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Synthesis and Properties of the First All-aza Analogue of a Biologically Active Peptide

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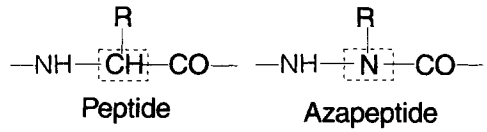
Abstract: The synthesis of the first all-aza-amino acid analogue (**2**) of a peptidic renin inhibitor is described. The X-ray structural analysis and molecular modelling investigations of this novel compound reveal interesting conformational features which have a significant impact on its biological activity. In addition, insight into conformational features of azapeptides in general in comparison with the corresponding purely peptidic compounds is given.

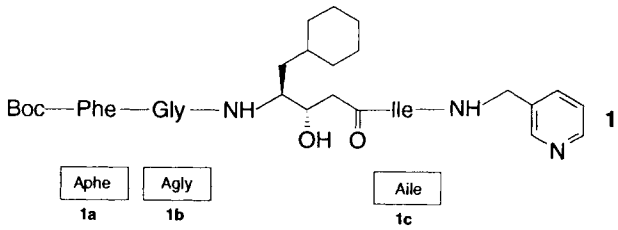
Keywords: All-Aza peptide; renin inhibitor analogue; X-ray; conformation

The isoelectronic replacement of an α-CH-group by an N-atom in one or more amino acid residues within a peptide backbone results in an 'azapeptide':



This class of peptide analogues was first described in 1962 [1–3]. Since then, these compounds have gained attention as analogues of biologically active peptides with promising therapeutic potential [4]. This interest is based on their enhanced activity compared with the corresponding original peptides in many cases. Additionally, since they are less susceptible to enzymatic breakdown by proteases, they may possibly lead to orally active drugs with longer durations of action.

Some years ago we synthesized analogues of the potent renin inhibitor **1** [5]:



with single amino acid residues being replaced with the corresponding aza-amino acids (**1a–1c**)[6–8]. These analogues had lower biological activity than the native compound but could be shown – in contrast to **1** – to be resistant to enzymatic degradation. Our present aim was to investigate the influence of *multiple* aza-replacement in the same molecule on the biological activity. We therefore built up the peptide analogue **2** with all three positions of natural amino acids simultaneously occupied by their aza-counterparts. To our knowledge, this is the first

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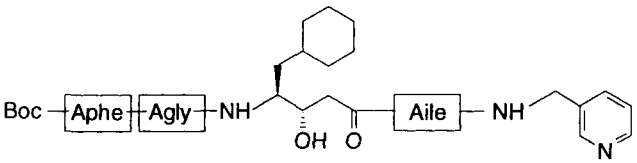
synthesis of an all-aza-analogue of a biologically active peptide.



2

The all-aza compound **2** was synthesized by following the reaction sequence of Figure 1: *N*-Boc-*N'*-benzylhydrazine **3** [9] was reacted with the 4-nitrophenylester **4** to the activated ester **5**. This compound was converted to the hydrazide **6** from which the ester **7** was obtained by reaction with compound **4**. Reaction of **7** with the Aile derivative **8** [8] gave the target compound **2**.

In comparison to the native molecule **1** which was active in the nanomolar range, compound **2** had very low renin-inhibiting activity (IC_{50}: $> 10^{-5}$M).

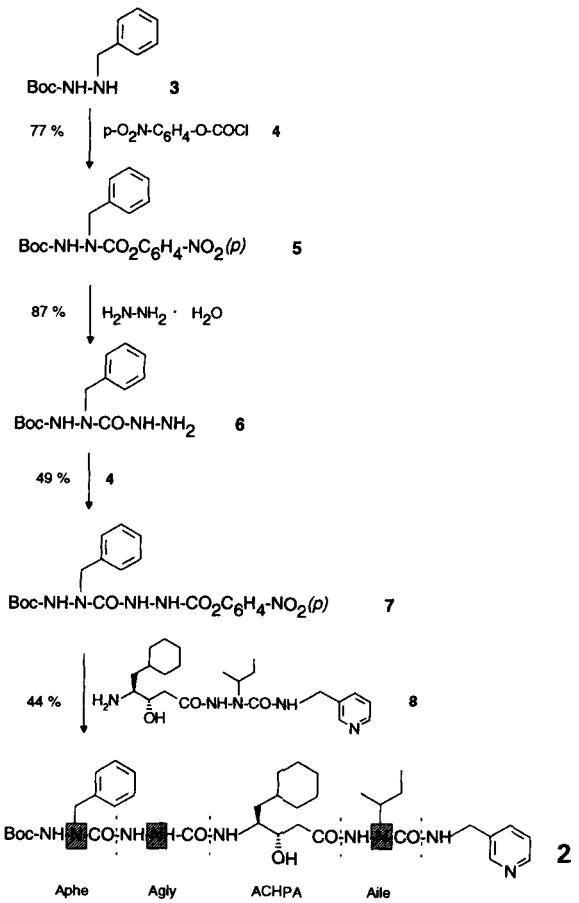


Figure 1 Synthesis of all-aza peptide **2**.

X-ray structure of 2 and molecular modelling investigations

To investigate the influence of the aza-group on molecular shape and flexibility we performed an X-ray structure analysis (Figure 2). Crystallization was successful from acetone, yielding orthorhombic crystals of space group $P2_12_12_1$ (No. 19) with cell parameters: $a = 9.162(3)$ Å, $b = 18.329(4)$ Å and $c = 27.941(4)$ Å, with *R*-factor = 0.096.

A detailed description of the studies of the structure determination of **2** will be published elsewhere [10] and supplementary information on the X-ray structure will be deposited at the Cambridge Crystallographic Data Centre in conjunction with this report.

As can be seen from Figure 2, **2** shows a bend conformation in the crystal with an internal H-bond between the carbonyl oxygen of Agly and the amide hydrogen of the 3-pyridylmethyl group (distance N···O: 2.80 Å). Additionally, the turn is held by π interactions between the phenyl ring of Aphe and the pyridine ring of the 3-pyridylmethyl group (distance between ring centroids: 3.93 Å).

The observed torsion angles and out-of-plane angles of the three aza residues in **2** are listed in Table I and indicate that: (i) introduction of the aza-entity into the peptide chain enforces planarity at the position analogous to C^{α}, (ii) torsion angle 'φ' shows no preference for a certain value, (iii) torsion angles 'ψ' and 'ω' tend to an up to 10° deviation from 0° or 180°, and (iv) the bend of the structure is caused mostly by the chain torsion angles of the ACHPA moiety and the Aile residue.

In summary, this shows that the substitution of the C^{α} atom by a nitrogen introduces rigidity. Thus, the all-aza peptide is considerably more rigid than a normal peptide.

Similar results concerning aza amino acids have been obtained by X-ray structure determinations of Aala and Apro containing peptides by Benatalah *et al.* [11] and Lecoq *et al.* [12], respectively. Their determinations revealed torsion angles of 70.2°,

Table I Torsion Angles in the X-ray Structure of **2**

	φ	ψ	ω	δ^a
Aphe	−109.0	11.1	−174.1	2.5
Agly	64.6	−167.7	169.4	-
Aile	−86.9	−4.4	−169.5	3.7

[a] Out-of-plane angle N, N^{α}, C', C^{β}.

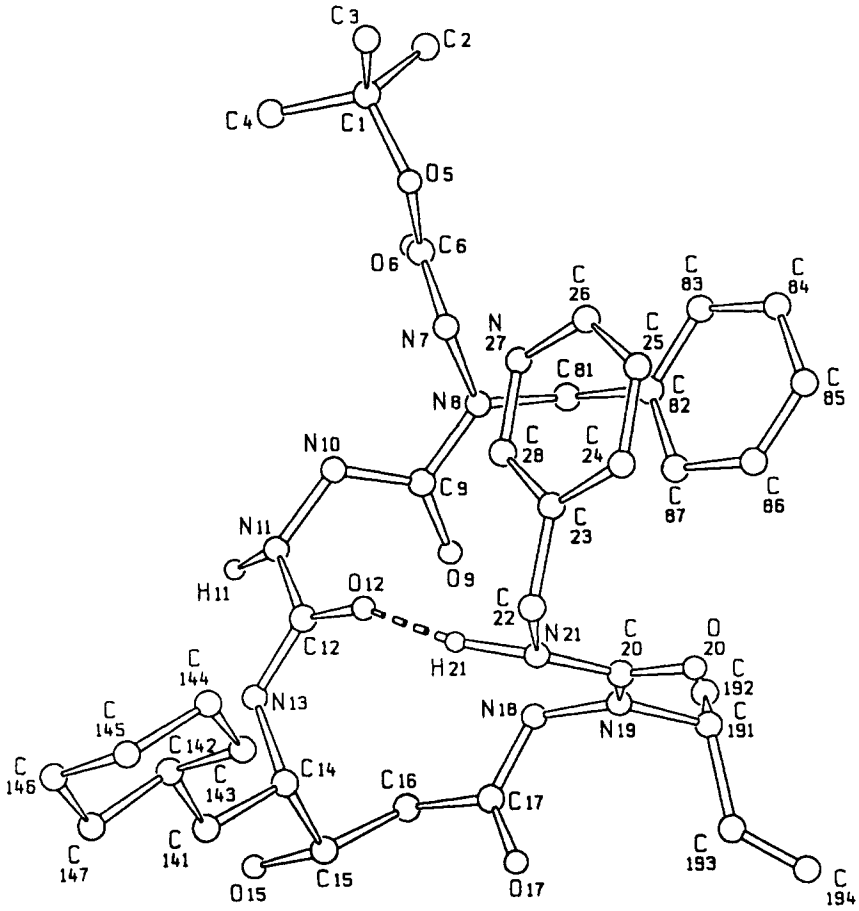


Figure 2 Crystal structure of **2** showing the internal H-bond and parallel orientation of the phenyl ring of Aphe and the pyridine ring of the 3-pyridylmethyl group.

111.2° and −113.3° for 'φ' and 16.0°, −22.9° and 16.1° for 'ψ', respectively. Equally interesting results were obtained by molecular modelling of the conformations and H-bonding capabilities of the compounds **1** and **2** at the active site of renin. Thus, the structural parameters of **1** and **2** might be correlated with the different biological activities.

In 1991 the X-ray structure of a co-crystal of human renin with a transition state analogue at 2.4 Å resolution (1RNE) [13] became available. There the importance and shape of the hydrogen bonding pattern can be directly seen. To understand the influence of the aza group on binding to renin in our compounds, we tried to find conformations of **1** and **2** which fit best to the inhibitor in 1RNE. For this purpose, the structures were model built and their torsion angles were modified manually to give the best superposition with the inhibitor of 1RNE. The inhibitor of 1RNE was replaced with the modelled compounds **1** and **2** (Figure 3). Both model complexes as well as the crystal structure complex were energy-minimized. All structural manipulations as well as the energy minimization were performed with the molecular modelling package SYBYL Version 5.5, Tripos Ass., St Louis, MO.

Not surprisingly the 'native' molecule **1** which does not contain an aza amino acid fits best to the inhibitor of 1RNE with respect to the hydrophobic sidechains of phenylalanine and ACHPA (4-(S)-amino-5-cyclohexyl-3(S)-hydroxypentanoic acid) and the trace of the backbone (Figure 2). Therefore, **1** can fill the hydrophobic pocket and fulfil the hydrogen bonding requirements. It also has most of the H-bonds found in the X-ray structure analysis of the renin inhibitor complex as can be seen from Table II.

Looking at **2**, fitting to the inhibitor of 1RNE can only be achieved with respect to the sidechains. As a

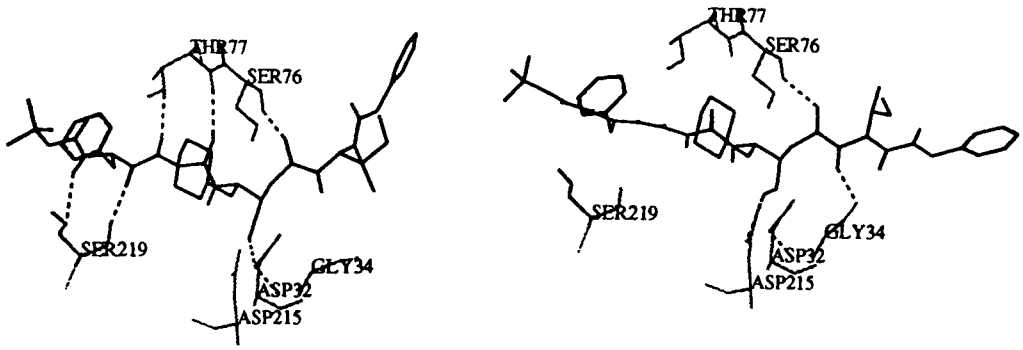


Figure 3 H-bond interactions of modelled complexes of renin and compounds **1** and **2**, respectively.

Table II H-bonds in Models of Renin and Inhibitors **1** and **2** in comparison with 1RNE

Inhibitor residue	Renin	**1**	**2**	X- ray (1RNE)
Aphe/Phe	NH···OH Ser 219	×		
	CO···NH Ser 219	×		×
Agly/Gly	NH···OH Thr 77	×		×
	CO···NH Thr 77	×		×
ACHPA	OH···COO Asp 32,215	×	×	×
	CO···NH Ser 76	×	×	×
Aile/Ile	NH···CO Gly 34		×	×

result, the hydrogen bonding pattern found in the co-crystal structure could only be built up for the part containing the ACHPA unit. All these observations agree with the low activity of **2**, which may be due to the enhanced planarity around the 'aza-atoms' which is clearly visible in this model as in the X-ray structural analysis (Figure 2).

In conclusion, these findings show that the rigidity caused by the aza moiety in the all-azapeptide **2** leads to worse fitting and fewer H-bond interactions within the active site, and therefore reduced binding.

EXPERIMENTAL

Melting points were determined with a Mettler FP 62 melting point apparatus and are uncorrected. Specific rotations were measured with a Perkin Elmer 241 MC polarimeter. NMR and mass spectra were recorded on a Bruker AC 200, WM 250 or AM 500 (TMS as internal standard) and a Vakkuum Generator VG 70-250 SE. Microanalyses were obtained with a Perkin Elmer 240 B CHN analyser. Thin-layer chromatography (TLC) was carried out on precoated silica gel F_{254} plates with a layer thickness of 0.25 mm from E. Merck, Darmstadt. Visualization was done with UV and I_2.

Boc-Aphe-4-Nitrophenyl Ester (5)

Compound **4** (1.8 g, 9 mmol) was added to a cooled (0°C) solution of **3** [9] (2.0 g, 9 mmol) and TEA (1.24 ml, 9 mmol) in 30 ml dry THF. After stirring for 15 h and evaporation of the solvent, water was added to the residue and the solution extracted by EtOAc. After drying the organic layer with Na_2SO_4 and evaporation, the remaining oil was chromatographed on silica gel with toluene/EtOAc (85/15) as eluent. 2.67 g (77%) of **5** were obtained as a white solid: mp 111°C; ^{1}H-NMR (200.13 MHz, $CDCl_3$) δ 1.42 (s, 9H, *t*-butyl), 4.77 (mbr, 2H, CH_2), 6.56 (br, 1H, NH), 7.21–7.41 (m, 7H, aromatic H), 8.25 (d, J = 8.5 Hz, 2H, aromatic H); FAB MS (M^+ +H) 388.

$C_{19}H_{21}N_3O_6$ (387.43); calculated, C 58.90, H 5.47, N 10.85; found, C 59.10, H 5.71, N 10.92.

Boc-Aphe-hydrazide (6)

The activated ester **5** (3 g, 7.8 mmol) was added to a solution of hydrazine hydrate (100%, 1.9 ml,

39 mmol) in 30 ml THF and the solution was stirred overnight. After evaporation to a small volume, water and EtOAc were added, and the reaction product was extracted into the organic phase. The solvent was dried (Na_2SO_4) and evaporated, and the remaining oil was purified over a silica gel column using CH_2Cl_2/acetone (93/7) as the eluent system. Compound **6** was obtained as an oil (1.9 g, 87%). ^{1}H-NMR (200.13 MHz, DMSO-d_6) δ 1.35 (s, 9H, *t*-butyl), 3.95 (sbr, 2H, NH_2), 4.50 (br, 2H, CH_2), 7.30 (m, 5H, aromatic H), 7.70 (s, 1H, NH), 8.88 (sbr, 1H, NH); FAB MS (M^+ +H) 280.

$C_{13}H_{20}N_4O_3$ (280.37): calculated, C 55.69, H.7.20, N 19.99; found, C55.27, H 7.41, N 19.67.

Boc-Aphe-Agly-4-Nitrophenylester (7)

Triethylamine (0.44 ml, 3.2 mmol) was added to a solution of the hydrazide **6** (0.9 g, 3.2 mmol) and compound **4** (0.64 g, 3.2 mmol) in 20 ml THF. After stirring overnight at room temperature, the solvent was evaporated. After addition of water the mixture was extracted with EtOAc. The organic phase was washed with water, dried (Na_2SO_4) and evaporated. The remaining oil was purified over a silica gel column (CH_2Cl_2/acetone, 97:3). The product (**7**) was obtained as an oil (0.9 g, 49%). ^{1}H-NMR (200.13 MHz, DMSO-d_6) δ 1.35 (s, 9H, *t*-butyl), 4.48 (sbr, 2H, CH_2), 6.91 (d, J = 8.5 Hz, 2H, aromatic H), 7.30 (m, 5H, aromatic H), 8.10 (d, J = 8.5 Hz, 2H, aromatic H), 9.44 (sbr, 1H, NH), 10.70 - 12.00 (br, 2H, NH); FAB MS (M^+ +H) 446.

$C_{20}H_{33}N_5O_7$ (445.48): calculated, C 53.92, H 5.21, N 15.72; found, C 53.48, H 5.48, N 15.30.

Boc-Aphe-Agly-ACHPA-Aile-3-pyridylmethylamide (2)

A solution of the activated ester **7** (0.7 g, 1.6 mmol), compound **8** [8] (0.77 g, 1.6 mmol), and triethylamine (0.43 ml, 3.2 mmol) in 15 ml THF was kept at room temperature for 15 h. After evaporation to dryness, water and EtOAc were added, and the product was extracted into the organic phase. This layer was washed with water, dried over Na_2SO_4 and brought to dryness. The oily residue was chromatographed with a silica gel column (CH_2Cl_2/MeOH; 95:5). After recrystallization from hexane, 0.5 g (44%) of compound **2** (white crystals) was obtained: mp 126°C; $[\alpha]_{20}^{d}$ + 14.1° (c 0.54, MeOH); ^{1}H-NMR (500.13 MHz, DMSO-d_6 + TFA-d_1, 353 K) δ 0.65–1.70 (m,30H, aliphat.H), 2.37 (m,1H, $-CO-CH_aH_b-$), 2.49 (m, 1H, $-CO-CH_aH_b-$), 3.74 (m, 1H, CH), 3.95 (m, 1H, CH), 4.17 (m, 1H, CH), 4.36–4.72 (m, 4H, 2 CH_2), 7.26 (m, 5H, Phenyl), 8.01 (m, 1H, Pyridin-H-5), 8.55 (m, 1H, Pyridin-H-4), 8.76 (m, 2H, Pyridin-H-1, -H-6); FAB MS (M^+ + H) 726.

$C_{36}H_{55}N_9O_7$ (726.00) calculated, C 59.55, H 7.65, N 17.37; found, C 59.29, H 7.92, N 17.01.

Biological Methods. *In Vitro* Enzyme Inhibition

The renin IC_{50} data were obtained with human EDTA plasma, utilizing the endogenous renin and angiotensinogen. **2** was dissolved in DMSO and the obtained solution was diluted so that prior to the addition to the assay system the solution was 10% in DMSO. At least three different concentrations of the inhibitor that bracketed the IC_{50} were used for the determination of IC_{50}. Final incubation mixture (750 µl): plasma, 100 µl; maleate buffer, pH 5.5, 0.1 M; EDTA, 7.2 mM; DMSO 1%; 8-hydroxyquinoline, 8.3 nM.

Samples were incubated at 37°C for 2 h and then placed on ice; an aliquot was analysed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percentage inhibition of the reaction was determined, and the IC_{50} was calculated.

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