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Comparison of Three γ-Turn Mimetic Scaffolds Incorporated into Angiotensin II

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Abstract—Rigidification of peptides by cyclization and iterative incorporation of well-defined secondary structure mimetics constitutes one approach to the design of non-peptidergic structures with better defined conformations. We herein present the synthesis of a potential γ-turn mimetic scaffold, and its incorporation in the 3–5 position of angiotensin II. Two analogues of angiotensin II (Ang II) incorporating this 1,3,5-trisubstituted benzene γ-turn scaffold were synthesized. Evaluation of the compounds in a radioligand binding assay showed that they lacked affinity to the AT_1 receptor. To rationalize these results a geometrical and electrostatical comparison with Ang II analogues encompassing a bicyclic scaffold that delivered inactive pseudo peptides and an azepine scaffold producing highly active ligands was made. This analysis did not provide a clear rationale for the inactivity of the benzene γ-turn scaffolds.

Introduction

Determination of the bioactive conformation(s) of important peptides remains an exciting challenge. In drug discovery processes where such peptides serve as starting points for design, the ultimate goal is to identify non-peptidic molecules with high bioavailability and with retained biological activity. Since no three-dimensional structural data are available for peptides interacting with G-protein coupled receptor targets, crucial insights into the topological requirements within a peptide–receptor complex have to be gained in an indirect fashion. One common strategy is to enforce the recognition elements into the correct region of space by the iterative introduction of various conformational constraints at selected sites of the peptide. Monocyclization by disulfide or amide bond formation constitutes experimentally facile procedures that efficiently restrict the flexibility of linear peptides.[1] These methods should preferably be exploited prior to the initiation of often-tedious synthetic programs aimed at more complex organic scaffolds. Thus, after the identification of a constrained peptide with the desired properties, subsequent conformational analyses of the ring systems will guide the selection of a suitable organic scaffold to substitute and to mimic the secondary structure motif.[2–6]

Cyclization between the i and $i+2$ residues with penicillamine (Fig. 1) has been of particular interest to us for two reasons. Firstly, incorporation of c[Pen^{3,5}] in angiotensin II (Ang II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), see Figure 2, delivered a full agonist with contractile activity in isolated rabbit aortic strips and with high binding affinity to the AT_1 receptor.[7,8] Secondly, theoretical analyses and NMR studies of the 11-membered c[Pen^{3,5}] ring system (Fig. 1) suggested a strong preference for the adoption of an inverse γ-turn-like conformation. Therefore, it seemed that γ-turn mimetics[7,9–19] should be the prime candidates for incorporation into peptides where 1,3-penicillamine cyclization affords desirable biological responses.[7]

We aim to develop γ-turn mimetics that are easy to prepare and that allow introduction of a large variety of side-chains, including non-natural, from a common precursor. Ang II, with a suggested turn centered around Tyr-4,[8,20–26] was identified as a suitable target peptide for biological evaluation of diverse turn mimetics.[7,27,28] We previously

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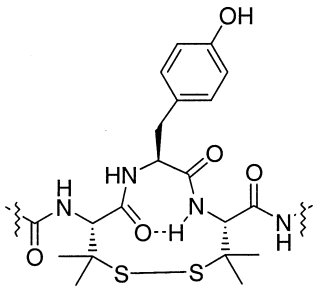


Figure 1. c[Pen^{3,5}] ring system in Ang II adopting a γ-turn conformation.

replaced amino acids 3–5 in c[Pen^{3,5}]Ang II with an azepine γ-turn mimetic, giving **1** and **2** (Fig. 2).[7] The synthesized diastereomers exerted similar affinity as c[Pen^{3,5}]Ang II for the AT_1 receptor. Interestingly, one diastereomer was an agonist with full contractile activity and almost equipotent with c[Pen^{3,5}]Ang II. Unfortunately, this azepine γ-turn mimetic required considerable efforts to synthesize.

When an alternative bicyclic γ-turn mimetic was introduced instead of amino acid residues 3–5 in c[Pen^{3,5}]Ang II to give **3** and **4** (Fig. 2), no receptor binding affinity was observed.[7] We speculated that the lack of affinity was due to the extra steric bulk of the bicyclic ring system, which may give rise to a negative steric interaction with the receptor. To challenge this hypothesis we have now designed and synthesized the geometrically smaller and synthetically more accessible 1,3,5-trisubstituted benzene γ-turn scaffold and incorporated this into Ang II to give **5** and **6**. We herein present the synthesis of these compounds and a comparison of the benzene scaffold in **5** and **6**, the bicyclic scaffold in **3** and **4** and the azepine scaffold in **1** and **2** as γ-turn mimetics.

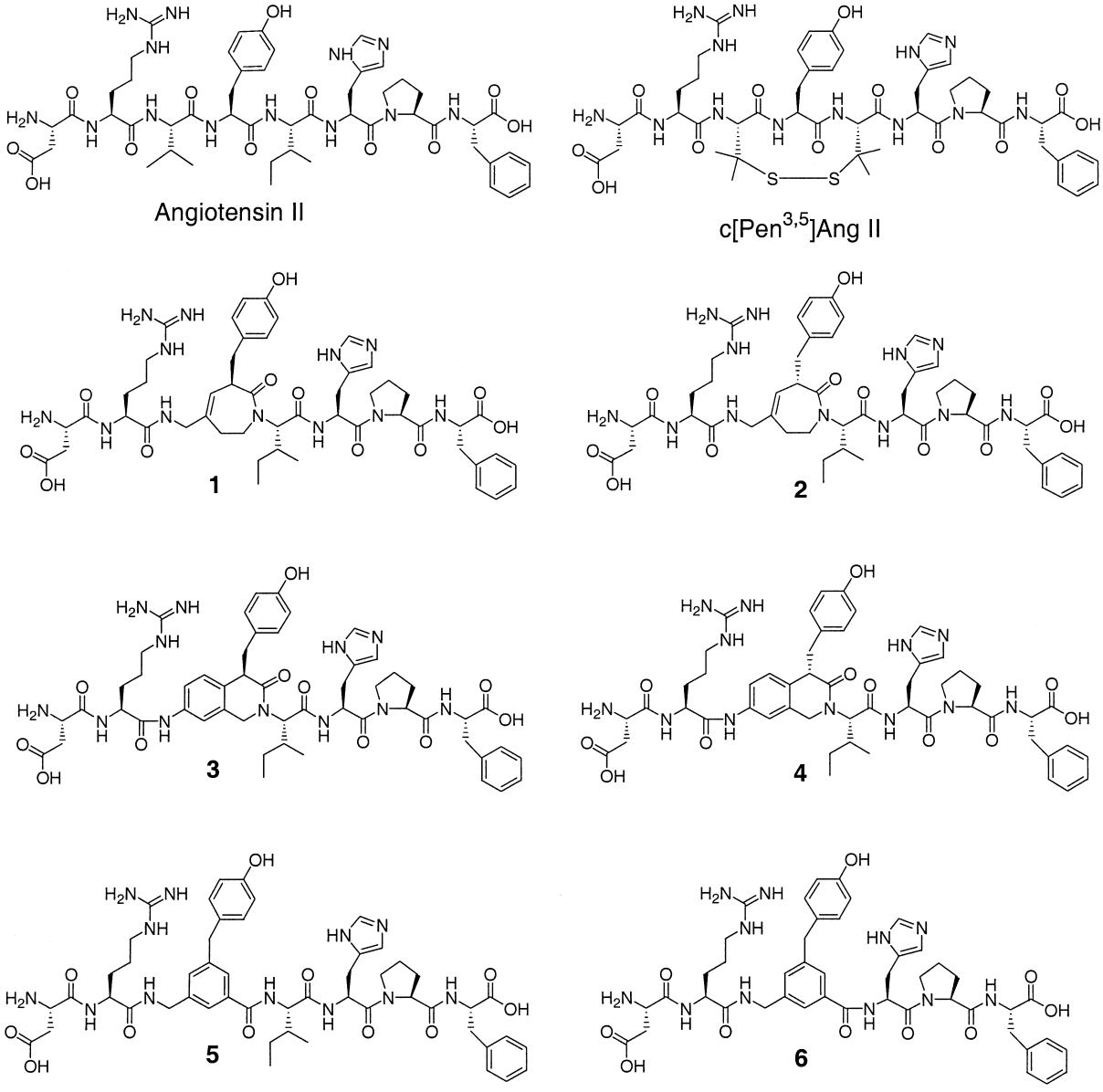
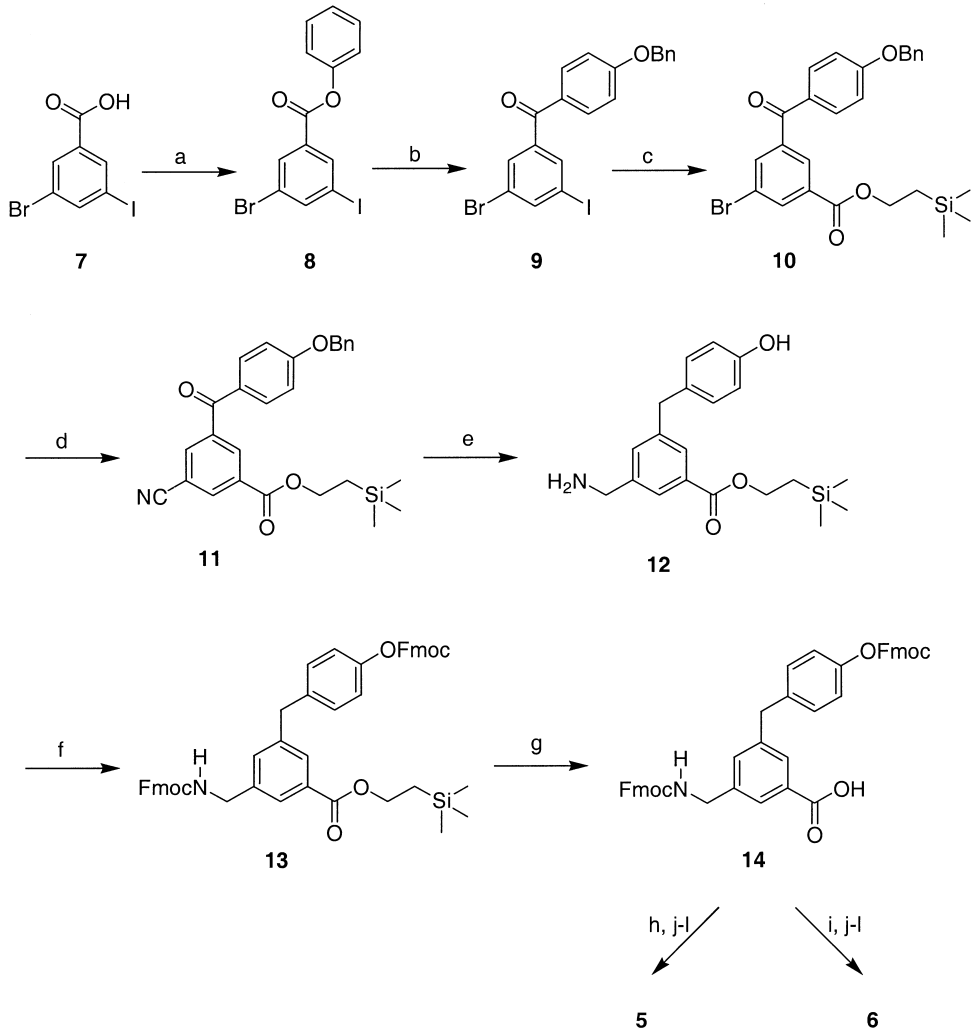


Figure 2. Angiotensin II and analogues incorporating potential γ-turn scaffolds.

Chemistry

The γ-turn mimetic scaffolds in compounds **1** and **2** were prepared essentially according to the procedure outlined by Huffman[11–13] and relied on Birch reduction, ozonolysis, lactam-cyclization and alkylation as key transformations.[7] The synthesis of the bicyclic scaffold in **3** and **4** were smoothly conducted from 3-isochromanone via alkylation, lactone-ring opening and cyclization.[7] The preparation of the aromatic scaffold of **5** and **6** is outlined in Scheme 1. The commercially available 3-bromo-5-iodobenzoic acid **7** underwent esterification[29] with phenol and *N,N′*-dicyclohexylcarbodiimide under standard conditions to deliver **8**. Fries reaction[30,31] afforded under the conditions applied predominantly *p*-benzoylation and after benzylation **9** was isolated. Direct Friedel–Crafts benzoylation of benzyl phenyl ether and **7** with polyphosphoric acid, or alternatively with $AlCl_3$ starting from the corresponding acyl chloride, was initially attempted but met with failure, despite the fact that in a control experiment with benzoic acid or with benzoyl chloride the reaction proceeded smoothly. The carbonylation[32,33] of **9** was conducted at 1 atmosphere with palladium acetate as precatalyst and dppp[34] as ligand and in the presence of an excess of trimethylsilyl ethanol to furnish the TMSE ester[35] **10** in 69% yield. The cyanation was thereafter performed with palladium tetrakistriphenylphosphine as catalyst and with zinc cyanide as additive in DMF according to a procedure developed by Tschaen,[36] and **11** was isolated in 87% yield. By hydrogenation on Pd/C using the classical conditions for hydrogenolysis of benzylic ketones,[37] debenzylation and reduction of the nitrile and carbonyl groups were performed and **12** was obtained in a single step. Fmoc protection of the amine and the phenolic hydroxyl groups preceded a TFA mediated liberation of the carboxy group and the building block **14** was obtained. Subjection to standard solid phase chemistry provided **5** and **6**. In the preparation of Ang II analogue **6,** His was protected as the less bulky Boc-derivative instead of Trt in order to diminish the steric hindrance during coupling.



Scheme 1. Reagents: (a) Phenol, DCC, DMAP, Et_2O, 73%; (b) (i) $AlCl_3$; (ii) BnBr, K_2CO_3, acetone, 33%; (c) CO, $Pd(OAc)_2$, dppp, Et_3N, $(CH_3)_3SiCH_2CH_2OH$, DMF, 69%; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 87%; (e) H_2, Pd/C, HCl, EtOH, EtOAc, 37%; (f) Fmoc-Cl, Et_3N, CH_2Cl_2, 64%; (g) TFA, CH_2Cl_2, 81%; (h) (i) Ile-His(Trt)-Pro-Phe-Wang resin, PyBOP, HOBt, DIEA, DMF, (ii) piperidine, DMF; (i) (i) His(Boc)-Pro-Phe-2-chlorotrityl resin, PyBOP, HOBt, DIEA, DMF; (ii) piperidine, DMF; (j) (i) Fmoc-Arg(Pbf), PyBOP, HOBt, DIEA, DMF, (ii) piperidine, DMF; (k) (i) Fmoc-Asp(O′Bu), PyBOP, HOBt, DIEA, DMF, (ii) piperidine, DMF; (l) TFA:H_2O:triethylsilane (90:5:5).

Results and Discussion

γ-Turns are defined by three adjoining amino acid residues forming a pseudo-seven-membered ring. Two types of γ-turns have been defined, the inverse γ-turn which is the most common in proteins[38,39] and the classic γ-turn. In the inverse γ-turn conformation the $i+1$ substituent assumes a pseudo equatorial position while in the classic γ-turn conformation the $i+1$ substituent is pseudo-axially oriented (Fig. 3).[40,41]

The general framework of the 1,3,5-trisubstituted benzene scaffold fits well within the dimensions defined for the inverse and classic γ-turn[42] (Fig. 3a and b). The overall fit is better to the inverse γ-turn, since in both the 1,3,5-trisubstituted benzene scaffold and the inverse γ-turn the $i+1$ substituent is positioned in a pseudo-equatorial orientation.

Further inspection of Figure 3 shows that the atom types in the C-terminal end of the γ-turn and the aromatic γ-turn mimetic are out of phase. The $C_{\alpha i+2}$ atom of the γ-turn is superimposed on the carbonyl carbon of the turn mimetic. This creates a problem concerning what amino sequence should be coupled to the C-terminal end of the scaffold in order to mimic Ang II. If $C_{\alpha i+2}$ of the mimetic is hypothesized to correspond to the atom after the benzamide moiety then Ile-His-Pro-Phe should be used. However, if $C_{\alpha i+2}$ corresponds to the aromatic carbon of the benzene ring then His-Pro-Phe should be used. In the latter case the Ile side-chain is assumed to be mimicked by a part of the benzene ring. We synthesized both of the alternative compounds, i.e. **5** and **6**.

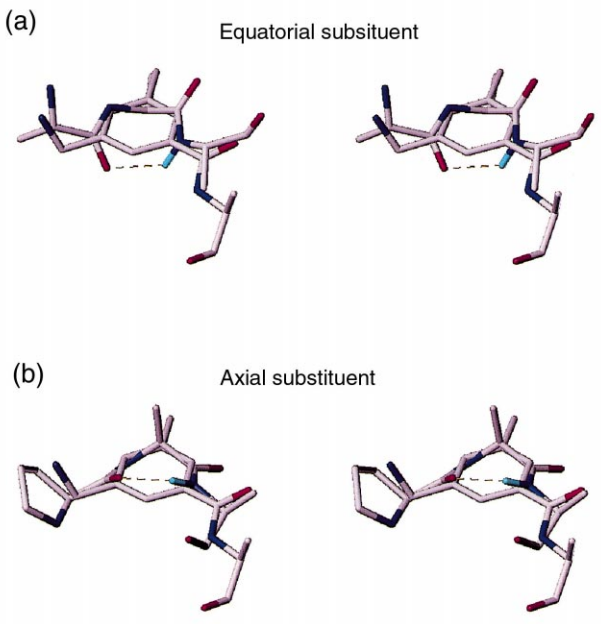


Figure 3. (a) Stereo image of the rms best fit of the 1,3,5-substituted benzene scaffold and an inverse γ-turn ($i+1$ substituent pseudo-equatorial).[42] (b) Stereo image of the rms best fit of the 1,3,5-substituted benzene scaffold and a classic γ-turn ($i+1$ substituent pseudo-axial).[42] In both Figure (a) and (b), C_i(O), N_{i+1}, $C_{\alpha i+1}$, C_{i+1}(O), N_{i+2}, $C_{\alpha i+2}$, C_{i+2}(O) of the γ-turn and the corresponding atoms in the benzene ring were included in the fitting procedure. For clarity, the hydrogens are omitted and only the C_β atom of the side-chains is displayed.

Furthermore, in accordance with the design and synthesis of the analogues **1** and **2**[7] we substituted Val-3 by Gly in **5** and **6**.

Compounds **5** and **6** were evaluated in a radioligand binding assay based on displacement of [^{125}I]-Ang II from AT_1 receptors in rat liver membranes. Disappointingly, both compounds lacked affinity for the AT_1 receptor ($IC_{50} > 10\,\mu M$). Ang II displayed an affinity with a K_i-value of 0.9 nM. There may be several reasons for the lack of binding affinity of compounds **5** and **6** as compared to **1** and **2.** We decided to focus on the following: (a) they are not geometrical, similar and/or (b) the electronic character of the benzene scaffold differs from that of the azepine scaffold.

It has already been demonstrated by the use of X-ray crystallography[43] and theoretical calculations[9] that there is a good overall geometrical similarity between the azepine γ-turn mimetic incorporated in **1** and **2** and the inverse γ-turn. We therefore used the azepine mimetic as our common frame of reference. The similarity between the γ-turn mimetic scaffolds compared in this study was assessed by recording the bond distances and angles of the AM1[44] minimized truncated model compounds **15**–**18** shown in Figure 4. We focused on the distances and angles between the three C_α-atoms within the γ-turn moiety. The γ-turn mimetic scaffolds in compounds **15** and **16** can adopt both the classic and inverse γ-turn, while the scaffold in **17** can only adopt the inverse γ-turn. In these comparisons we considered only the inverse γ-turn conformation. The measured distances are given outside and angles inside the triangles in Figure 4.

In the comparison between model compounds **15** and **16** the largest deviation is found in the distance from $C_{\alpha i}$ to $C_{\alpha i+1}$ and $C_{\alpha i}$ to $C_{\alpha i+2}$ ($C_{\alpha i}$ in **16** is assumed to correspond to the aromatic carbon of the bicyclic system as depicted in Figure 4). The other distances and angles compare well and a likely reason for the inactivity of **3** and **4** is thus the extra steric bulk of the bicyclic system.

In the comparison between the model compounds **15** and **17** it is evident that the γ-turn moieties are different. For example, the $C_{\alpha i}$ to $C_{\alpha i+2}$ and $C_{\alpha i+1}$ to $C_{\alpha i+2}$ distances are significantly shorter, which is also demonstrated in Figures 4 and 5d. In **18** the flexibility of the C-terminal side-chain prevents the unambiguous measurement of distances and angles. However, visual inspection of Figure 4 and preliminary conformational analysis studies shows that the $C_{\alpha i}$ to $C_{\alpha i+1}$ and $C_{\alpha i}$ to $C_{\alpha i+2}$ distances are longer in **18** than in **15**. Thus, due to the unfavorable shift in the C-terminal end of the 1,3,5-trisubstituted benzene scaffold, as compared to the γ-turn, a sub-optimal geometrical fit is obtained, making the γ-turn moiety either too wide or too compact.

The electronic properties of the three γ-turn mimetic scaffolds of the model compounds of **1, 3** and **6** were also compared using the AM1 optimized geometries shown in Figure 4. The electrostatic potential was mapped onto the molecular surface of the model compounds **15**, **16** and **17** of the mimetics using MOLCAD within

SYBYL.[45] As is obvious from Figure 5 there are similarities but also differences between the scaffolds studied. For example, there is an electronegative potential in the center of the ring systems although this is somewhat less pronounced in the azepine scaffold. However, taken together we conclude that a comparison of the electrostatic potential of the molecular surface does not provide a rationale for the inactivity of **3**–**6**.

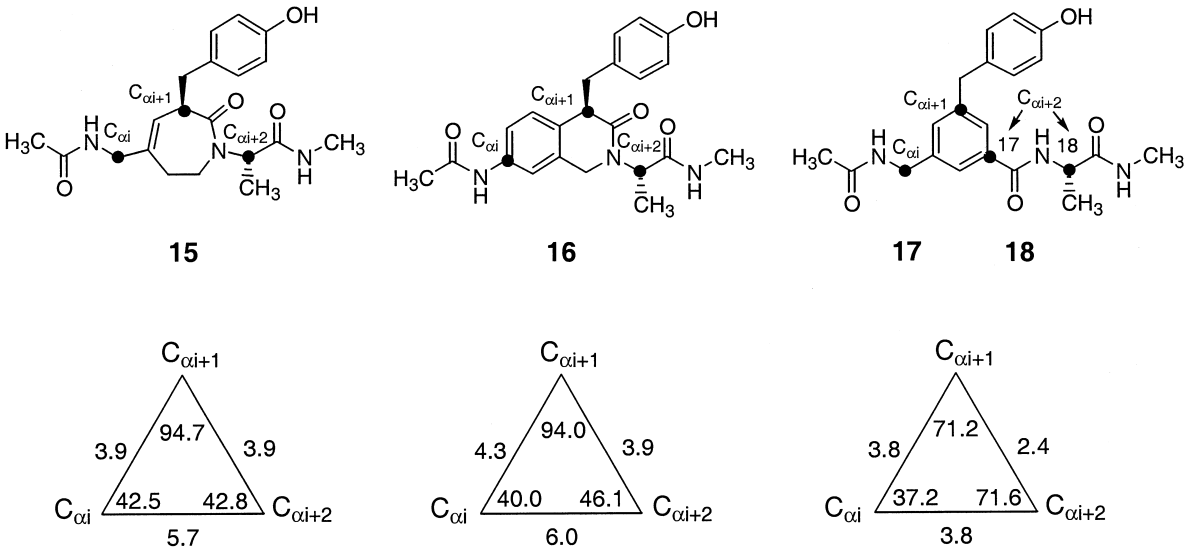


Figure 4. Distances and angles between C_α atoms of model compounds of 1 (**15**), 3 (**16**) and 6 (**17**) incorporating potential γ-turn scaffolds. Compounds were minimized with the AM1 method.[44] Distances are given outside the triangle and angles inside the triangle. C_α atoms are highlighted with a filled circle.

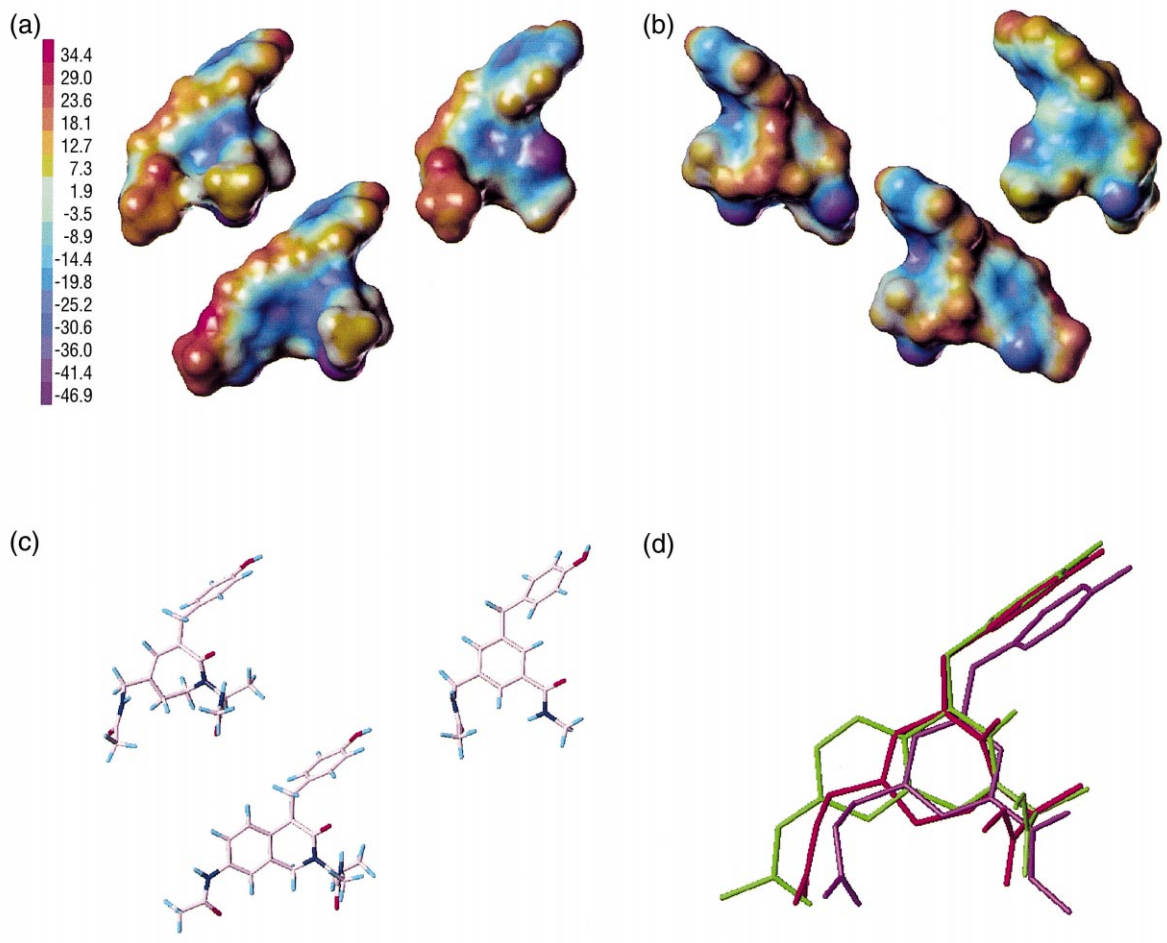


Figure 5. (a) Electrostatic potential mapped onto the Connolly surface of **15**, **16** and **17**. Negative charge on the surface corresponds to blue colors and positive charge to red colors. (b) Same molecules as in Figure 5(a) but they have been rotated 180 degrees along the Y-axis. (c) Same as in Figure 5(a) but only the molecules are shown. (d) Superimposition of molecules **15** (red), **16** (green) and **17** (violet). The C_α atoms were used in the molecular fit.

Conclusion

Previous attempts to substitute amino acids 3–5 in Ang II with an azepine γ-turn mimetic delivered analogues which had high affinity and agonistic activity, but were laborious to synthesize. When an alternative, slightly more bulky bicyclic mimetic was introduced in Ang II in the same position no binding affinity was observed. It was assumed that the lack of affinity was attributed to the extra steric bulk and therefore we incorporated a smaller and synthetically more accessible 1,3,5-substituted benzene scaffold in the 3–5 position of Ang II giving two analogues, which also lacked affinity for the AT_1 receptor. This may be due to a sub-optimal similarity of the benzene scaffold with the azepine scaffold and/or to the shift in amino acid sequence that occurs between c[Pen^{3,5}]Ang II and compounds **5** and **6.** An alternative explanation may be that the different geometry of the benzene scaffolds compared to the azepine scaffold prevents the pharmacophore groups from adopting the bioactive conformation. Taken together, the results presented here and in previous studies[7,8,27,28] indicate that modifications of Ang II in the 3–5 region have a dramatic effect on its interaction with the AT_1 receptor.

Experimental

Chemistry

General comments. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM EX270 spectrometer at 270 and 67.8 MHz respectively. Spectra were recorded at ambient temperature. Chemical shifts are reported as δ values (ppm), referenced to Me_4Si. Low-resolution electron-impact MS spectra were measured at an ionization potential of 70 eV. The mass detector was interfaced with a gas chromatograph equipped with an HP-1 (25 m×0.20 mm) column. Infrared spectra were recorded as solids on a Perkin–Elmer Model 1605 FT-IR instrument mounted with a Microfocus Beam Condenser with ZnSe lenses in a Diasqueeze Plus Diamond Compressor Cell (Graseby Specac Inc., Smyrna, USA) and are reported as ν_{max} (cm^{-1}). Melting points (uncorrected) were determined in open glass capillaries in a melting point microscope. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden and were within 0.4% of the calculated values. Column chromatography was performed using Merck silica gel 60 (40–63 μm). Thin-layer chromatography (TLC) was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). Chromatographic spots were visualized by UV and/or spraying with an acidic, ethanolic solution of *p*-anisaldehyde or an ethanolic solution of ninhydrin followed by heating. 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 hydrolysates with an LKB 4151 alpha plus analyzer, using ninhydrin detection.

Materials. 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. 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) were purchased from Richelieu Biotechnologies (St Hyacinthe, QC, Canada). (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) was purchased from Calbiochem-Novabiochem (Läufelfingen, Switzerland). 3-Bromo-5-iodobenzoic acid was obtained from Aldrich. THF was distilled from sodium/benzophenone before use. All other reagents were obtained from commercial sources and used as received.

Solid-phase peptide synthesis (SPPS). The starting resins were synthesized on a 150 μmol scale with a Symphony instrument (Protein Technologies Inc., Tucson, AZ) using Fmoc/*tert*-butyl protection. The side-chain protecting groups were as follows: Asp(O′Bu), Arg(Pbf) and His(Trt) or His(Boc). Removal of the Fmoc group was achieved by reaction with 20% piperidine in DMF for 5 + 10 min. Coupling of the amino acids (200 μmol) was done in DMF (2.5 mL) using HBTU (200 μmol) in the presence of *N*-methylmorpholine, NMM (500 μmol). Double couplings (2×30 min) were used for all amino acids. After the introduction of each amino acid, remaining amino groups were capped by addition of 20% acetic anhydride in DMF (2.5 mL) to the coupling mixture and allowing the reaction to proceed for 5 min. The couplings of the aromatic building block and the subsequent amino acids (3 equiv) were done manually with PyBOP (3 equiv) in the presence of HOBt (3 equiv) and diisopropylethylamine, DIEA (6 equiv), in DMF (0.7 mL). 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. The peptides were cleaved and deprotected by TFA:H_2O:triethylsilane (90:5:5), precipitated and purified by preparative RP-HPLC. Yields for the purified Ang II analogues were corrected for peptide content.

Phenyl(3-bromo-5-iodo)benzoate (8). Phenol (2.26 g, 24 mmol), *N,N′*-dicyclohexylcarbodiimide (DCC) (4.95 g, 24 mmol) and 4-dimethylaminopyridine (0.24 g, 2 mmol) were added to a solution of 3-bromo-5-iodobenzoic acid **7** (6.54 g, 20 mmol) in diethyl ether (250 mL) at 0 °C. The reaction mixture was allowed to reach rt overnight. The *N,N′*-dicyclohexyl urea was filtered off and the filtrate was washed with water (3×180 mL), 5% aqueous acetic acid (3×180 mL), water (3×180 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (isohexane:EtOAc, 9:1, to EtOAc) to give **8** (5.86 g, 73%) TLC R_f 0.61 (isohexane:EtOAc, 9:1); mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 7.15–7.23 (m, 2H, Ar), 7.25–7.34 (m, 1H, Ar), 7.39–7.49 (m, 2H, Ar), 8.11 (dd, 1H, Ar), 8.29 (dd, 1H, Ar), 8.45 (dd, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 94.1, 121.4, 123.2, 126.3, 129.6, 132.4, 132.7, 137.6, 144.4, 150.4, 162.4; IR (solid)

1725 cm^{-1}; MS [IP 70 eV; *m*/*z* (% rel int)] 404 ($M^+ + 2$, 18), 402 (M^+, 18), 311 (100), 309 (100). Anal. ($C_{13}H_8BrIO_2$) C, H.

3-Bromo-5-iodo-4′-benzyloxybenzophenone (9). Phenyl (3-bromo-5-iodo)benzoate **8** (3.19 g, 7.91 mmol) and $AlCl_3$ (2.11 g, 15.8 mmol) were vigorously stirred and heated in a preheated oil bath (145 °C). The reaction mixture melted and a white fume developed. When a red fume started to develop after about 30 min the reaction was stopped and the reaction mixture was allowed to reach rt. The solid was dissolved in EtOAc (50 mL) and 1 M HCl (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2×30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was filtered through silica (isohexane:EtOAc, 9:1, to EtOAc) to give a residue (1.05 g), which was dissolved with acetone (25 mL) and K_2CO_3 (0.38 g, 2.8 mmol) and benzyl bromide (0.52 g, 3.04 mmol) was added. The reaction mixture was heated to reflux. After 5 h the reaction mixture was cooled and diluted with ether (50 mL). The organic layer was washed with H_2O (50 mL), 2 M NaOH (3×50 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (isohexane:EtOAc, 9:1, to EtOAc) to give **9** as a solid (1.10 g, 28%). TLC R_f 0.50 (isohexane:EtOAc, 9:1); mp 104–106 °C; ^{1}H NMR ($CDCl_3$) δ 5.16 (s, 2H, OCH_2Ph), 7.06 (dm, J = 8.9 Hz, 2H, 3′), 7.34–7.47 (m, 5H, BnAr), 7.76 (dd, 1H, Ar), 7.80 (dm, J = 8.9 Hz, 2H, 2′), 7.97 (dd, 1H, Ar), 8.04 (dd, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 70.2, 94.2, 114.7, 122.9, 127.4, 128.3, 128.7, 129.0, 131.7, 132.5, 135.9, 136.8, 141.4, 142.5, 162.9, 192.0; IR (solid) 1648 cm^{-1}; MS [IP 70 eV; *m*/*z* (% rel int)] 494 ($M^+ + 2$, 4), 492 (M^+, 4), 91 (100). Anal. ($C_{20}H_{14}BrIO_2$) C, H.

[2-(Trimethylsilyl)ethyl] 3-(4′-benzyloxy)benzoyl-5-bromobenzoate (10). $Pd(OAc)_2$ (14.6 mg, 0.065 mmol) and dppp (26.9 mg, 0.065 mmol) were mixed in DMF (2 mL) under a stream of nitrogen. The yellow mixture was stirred for 10 min before the addition of a solution of **9** (1.07 g, 2.17 mmol) in DMF (8 mL), triethylamine (0.36 mL, 2.6 mmol) and trimethylsilylethanol (2.56 g, 21.7 mmol). The reaction mixture was stirred and CO was bubbled through the solution at rt for 20 min before the dark brown–red solution was heated to 85 °C. After 5 h CO-bubbling, the mixture was placed under 1 atm of CO. After 2 days the reaction mixture was cooled and concentrated. The residue was dissolved in ether (50 mL), washed with brine (2×40 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (isohexane:EtOAc, 9:1) to give **10** (0.75 g, 69%). TLC R_f 0.36 (isohexane:EtOAc, 9:1); ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 9H, Me_3), 1.14 (m, 2H, CH_2Si), 4.44 (m, 2H, OCH_2), 5.17 (s, 2H, OCH_2Ph), 7.06 (dm, J = 8.9 Hz, 2H, 3′), 7.32–7.48 (m, 5H, Bn), 7.80 (dm, J = 8.9 Hz, 2H, 2′), 8.06 (dd, 1H, Ar), 8.28 (dd, 1H, Ar), 8.35 (dd, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ −1.5, 17.4, 64.1, 70.2, 114.7, 122.6, 127.4, 128.3, 128.7, 129.0, 129.2, 132.55, 132.56, 135.4, 136.0, 136.2, 140.3, 162.9, 164.7, 192.9; IR (solid) 1722, 1658 cm^{-1}; MS [IP 70 eV; *m*/*z* (% rel int)] (M^+ missing) 484/482 ($M^+ - 28$, 1:1), 91 (100), 73 (8). Anal. ($C_{26}H_{27}BrO_4Si$) C, H.

[2-(Trimethylsilyl)ethyl]3-(4′-benzyloxy)benzoyl-5-cyanobenzoate (11). $Zn(CN)_2$ (31.3 mg, 0.267 mmol) and $Pd(PPh_3)_4$ (26.4 mg, 0.023 mmol) were mixed in DMF (1 mL) under a stream of nitrogen. Compound **10** (195 mg, 0.381 mmol) dissolved in DMF (1 mL) was added to the mixture. The heavy-walled Pyrex tube was sealed with a screw cap fitted with a Teflon gasket, and the reaction mixture was stirred at 80 °C for 6 h. After cooling, the black mixture was diluted with EtOAc (20 mL), washed with aqueous saturated Na_2CO_3 (3×15 mL) and brine (3×15 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (isohexane: EtOAc, 9:1) to give **11** as a solid (138 mg, 87%). TLC R_f 0.28 (isohexane:EtOAc, 9:1); mp 73–75 °C; ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 9H, Me_3), 1.16 (m, 2H, CH_2Si), 4.47 (m, 2H, OCH_2), 5.18 (s, 2H, OCH_2Ph), 7.08 (dm, J = 9.1 Hz, 2H, 3′), 7.25–7.66 (m, 5H, Bn), 7.79 (dm, J = 9.1 Hz, 2H, 2′), 8.18 (dd, 1H, Ar), 8.48 (dd, 1H, Ar), 8.58 (dd, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ −1.5, 17.5, 64.6, 70.3, 113.2, 114.9, 117.2, 127.4, 128.4, 128.68, 128.71, 132.2, 132.6, 134.0, 135.5, 135.8, 136.4, 139.8, 163.2, 164.1, 192.1; IR (solid) 2238, 1713, 1655 cm^{-1}. Anal. ($C_{27}H_{27}NO_4Si$) C, H, N.

[2-(Trimethylsilyl)ethyl]3-aminomethyl-5-(4′-hydroxybenzyl)benzoate (12). A mixture of compound **11** (150 mg, 0.546 mmol) and 10% Pd(C) (50 mg, 0.047 mmol Pd) in absolute ethanol (10 mL), EtOAc (4 mL) and 3 M HCl (10 mL) was stirred under H_2 (1 atm) overnight. The mixture was adjusted to pH 8 using aqueous saturated $NaHCO_3$ and concentrated. The residue was dissolved in CH_2Cl_2:EtOH (95:5), filtered through a pad of Celite and concentrated. The residue was dissolved in water (10 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated and purified by column chromatography ($CHCl_3$:MeOH, 9:1, to $CHCl_3$:MeOH, 1:1) to give **12** as a solid (73 mg, 37%). TLC R_f 0.31 ($CHCl_3$: MeOH, 1:1); mp 111–113 °C; ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 9H, Me_3), 1.11 (m, 2H, CH_2Si), 3.75–3.89 (br s, 2H, NH_2), 3.85 (s, 2H, CH_2NH_2), 3.87 (s, 2H, CH_2Ph), 4.38 (m, 2H, OCH_2), 6.68 (dm, J = 8.5 Hz, 2H, 3′), 6.96 (dm, J = 8.5 Hz, 2H, 2′), 7.29 (dd, 1H, Ar), 7.77 (m, 2H, 2×Ar); ^{13}C NMR ($CDCl_3$) δ −1.5, 17.4, 40.8, 45.6, 63.4, 115.7, 126.1, 128.7, 129.9, 131.0, 131.6, 132.3, 141.9, 142.7, 155.0, 166.8; IR (solid) 3354, 3286, 3500–2300, 1713 cm^{-1}. Anal. ($C_{20}H_{27}NO_3Si$) C, H, N.

[2-(Trimethylsilyl)ethyl]3-[(9-fluorenylmethyloxycarbonyl)amino]methyl-5-[4′(9-fluorenylmethyloxycarbonyloxy)-benzyl]benzoate (13). Compound **12** (50 mg, 0.140 mmol) and Et_3N (21.2 mg, 0.210 mmol) were dissolved in CH_2Cl_2 (3 mL) and cooled to 0 °C. Fmoc-Cl (90.5 mg, 0.350 mmol), dissolved in CH_2Cl_2 (2 mL), was added dropwise, whereafter the reaction mixture was allowed to reach rt under continued stirring overnight. The mixture was concentrated and purified by column chromatography (isohexane:EtOAc, 3:1, to isohexane:EtOAc, 2:1) to give **13** as amorphous crystals (72 mg, 64%). TLC R_f 0.45 (isohexane:EtOAc, 2:1); ^{1}H NMR ($CDCl_3$) δ 0.06 (s, 9H, Me_3), 1.10 (m, 2H, CH_2Si), 3.99 (s, 2H, CH_2Ph), 4.22 (t, J = 7.0 Hz, 1H, CH Fmoc-N), 4.27–4.46 (m, 7H, CH_2O, CH_2 Fmoc, CH_2N, CH Fmoc-O), 4.47–4.52 (m,

2H, CH_2 Fmoc), 5.13 (br t, 1H, NH), 7.06–7.11 (m, 2H, 3′), 7.15–7.20 (m, 2H, 2′), 7.25–7.46 (m, 9H, CH Ar Fmoc-O, CH Ar Fmoc-N, Ar(1H)), 7.55–7.65 (m, 4H, CH Ar Fmoc-O, CH Ar Fmoc-N), 7.72–7.84 (m, 6H, CH Ar Fmoc-O, CH Ar Fmoc-N, Ar(2H)); ^{13}C NMR ($CDCl_3$) δ −1.5, 17.4, 41.0, 44.6, 46.7, 47.2, 63.4, 66.8, 70.4, 119.9, 120.1, 121.1, 125.0, 125.1, 126.4, 127.0, 127.1, 127.6, 127.9, 129.1, 129.8, 131.4, 132.4, 138.2, 139.1, 141.26, 141.29, 141.5, 143.1, 143.8, 149.6, 153.6, 156.3, 166.4; IR (solid) 3354, 1761, 1715 cm^{-1}. Anal. ($C_{50}H_{47}NO_7Si$) C, H, N.

3-[(9-Fluorenylmethyloxycarbonyl)amino]methyl-5-[4′(9-fluorenylmethyloxycarbonyloxy)benzyl]benzoic acid (14). Compound **13** (59 mg, 0.073 mmol) was treated with 50% TFA in CH_2Cl_2 (4 mL) at room temperature for 2 h. The reaction mixture was concentrated and purified by column chromatography ($CHCl_3$ to $CHCl_3$:MeOH, 9:1) to give compound **14** as a solid (41.8 mg, 81%). TLC R_f 0.43 ($CHCl_3$:MeOH, 9:1); mp 108–110 °C; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 2H, CH_2Ph), 4.19 (br t, 1H, CH Fmoc-N), 4.24–4.52 (m, 7H, CH Fmoc-O, 2×CH_2 Fmoc, CH_2N), 5.17 (br s, 1H, NH), 7.02–7.09 (m, 2H, 3′), 7.11–7.18 (m, 2H, 2′), 7.25–7.44 (m, 9H, CH Ar Fmoc-O, CH Ar Fmoc-N, Ar(1H)), 7.54–7.63 (m, 4H, CH Ar Fmoc-O, CH Ar Fmoc-N), 7.71–7.78 (m, 4H, CH Ar Fmoc-O, CH Ar Fmoc-N), 7.85 (m, 2H, 2×Ar); ^{13}C NMR ($CDCl_3$) δ 40.9, 44.5, 46.7, 47.1, 66.7, 70.4, 119.2, 120.1, 121.2, 125.0, 125.1, 126.6, 127.0, 127.2, 127.7, 127.9, 129.79, 129.81, 131.0, 133.2, 137.9, 139.4, 141.29, 141.31, 141.6, 143.1, 144.1, 149.7, 153.6, 156.6, 170.7; IR (solid) 3600–2400, 3448, 1757, 1720 cm^{-1}. Anal. ($C_{45}H_{35}NO_7\cdot\frac{1}{2}H_2O$) C, H, N.

Ang II analogue 5. The Fmoc-protected building block **14** (20.9 mg, 29.8 μmol), PyBOP (45.4 mg, 87.2 μmol), HOBt (129.5 mg, 218 μmol) and DIEA (37 μL, 218 μmol) were dissolved in DMF (0.5 mL) and added to preswollen Ile-His(Trt)-Pro-Phe-Wang resin (75 mg, 38 μmol) (see SPPS) in a 2 mL disposable syringe equipped with a porous polyethylene filter. After mixing by slow rotation at rt for 20 h the resin was filtered off and washed with DMF (5×1 mL), deprotected with 20% piperidine in DMF (3×1 mL, 1 + 5 + 10 min) and washed with DMF (6×1 mL). The peptide was further elongated by reaction with, in turn, Fmoc-Arg(Pbf)-OH (19 h) and Fmoc-Asp(O^{t}Bu)-OH (1.5 h) as described above (SPPS). Fmoc deprotection, washing with DMF, and drying in air and in vacuo afforded the partially protected peptide resin (100.9 mg). The resin was treated with TFA:H_2O:triethylsilane (90:5:5) (1 mL) for 1.5 h and the mixture was filtered through a small plug of glass wool in a Pasteur pipette. After washing with TFA (3×0.3 mL), the product was precipitated by the addition of cold, anhydrous ether (13.5 mL). The precipitate was collected by centrifugation, washed with ether (4×4.5 mL) and dried to furnish 50.5 mg of crude peptide.

The peptide was dissolved in 0.1% aqueous TFA (12 mL) and purified in two runs by preparative RP-HPLC on a Vydac 10-μm C18 column (2.2×25 cm) using a 120 min gradient of 20–50% CH_3CN in 0.1% aqueous TFA at a flow rate of 4 mL/min. The separation was monitored at 230 nm and selected fractions were analyzed by analytical RP-HPLC (220 nm) and by PDMS. One compound of the expected mass was isolated. The final yield of **5** was 24.6 mg (59%). Amino acid analysis: Asp 1.00, Arg 1.01, Ile 1.01, His 1.02, Pro 0.94, Phe 1.03 (73% peptide); PDMS (M_W 1023.2): 1024.7 ($M + H^+$).

Ang II analogue 6. The peptide was synthesized according to the procedure used for **5**, but with His(Boc)-Pro-Phe-2-chlorotrityl-resin (123 mg, 38 μmol) as the starting polymer. The partially protected peptide resin (133.5 mg) was cleaved as described above to yield 38 mg of crude peptide. The peptide was dissolved in 0.1% aqueous TFA (12 mL) and purified in two runs by preparative RP-HPLC on a Vydac 10-μm C18 column (2.2×25 cm) as described above. One compound of the expected mass was isolated. The final yield of **6** was 21.5 mg (57%). Amino acid analysis: Asp 1.00, Arg 1.01, His 1.01, Pro 0.98, Phe 1.01 (72% peptide); PDMS (M_W 910.0): 910.3 ($M + H^+$).

Rat liver membrane AT_1 receptor binding assay. Rat liver membranes were prepared according to the method of Dudley et al.[46] 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 liver homogenate corresponding to 5 mg of the original tissue weight, [^{125}I]-Ang II (70 000 cpm, 0.03 nM), and variable concentrations of 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 gamma counter. Non-specific binding was determined in the presence of 10 μM saralasin. All experiments were performed in duplicate.

Theoretical calculations. The minimizations of **15–18** were performed using the AM1[44] method as implemented in SYBYL[45] using the keywords PRECISE, XYZ and NOMM. Graphical manipulations were carried out within SYBYL 6.6. The electrostatic potential was mapped onto the calculated Connolly surfaces (MOLCAD module). Default parameters were used. To force the electrostatic potential colors of each molecule into common borders, the option global was selected.

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