar Fmoc), 7.72 (d, J = 7.2 Hz, 1H, CH Ar Fmoc), 7.73 (d, J = 7.2 Hz, 1H, CH Ar Fmoc), 7.87 (d, J = 7.5 Hz, 2H, CH Ar Fmoc). ^{13}C NMR (acetone-d_6): δ 35.5 (3), 47.9 (CH Fmoc), 51.6 (2), 53.4, 53.6 (OCH_3), 67.1 (CH_2 Fmoc), 102.8 (4), 120.7, 126.0, 127.8, 128.4 (CH Ar Fmoc), 142.0, 144.8, 145.0 (ipso Fmoc), 156.9 (CO Fmoc), 173.7 (1). IR (KBr): 3320, 1721. Anal. ($C_{21}H_{23}NO_6$) C, H, N.

Method B. Aldehyde **8**[27−29] was treated with essentially the same reaction conditions and workup procedure as described for method A, to afford pure product in 42% yield.

Ang II Analogue 11. Fmoc-Phe-Wang resin (125 mg, 75 μmol) was reacted as described above (general procedure) to yield 238 mg of the partially protected peptide resin (99% according to weight increase). A portion of the resin (222 mg, 69 μmol) was treated with TFA/H_2O/thioanisole (90:5:5; 2.0 mL) for 2 h. The mixture was filtered through a small plug of glass wool in a Pasteur pipet and the resin washed with TFA (3 × 0.5 mL). The combined filtrates were evaporated in a stream of dry nitrogen to ca. 2 mL, and the product was precipitated by the addition of cold anhydrous ether (12 mL). The precipitate was collected by centrifugation, washed with ether (3 × 5 mL), and dried. Plasma desorption mass spectroscopy (PDMS) analysis revealed that a substantial part of the crude material was still monotritylated. Detritylation was accomplished by renewed treatment with TFA/H_2O/thioanisole (2 mL) for 1 h. Precipitation and washing with ether as described above furnished 83.9 mg of the crude bicyclic Ang II analogue **11**. The peptide was dissolved in 0.1% aqueous TFA (12 mL) and purified by preparative RP-HPLC on a Vydac 10 μm C18 column (2.2 × 25 cm) with an 80 min gradient of 0−60% MeCN in 0.1% aqueous TFA at a flow rate of 4 mL/min. The separation was monitored at 230 nm and by PDMS. The yield of purified **11** was 17.1 mg (18%). Amino acid analysis: Asp, 0.97; Arg, 0.98; Tyr, 0.07; His, 0.96; Pro, 1.07; Phe, 1.02; Cys, not determined (nd) (73% peptide). PDMS (MW 1018.1): 1019.1 ($M + H^+$).

Ang II Analogue 12. The partially protected peptide resin was recovered in 97% yield according to the weight increase. A portion of the resin (95 mg, 30 μmol) was treated with TFA/H_2O/thioanisole using the two-step procedure described for **11** above to give 42.2 mg of the crude material. Preparative RP-HPLC, as described for **11**, but using a 60 min gradient of 0−40% MeCN furnished 15.2 mg (40%) of pure **12**. Amino acid analysis: Asp, 1.02; Arg, 0.99; Val, 1.00; Tyr, 0.99; His, 0.70; Phe, 1.01; Cys, nd (80% peptide). PDMS (MW 1020.0): 1021.7 ($M + H^+$), 1043.3 ($M + Na^+$).

Ang II Analogue 13. Automated SPPS on an 80 μmol scale provided 244 mg (90%) of the partially protected peptide resin. Part of this resin (227 mg, 71 μmol) was reacted with TFA/H_2O (95:5; 2.5 mL) for 2 h, and the product was isolated as described above for **11**. To a solution of the precipitate in TFA/H_2O (99:1; 0.75 mL) was added TFA/triethylsilane (95:5; 0.75 mL). After 10 min the peptide was precipitated and isolated in the usual way to yield 63.3 mg of crude product. The crude product was purified by RP-HPLC (three runs) on a 10 μm Vydac C18 column (1.0 × 25 cm) with a 60 min gradient of 10−40% MeCN in 0.1% TFA at a flow rate of 3 mL/min. The yield of pure **13** was 20.4 mg (21%). Amino acid analysis: Asp, 1.02; Arg, 0.99; Tyr, 0.02; His, 0.99; Pro, 1.00; Phe, 1.14 (overlapping with an impurity derived from Hcy); Hcy, nd (74% peptide). PDMS (MW 1032.1): 1033.6 ($M + H^+$), 1055.9 ($M + Na^+$).

Ang II Analogue 14. The partially protected peptide resin was synthesized in 88% yield according to the weight increase. The resin (223 mg, 69 μmol) was subjected to cleavage and cyclization using the conditions described for **13** to furnish 55.9 mg of the crude product. The peptide was purified (three runs) on the small C18 column using the chromatographic conditions given above (**13**). The yield of pure **14** was 15.8 mg (16%). Amino acid analysis: Asp, 1.01; Arg, 0.99; Val, 1.01; Tyr, 0.98; His, 0.72; Phe, 1.15 (overlapping with an impurity derived from Hcy); Hcy, nd (74% peptide). PDMS (MW 1034.0): 1035.7 ($M + H^+$), 1058.0 ($M + Na^+$).

Ang II Analogue 16. The partially protected resin (101 mg, 29 μmol) obtained in 97% yield was treated with TFA/H_2O/thioanisole (90:5:5; 1.5 mL) for 2 h using the two-step procedure described for **11** to provide 43.3 mg of crude product. After preparative RP-HPLC, using the conditions given for **11**, 15.0 mg (38%) of pure **16** was obtained. ^{1}H NMR spectra were obtained from a sample that was kept in DMSO for several hours, during which signal positions were altered, most likely due to dimerization of **16**. This was also supported by PDMS data (2047.2 for $M + H^+$). Amino acid analysis: Asp, 1.00; Arg, 1.00; Tyr, 1.00; His, 0.73; Phe, 1.00; Cys, nd (76% peptide). PDMS (MW 1024.0): 1024.9 ($M + H^+$).

Conformational Energy Calculations. The calculations of **17** and **18** were performed using the Amber* all-atom force field and the all-atom charge set as implemented in the program Macromodel version 5.5.[41] The generalized Born/surface area (GB/SA) method for water developed by Still[40] 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 ($n = 8$ in **17** and $n = 9$ in **18**). All torsion angles, except ω_2 and ω_5, were defined as rotatable. Conformational searches were conducted by use of the systematic unbound multiple minimum search (SUMM) method[61] in the Batchmin program (command SPMC). Ten thousand-step runs were performed, and those conformations within 5 kcal/mol of the global minimum were kept. Two ring closure bonds were defined for the bicyclic system. Torsional memory and geometrical preoptimization were used. Truncated Newton conjugated gradient (TNCG) minimization with a maximum of 5000 iterations was used in the conformational search with the derivate convergence set to a value of 0.001 (kJ/mol)/Å.

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Supporting Information Available: ^{1}H NMR (DMSO-d_6) spectral data and assignments of compounds **11**−**14** and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Smith, J. A.; Pease, L. G. Reverse Turns in Peptides and Proteins. *Crit. Rev. Biochem.* **1980**, *8*, 315−399.
(2) Rose, G. D.; Gierasch, L. M.; Smith, J. A. Turns in Peptides and Proteins. *Adv. Protein Chem.* **1985**, *37*, 1−109.
(3) Ball, J. B.; Alewood, P. F. Conformational Constraints: Nonpeptide β-Turn Mimics. *J. Mol. Recognit.* **1990**, *3*, 55−64.
(4) Hölzemann, G. Peptide Conformation Mimetics. *Kontakte (Darmstadt)* **1991**, 3−12.
(5) Hölzemann, G. Peptide Conformation Mimetics. *Kontakte (Darmstadt)* **1991**, 55−63.
(6) Giannis, A.; Kolter, T. Peptidomimetics for Receptor Ligands − Discovery, Development and Medical Perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.
(7) Liskamp, R. M. J. Conformationally Restricted Amino Acids and Dipeptides, (Non)peptidomimetics and Secondary Structure Mimetics. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1−19.
(8) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V. S.; Cook, C. M. Design and Synthesis of a Protein β-Turn Mimetic. *J. Am. Chem. Soc.* **1990**, *112*, 323−333.
(9) Gardner, B.; Nakanishi, H.; Kahn, M. Conformationally Constrained Nonpeptide β-Turn Mimetics of Enkephalin. *Tetrahedron* **1993**, *49*, 3433−3448.
(10) Newlander, K. A.; Callahan, J. F.; Moore, M. L.; Tomaszek, T. A., Jr.; Huffman, W. F. A Novel Constrained Reduced-Amide Inhibitor of HIV-1 Protease Derived from the Sequential Incorporation of γ-Turn Mimetics into a Model Substrate. *J. Med. Chem.* **1993**, *36*, 2321−2331.
(11) Schmidt, B.; Lindman, S.; Tong, W.; Lindeberg, G.; Gogoll, A.; Lai, Z.; Thörnwall, M.; Synnergren, B.; Nilsson, A.; Welch, C. J.; Sohtell, M.; Westerlund, C.; Nyberg, F.; Karlén, A.; Hallberg, A. Design, Synthesis, and Biological Activities of Four Angiotensin II Receptor Ligands with γ-Turn Mimetics Replacing Amino Acid Residues 3−5. *J. Med. Chem.* **1997**, *40*, 903−919.
(12) Brickmann, K.; Somfai, P.; Kihlberg, J. An Approach to Enantiomerically Pure Inverse γ-Turn Mimetics for Use in Solid-phase Synthesis. *Tetrahedron Lett.* **1997**, *38*, 3651−3654.
(13) Freidinger, R. M.; Veber, D. F.; Schwenk Perlow, D.; Brooks, J. R.; Saperstein, R. Bioactive Conformation of Luteinizing Hormone-Releasing Hormone: Evidence from a Conformationally Constrained Analogue. *Science* **1980**, *210*, 656−658.
(14) Nagai, U.; Sato, K. Synthesis of a Bicyclic Dipeptide with the Shape of β-Turn Central Part. *Tetrahedron Lett.* **1985**, *26*, 647−650.

(15) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. Design and Synthesis of a Novel Peptide β-Turn Mimetic. *J. Chem. Soc., Chem. Commun.* **1988**, 1447−1449.
(16) Genin, M. J.; Johnson, R. L. Design, Synthesis, and Conformational Analyses of a Novel Spiro-Bicyclic System as a Type II β-Turn Peptidomimetic. *J. Am. Chem. Soc.* **1992**, *114*, 8778−8783.
(17) Gramberg, D.; Weber, C.; Beeli, R.; Inglis, J.; Bruns, C.; Robinson, J. A. Synthesis of a Type-VI β-Turn Peptide Mimetic and Its Incorporation into a High-Affinity Somatostatin Receptor Ligand. *Helv. Chim. Acta* **1995**, *78*, 1588−1606.
(18) Subasinghe, N. L.; Bontems, R. J.; McIntee, E.; Mishra, R. K.; Johnson, R. L. Bicyclic Thiazolidine Lactam Peptidomimetics of the Dopamine Receptor Modulating Peptide Pro-Leu-Gly-NH_2. *J. Med. Chem.* **1993**, *36*, 2356−2361.
(19) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. Bicyclic Turned Dipeptide (BTD) as a β-Turn Mimetic; its Design, Synthesis and Incorporation into Bioactive Peptides. *Tetrahedron* **1993**, *49*, 3577−3592.
(20) Tran, T.-A.; Mattern, R.-H.; Zhu, Q.; Goodman, M. A Novel RGD Containing Dodecapeptidomimetic which Exhibits Selective Binding to the $\alpha_v\beta_3$ Receptor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 997−1002.
(21) Viles, J. H.; Patel, S. U.; Mitchell, J. B. O.; Moody, C. M.; Justice, D. E.; Uppenbrink, J.; Doyle, P. M.; Harris, C. J.; Sadler, P. J.; Thornton, J. M. Design, Synthesis and Structure of a Zinc Finger with an Artificial β-turn. *J. Mol. Biol.* **1998**, *279*, 973−986 and references therein.
(22) Wagner, J.; Kallen, J.; Ehrhardt, C.; Evenou, J.-P.; Wagner, D. Rational Design, Synthesis, and X-ray Structure of Selective Noncovalent Thrombin Inhibitors. *J. Med. Chem.* **1998**, *41*, 3664−3674 and references therein.
(23) Sheehan, J. C.; Henery-Logan, K. R. The Total Synthesis of Penicillin V. *J. Am. Chem. Soc.* **1959**, *81*, 3089−3094.
(24) 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.
(25) 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.
(26) Johnston, C. I. Biochemistry and Pharmacology of the Renin-Angiotensin System. *Drugs* **1990**, *39*, 21−31.
(27) Ramsamy, K.; Olsen, R. K.; Emery, T. Synthesis of *N*-*t*-Boc-L-α-aminoadipic Acid 1-*t*-Butyl 6-Ethyl Ester from L-Aspartic Acid: A New Route to L-α-Aminoadipic Acid. *Synthesis* **1982**, 42−43.
(28) Wernic, D.; DiMaio, J.; Adams, J. Enantiospecific Synthesis of L-α-Aminosuberic Acid. Synthetic Applications in Preparation of Atrial Natriuretic Factor Analogues. *J. Org. Chem.* **1989**, *54*, 4224−4228.
(29) Bergmeier, S. C.; Cobás, A. A.; Rapoport, H. Chirospecific Synthesis of (1S,3R)-1-Amino-3-(hydroxymethyl)cyclopentane, Precursor for Carbocyclic Nucleoside Synthesis. Dieckmann Cyclization with an α-Amino Acid. *J. Org. Chem.* **1993**, *58*, 2369−2376.
(30) Carpino, L. A.; Han, G. Y. The 9-Fluorenylmethoxycarbonyl Amino-Protecting Group. *J. Org. Chem.* **1972**, *37*, 3404−3409.
(31) Jung, M. E.; Andrus, W. A.; Ornstein, P. L. Non-Aqueous Conversion of Ketals to Ketones via Treatment with Trimethylsilyl Iodide. *Tetrahedron Lett.* **1977**, 4175−4178.
(32) Slusarchyk, W. A.; Robl, J. A.; Taunk, P. C.; Asaad, M. M.; Bird, J. E.; DiMarco, J.; Pan, Y. Dual Metalloprotease Inhibitors. V. Utilization of Bicyclic Azepinonethiazolidines and Azepinonetetrahydrothiazines in Constrained Peptidomimetics of Mercaptoacyl Dipeptides. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 753−758.
(33) Mueller, L. P.E.COSY, a Simple Alternative to E.COSY. *J. Magn. Reson.* **1987**, *72*, 191−196.
(34) Braunschweiler, L.; Ernst, R. R. Coherence Transfer by Isotropic Mixing: Application to Proton Correlation Spectroscopy. *J. Magn. Reson.* **1983**, *53*, 521−528.
(35) States, D. J.; Haberkorn, R. A.; Ruben, D. J. A Two-Dimensional Nuclear Overhauser Experiment with Pure Absorption Phase in Four Quadrants. *J. Magn. Reson.* **1982**, *48*, 286−292.
(36) Wider, G.; Macura, S.; Kumar, A.; Ernst, R. R.; Wüthrich, K. Homonuclear Two-Dimensional ^{1}H NMR of Proteins. Experimental Procedures. *J. Magn. Reson.* **1984**, *56*, 207−234.
(37) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; 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.
(38) Summers, M. F.; Marzilli, L. G.; Bax, A. Complete ^{1}H and ^{13}C Assignments of Coenzyme B_{12} through the Use of New Two-Dimensional NMR Experiments. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.
(39) Kövér, K. E.; Prakash, O.; Hruby, V. J. Improved 2D Inverse Proton Detected C,H Correlation NMR Techniques for the Total Assignment of Carbon Resonances of a Highly Delta Opioid Receptor Agonist Peptide. *Magn. Reson. Chem.* **1993**, *31*, 231−237.
(40) 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.
(41) 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.
(42) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. Chain Reversals in Proteins. *Biochim. Biophys. Acta* **1973**, *303*, 211−229.
(43) Richardson, J. S. The Anatomy and Taxonomy of Protein Structure. *Adv. Protein Chem.* **1981**, *34*, 167−399.
(44) Hutchinson, E. G.; Thornton, J. M. A Revised Set of Potentials for β-Turn Formation in Proteins. *Protein Sci.* **1994**, *3*, 2207−2216.
(45) Sibanda, B. L.; Thornton, J. M. β-Hairpin Families in Globular Proteins. *Nature* **1985**, *316*, 170−174.
(46) Dyson, H. J.; Rance, M.; Houghten, R. A.; Lerner, R. A.; Wright, P. E. Folding of Immunogenic Peptide Fragments of Proteins in Water Solution. *J. Mol. Biol.* **1988**, *201*, 161−200.
(47) 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.
(48) 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.
(49) Perlman, S.; Schambye, H. T.; Rivero, R. A.; Greenlee, W. J.; Hjorth, S. A.; Schwartz, T. W. Non-peptide Angiotensin Agonist. *J. Biol. Chem.* **1995**, *270*, 1493−1496.
(50) CHO cells stably expressing the rat AT_1 receptor were generously provided by Professor Thue Schwartz, University Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark.
(51) Sugg, E. E.; Dolan, C. A.; Patchett, A. A.; Chang, R. S. L.; Faust, K. A.; Lotti, V. J. Cyclic Disulfide Analogues of [Sar^1,Ile^8]-angiotensin II. In Peptides: Chemistry, Structure and Biology: Proceedings of the Eleventh American Peptide Symposium; Rivier, J. E., Marshall, G. R., Eds.; ESCOM Publishers: Leiden, 1990; pp 305−306.
(52) 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 Analogues Having High Potency. *J. Med. Chem.* **1990**, *33*, 1935−1940.
(53) 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.
(54) Zhang, W.-J.; Nikiforovich, G. V.; Pérodin, J.; Richard, D. E.; Escher, E.; Marshall, G. R. Novel Cyclic Analogues of Angiotensin II with Cyclization between Positions 5 and 7: Conformational and Biological Implications. *J. Med. Chem.* **1996**, *39*, 2738−2744.
(55) Vlahakos, D. V.; 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 Analogue [Sar^1,Lys^3,Glu^5]ANG II. *Lett. Pept. Sci.* **1996**, *3*, 191−194.
(56) 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.
(57) 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.
(58) Nikiforovich, G. V.; Kao, J. L.-F.; Plucinska, K.; Zhang, W.-J.; Marshall, G. R. Conformational Analysis of Two Cyclic Analogues of Angiotensin: Implications for the Biologically Active Conformation. *Biochemistry* **1994**, *33*, 3591−3598.
(59) Joseph, M.-P.; Maigret, B.; Scheraga, H. A. Proposals for the Angiotensin II Receptor-Bound Conformation by Comparative Computer Modeling of AII and Cyclic Analogues. *Int. J. Pept. Protein Res.* **1995**, *46*, 514−526.
(60) Balodis, J.; Golbraikh, A. Conformational Analysis of Cyclic Angiotensin II Analogues. *Lett. Pept. Sci.* **1996**, *3*, 195−199.
(61) Goodman, J. M.; Still, W. C. An Unbounded Systematic Search of Conformational Space. *J. Comput. Chem.* **1991**, *12*, 1110−1117.

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