ORGANIC LETTERS

2001
Vol. 3, No. 24
3843−3846

Unexpected Stability of the Urea *cis–trans* Isomer in Urea-Containing Model Pseudopeptides

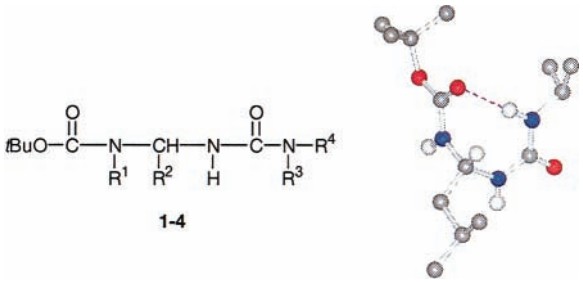
Vincent Semetey,[†] Christine Hemmerlin,[‡] Claude Didierjean,[§] Arnaud-Pierre Schaffner,[†] Ana Gimenez Giner,[†] André Aubry,[§] Jean-Paul Briand,[†] Michel Marraud,[‡] and Gilles Guichard*,[†]

Immunologie et Chimie Thérapeutiques, UPR CNRS 9021, IBMC, 15, rue Descartes, F-67084 Strasbourg, France, LCPM, UMR CNRS-INPL 7568, ENSIC-INPL, BP 451, F-54001 Nancy, France, and LCM3B, ESA-CNRS 7036, UHP, BP 239, F-54506 Vandœuvre, France

g.guichard@ibmc.u-strasbg.fr

Received August 27, 2001

ABSTRACT



In contrast to the situation observed in the crystal state, the urea moiety in *N*-Boc-*N′*-carbamoyl-*gem*-diaminoalkyl derivatives (single-residue ureidopeptides) 1–4 exclusively assumes a *cis–trans* conformation in solution. When R^3 = H, the resulting structure can be further stabilized by an intramolecular hydrogen bond that closes an eight-membered pseudocycle. The root-mean-square deviation calculated for heavy atoms between a peptide γ-turn and the folded conformation that we propose to call *urea turn* is 0.60 Å.

The use of peptide backbone mimetics for forming or stabilizing defined secondary structures, i.e., turns, helices, or sheets, via noncovalent interactions is an area of major interest in peptidomimetic chemistry. In this context, unsymmetrical ureas, because they contain both hydrogen bond donors and acceptors, have recently emerged as a promising class of nonpeptide linkage for studying local folding propensity.[1,2] These studies, however, have focused mainly on trisubstituted ureas, and conformational preferences as well as hydrogen-bonding properties of peptidomimetics incorporating *N*,*N′*-disubstituted ureas in their backbone remain largely unexplored. In the crystal state[3] and in organic solution at high concentrations,[4] *N*,*N′*-disubstituted ureas exhibit two *trans* amide bonds, so that both N−H bonds are oriented in a direction opposed to that of the carbonyl, and hence, are often involved in self-complementary bidirectional intermolecular hydrogen bonds. However, as a result of competitive conjugation, the barriers of rotation of ureas being lower than those in corresponding amides, one might

[†] UPR CNRS 9021, Immunologie et Chimie Thérapeutiques.
[‡] UMR CNRS-INPL 7568, LCPM.
[§] ESA CNRS 7036, LCM3B.

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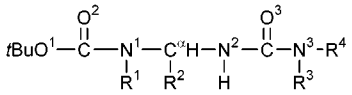
10.1021/ol0166552 CCC: $20.00
Published on Web 11/08/2001

expect a more complex isomer distribution in organic solvents at low concentration.[5] Herein, we report on the conformational preferences, both in the crystal state and in organic solvents ($CHCl_3$, CH_2Cl_2, DMSO), of simple ureidopeptides of formula **1**−**4** (Figure 1) obtained by substitu-



1 R^1 = H , R^2 = *i*Bu
2 R^1 = H , R^2 = Bn
3 R^1 = H , R^2 = CH_2OBn
4 R^1, R^2 = $(CH_2)_3$

a R^3= H, R^4 = Me; **b** R^3= H, R^4 = *i*Pr; **c** R^3= Me, R^4 = Me

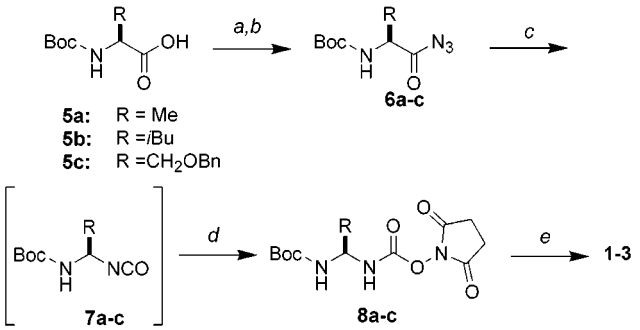
Figure 1. *N*-Boc-*N*′-carbamoyl-*gem*-diaminoalkyl derivatives **1**−**4** investigated.

tion of a ureido unit Ψ[NH−CO−NH] for an amide CO−NH peptide bond.

The first synthesis of ureido analogues of a biologically active peptide (angiotensin II) was described more than two decades ago by Chipens and co-workers.[6] The urea linkage has since been incorporated into gastrin[7] and enkephalin.[8] A more recent and noteworthy application includes the identification of potent orally active growth hormone secretagogues based on ureidotripeptides.[9] Structural studies of these derivatives, however, have been limited so far to the X-ray structure determination of two *N*-acetyl-*N*′-carbamoyl-*gem*-diaminoalkyl derivatives with side chains of Phe and Val.[10−12]

Model ureidopeptides **1**−**3** were prepared by reaction of succinimidyl carbamate derivatives[13,14] of *N*-Boc-*gem*-diamino residues **8** (bearing side chain of Leu, Phe, Ser-(Bn)) with methylamine (**1a**−**3a**), isopropylamine (**1b**−**3b**), or dimethylamine (**3c**, **4c**) in a manner analogous to that described previously for the synthesis of *N*,*N*′-linked oligoureas (Scheme 1).[15] Proline derivatives **4** were obtained

Scheme 1[a]



[a] EtCOOCl, NMM, -20 °C. [b]NaN_3, H_2O. [c]Toluene, 65 °C. [d]*N*-Hydroxysuccinimide, pyridine. [e]HNR^1R^2, DMF.

by direct amine treatment of the isocyanate resulting from Curtius rearrangment of the corresponding amino acyl azide Boc-Pro-N_3.

In the crystal state, X-ray diffraction on a single crystal of **1b** in the racemic form[16] reveals extended molecules exchanging intermolecular hydrogen bonds (Figure 2).[17] The urea moiety classically assumes the *all-trans* planar conformation. Rows along the [010] direction of molecules having the same chirality are connected in such a way that the three NH hydrogens of each molecule more or less closely interact with the two carbonyl oxygens of a neighboring molecule. Two of the intermolecular N···O distances are standard, and the other two are just above the limit generally considered for hydrogen bonding (Figure 2).[19]

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(12) In a study focused on related geminal bis-ureas as gelator molecules, aggregation behavior has been studied in solution at high concentration, in the gel state, and in the crystal state. See ref 4.
(13) The *N*-protected α-amino acid **5** (l equiv) was dissolved in THF (30 mL) under Ar and cooled to −20 °C. After addition of EtOCOCl (1.1 equiv) and NMM (1.1 equiv), the mixture was stirred at −20 °C for 20 min. The resulting white suspension was allowed to warm to −5 °C and was treated with an aqueous solution (5 mL) of NaN_3 (2.5 equiv). The mixture was stirred for 5 min, diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the acyl azide **6**, which was used without further purification. Toluene was added under Ar, and the resulting solution was heated to 65 °C under stirring. After the gas evolution had stopped (ca. 10 min), *N*-hydroxysuccinimide (1 equiv) and pyridine (1 equiv) were added. The mixture was stirred for 5 min at 65 °C and then cooled to room temperature. In all cases, compound **8** crystallized from the toluene solution and was collected by filtration. Recrystallization from toluene afforded pure **8**.
(14) Compound **8c**. Yield: 60%. White solid, mp 142 °C. $[\alpha]^{25}_D$ −4.5 (*c* 1.9, DMF). HPLC t_R 12.88 min (A: 0.1% aqueous TFA. B: 0.08% MeCN, linear gradient, 20−80% B, 20 min). ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 1.35 (s, 9H, $C(CH_3)_3$), 2.73 (s, 4H, CH_2CH_2), 3.46 (d, J = 6.6 Hz, 2H, $CHCH_2$), 4.46 (s, 2H, $OCH_2C_6H_5$), 5.07−5.21 (m, 1H, NHCHNH), 7.19−7.36 (m, 5H, H aromatic), 7.38 (br d, 1H, $NHCO_2C(CH_3)_3$), 8.67 (br d, J = 7.6, 1H, $NHCO_2Su$). ^{13}C NMR (DMSO-d_6, 50 MHz): δ = 25.2 (CH_2), 28.0 (CH_3), 59.0 (CH), 69.8 (CH_2), 72.0 (CH_2), 78.4 (C), 127.5 (CH), 128.1 (CH), 138.0 (C), 150.8 (C), 154.4 (C), 170.7 (C). MALDI-MS *m*/*e* 430.21 [M + Na]$^+$, 446.27 [M + K]$^+$.
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(16) *rac*-**1b** rather than (*S*)-**1b** gave good quality crystals suitable for single-crystal X-ray structure determination.
(17) Crystal data for *rac*-**1b**: $C_{14}H_{29}N_3O_3$, M_r = 287.40, colorless prism, crystal size 0.4 × 0.1 × 0.1 mm^3, a = 12.168 (6) Å, b = 9.397(3) Å, c = 16.467(2) Å, β = 103.86(3)°, V = 1828.1(11) Å^3, T = 293 K, monoclinic, space group $P2_1/c$, Z = 4, D_c = 1.044 g cm^{-3}, μ = 0.593 mm^{-1}. Nonius Mach3 diffractometer, λ = 1.54060 Å; 2408 measured reflections, 2408 unique, 2017 with $I > 2\sigma(F_o^2)$. The structure was solved by direct methods and refined by full-matrix least squares on F^2 for all data to R = 0.0624 [$I > 2\sigma(F_o^2)$], wR = 0.169 [$I > 2\sigma(F_o^2)$], 191 parameters.
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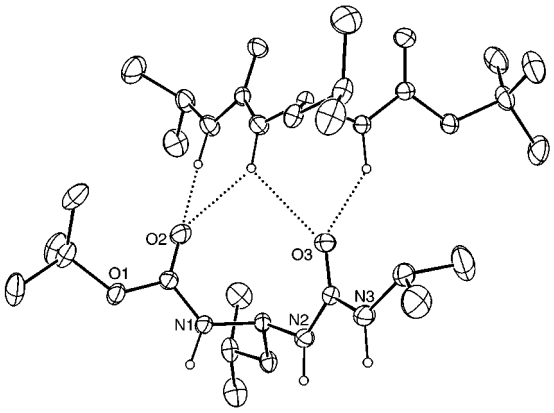

Figure 2. An ORTEP representation of the extended molecular structure of Boc-(*R*,*S*)-*g*Leu-CO-NH*i*Pr *rac*-**1b**, in the crystal state. The thermal ellipsoids are shown at the 40% probability level. Intermolecular H-bonds are indicated by dashed lines. Intermolecular distances: $N^1\cdots O^3 = 2.83$ Å, $N^2\cdots O^2 = 3.27$ Å, $N^2\cdots O^3 = 3.15$ Å, $N^3\cdots O^2 = 2.88$ Å.

The molecular structure and crystal packing of *rac*-**1b** are similar to those already observed in the two related enantiopure derivatives (*S*)-AcNH-CH(*i*Pr)-NHCONHMe[10] and (*S*)-AcNH-CH(Bn)-NHCONHMe.[11]

In contrast to the above structure, the urea moiety in the derivatives with $R^3 = H$ assumes the *cis*−*trans* conformation in $CDCl_3$. NOESY experiments indicate that the N^3H proton is much closer to the N^1H and the geminal $C^\alpha H$ protons than to the other urea N^2H proton (Figure 3).

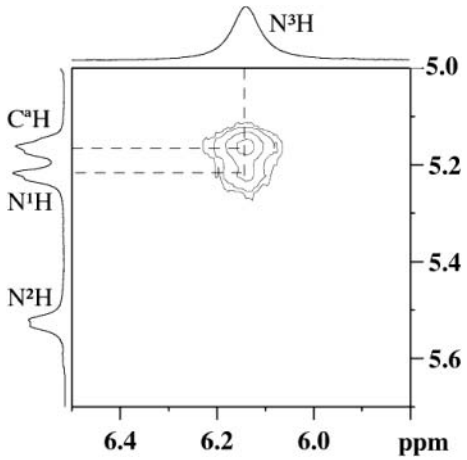

Figure 3. NOESY map (600 MHz) for **1a** in $CDCl_3$ showing the short N^3H to $C^\alpha H$ proton−proton distance typical of the *cis*−*trans* urea moiety.

Moreover, these molecules in $CHCl_3$ or CH_2Cl_2 are partially folded and stabilized by an intramolecular N^3H to CO^2 H-bond, closing an eight-membered pseudocycle that we propose to call a "urea turn" (Figure 4). The root-mean-square deviation calculated for heavy atoms between a peptide γ-turn and the *urea turn* is 0.60 Å. This conclusion is supported by the following observations. On one hand, the NH stretching absorption for **1a**−**4a** and **1b**−**4b** exhibits a low-frequency component (Table 1), typical of an H-bonded vibrator, which is sensitive to the Me or *i*Pr nature of R^4 and is absent from the spectrum for the homologous **3c** and **4c** derivatives.

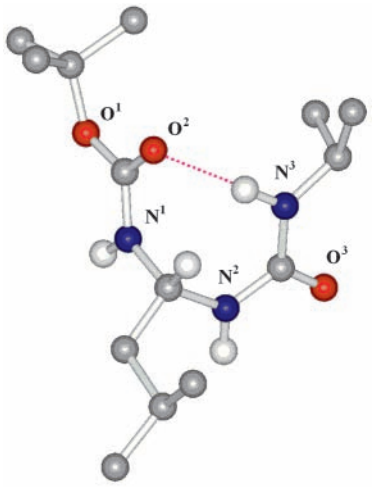

Figure 4. MM2 energy-minimized conformation of **1b**. The unessential hydrogens have been omitted for clarity. Main backbone torsion angles: $O^1-C-N^1-C = 179°$, $C-N^1-C^\alpha-N^2 = -110°$, $N^1-C^\alpha-N^2-C = 76°$, $C^\alpha-N^2-C-N^3 = 2°$, $N^2-C-N^3-C = -171°$.

It can be thus attributed to the H-bonded C-terminal N^3H group. On the other hand, compounds **3c** and **4c** give rise to a much higher CO^2 carbonyl absorption than the other compounds, indicating that this carbonyl is H-bonded in the latter case. Moreover, the solvent sensitivity of the N^3H proton resonance differs significantly from that for the other two NHs (Table 1) and attests to its H-bonded character. It has been actually demonstrated that a strong positive shift when going from $CDCl_3$ to DMSO-d_6 denotes a free NH whereas a weakly positive or a negative value is associated with an H-bonded NH.[20]

The comparison of the N−H stretching domain for the homologous **1a**−**4a** and **1b**−**4b** derivatives in CH_2Cl_2 also reveals that the more or less intense absorption at 3453 cm^{-1} is a contribution of the free N^3-H vibrator. It results that the above-mentioned folded structure (Figure 4) is in rapid exchange with an open conformation. However, the absence of Overhauser effect between the N^2H and N^3H urea protons on one hand and of resonance split-

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Table 1. IR and NMR Data for the Ureidopeptides **1**−**4**

					ν_{N-H} (cm^{-1})[a]		$\nu_{C=O}$ (cm^{-1})[a]		δNH (ppm)[b]			folded conformer
no.	R^1	R^2	R^3	R^4	N^1H/N^2H	N^3H	CO^2	CO^3	N^1H	N^2H	N^3H	(%)[c]
1a	H	*i*Bu	H	Me	3431	3453[sh]/**3350**[br]	1712[sh]/**1695**	1676	4.99 (2.04)	4.76 (1.22)	6.08 (−0.22)	55
1b	H	*i*Bu	H	*i*Pr	3431	3431/**3336**[br]	1712[sh]/**1695**	1667				
2a	H	Bn	H	Me	3431	3453/**3363**[br]	1708/**1699**	1680	5.03 (2.12)	5.21 (0.97)	6.15 (0.42)	30
2b	H	Bn	H	*i*Pr	3431	3431/**3344**[br]	1708/**1699**	1670				
3a	H	CH_2OBn	H	Me	3431	3453[sh]/**3354**[br]	1717[sh]/**1692**	1679	5.53 (1.57)	5.24 (0.84)	6.15 (−0.16)	55
3b	H	CH_2OBn	H	*i*Pr	3431	3431/**3336**[br]	1717[sh]/**1695**	1668				
3c	H	CH_2OBn	Me	Me		3444[br]	1714	1657				
4a	$(CH_2)_3$		H	Me	3431	3453/**3310**[br]	1696[sh]/**1673**	1673		4.54 (1.68)	7.30 (−1.74)	70
4b	$(CH_2)_3$		H	*i*Pr	3429[w]	3431/**3291**[br]	1696[sh]/**1670**	1670				
4c	$(CH_2)_3$		Me	Me		3455[br]	1693	1656				

[a] Stretching frequencies in CH_2Cl_2, 5 mM (no aggregation revealed by further dilution). br, broad; sh, shoulder; w, weak absorption. The low frequencies in bold type denote H-bonded vibrators. [b] NH proton resonance in $CDCl_3$, 5mM. The values in parentheses are the resonance shifts from $CDCl_3$ to DMSO-d_6. [c] Estimated from the residual MeN^3−H stretching absorption in CH_2Cl_2 for **1a**−**4a** after subtraction of the absorption for **1b**−**4b**.[18]

ting on the other hand,even at low temperatures down to −60 °C, indicates that the urea moiety adopts only the *cis*−*trans* conformation in both folded and open conformers.

The percentage of the folded conformer strongly depends on the *gem*-residue (Table 1) and may be estimated from the residual contribution of the free N^3H vibrator, obtained by subtraction two by two of the IR spectra for the homologous **1a**−**4a** and **1b**−**4b** derivatives.[17] Assuming that the free N^3H stretching has the same molar extinction coefficient as for peptides,[17] the folding ratio is about 70% for the constrained *g*Pro derivatives, 55% for the *g*Leu and *g*Ser derivatives, and only 30% for the *g*Phe derivatives.

We can therefore conclude that, in *N*-Boc-*N*′-carbamoyl-*gem*-diaminoalkyl derivatives bearing proteinogenic side chains, the urea moiety adopts a stable *cis*−*trans* conformation in low polar solvents, which can be further stabilized by an intramolecular hydrogen bond. We are currently evaluating the propensity for urea *cis*−*trans* isomerization and *urea turn* formation in longer chain ureidopeptides as well as in more polar solvents.

OL0166552