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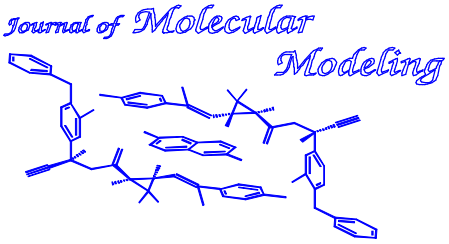

Reparametrisation of Force Constants in MOPAC 6.0/7.0 for Better Description of the Activation Barrier of Peptide Bond Rotations

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Abstract

We have reparametrised the force constants in AM1 and PM3 for better description of activation barriers of peptide bond rotations. A new keyword MMOP was introduced for special recognition of peptide bonds preceding a proline residue or other N-dialkyl substituted amides. The bug in the original MOPAC was corrected where in the case of amides where the nitrogen atom is linked to two hydrogens the force field correction term is counted twice. The new parametrisation of the force constants for peptide bond rotations leads to more realistic rotational barriers of peptide bond rotations. The PM3 optimised pyrrolidine ring in proline adopts no longer a pyramidalised nitrogen atom but a more sp^2-hybridised flat peptide bond geometry.

Keywords: proline, MOPAC, AM1, PM3, force field correction, peptide bond, rotational barrier.

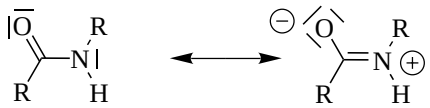
Introduction

The peptide bonds in peptides or proteins usually adopt a *trans* conformation. From peptide bonds preceding a proline residue, however, about 10% occur as a *cis* isomer in native proteins [1-3].

It has been shown that *cis-trans* isomerisation reactions can be the rate limiting step in protein folding processes [4-30]. Furthermore, *trans-cis* isomerisation has been suggested as essential step in enzyme catalysis mechanisms in proline specific proteases in particular for dipeptidyl peptidase IV [31].

The investigation of these mechanisms by means of theoretical calculations requires a correct description of the activation barrier for torsion of the peptide bond. Ab initio methods can be used for the calculation of such activation barriers for relatively small molecules only [32].

Semiempirical methods such as AM1 and PM3 [33-36] do not reflect the mesomery stabilisation of the peptide bond correctly (see Scheme 1) [36, 37].



Scheme 1: *Mesomery stabilisation of the peptide bond*

Therefore, additional force field correction terms ($E = k \cdot \sin^2(w)$) (Keyword MMOK) have been introduced in MOPAC for better description of the activation barrier for peptide bond isomerisation.

Despite these artificial improvements of the methods several problems remain by employing these methods for inves-

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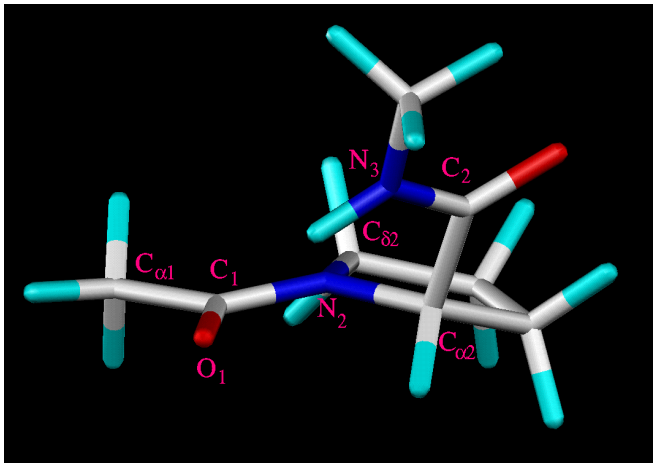
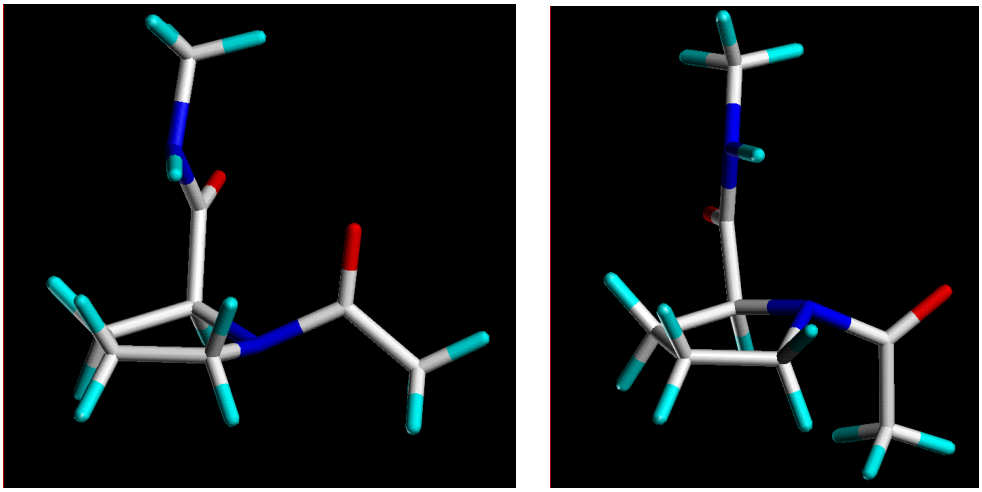
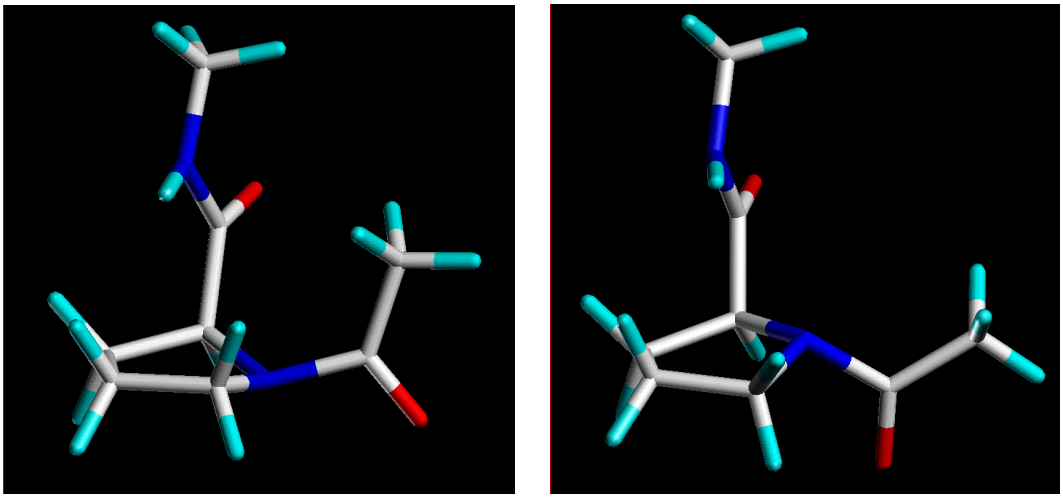


Figure 1: *Trans conformation of N-acetylproline-methylamide with marked atoms to define the considered atoms for peptide bond rotations:* $\zeta = C_{\alpha 1}\text{-}O_1\text{-}C_{\delta 2}\text{-}C_{\alpha 2}$; $\eta = C_1\text{-}C_{\alpha 2}\text{-}N_2\text{-}C_{\alpha 2}$; $\omega = C_{a1}\text{-}C_1\text{-}N_2\text{-}C_{\alpha 2}$; $\phi = C_1\text{-}N_2\text{-}C_{\alpha 2}\text{-}C_2$; $\psi = N_2\text{-}C_{\alpha 2}\text{-}C_2\text{-}N_3$; $\chi = C_{\alpha 2}\text{-}C_{\beta 2}\text{-}C_{\gamma 2}\text{-}C_{\delta 2}$

tigations in *trans-cis* or *cis-trans* isomerisation processes: (i) The keyword MMOK leads to the identification of peptide bonds possessing an amide group with at least one N-H bond. Thus, the additional force field correction is not applied for peptide bonds preceding a proline residue or other N-dialkyl substituted compounds (e.g. N,N-dimethyl acetamide). (ii) The conformation of the pyrrolidine ring in proline residues differs considerably from X-ray structures. That is, a pyramidal structure of the nitrogen (in particular with PM3 optimisation) and a too flat pyrrolidine ring conformation. (iii) Since the application of the keyword MMOK leads to the recognition of amide bonds when at least one nitrogen is linked to a hydrogen atom the force field correction term is counted twice for amides with two N-H bonds such as acetyl amide or peptidyl-amides. (iv) The activation barriers for rotation of the peptide bonds are too small even if the keyword MMOK is applied (see Table 7).



anti-endo anti-exo



syn-endo syn-exo

Figure 2: *The four possible transition states of peptide bond rotations.*

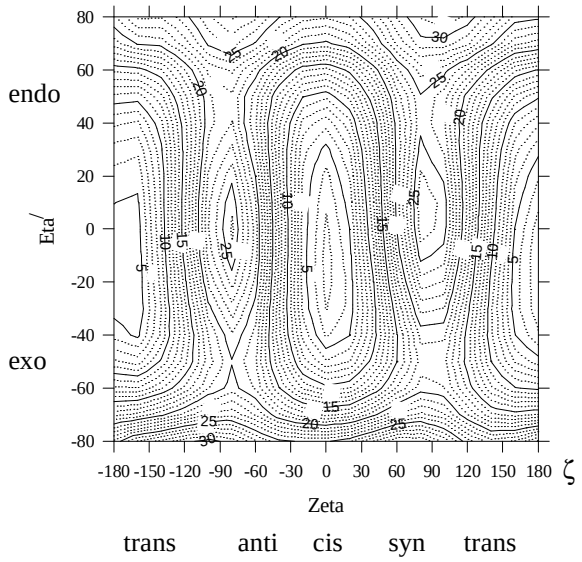


Figure 3. *Conformational η´-ζ rotation map for N-acetyl-(S)-proline-methylamide.*
η´ = 180° - η in correspondence to [32].

Table 1. *Comparison of barriers for trans-cis isomerisation in N-Acetyl-(S)-proline-methylamide calculated with AM1 and PM3*

barrier	AM1		PM3	
	ω-plot	**η–ζ-plot**	**ω-plot**	**η–ζ-plot**
anti	22.5	21.4	23.9	19.7
syn	22.4	18.5	21.7	18.6

energies in kcal/mol obtained with MMOP and HTYP (see below)

Methods

The semiempirical methods AM1 and PM3 embedded in MOPAC 6.0/7.0 were used to calculate and adjust the activation barriers for torsion of peptide bonds.

The rotation of a peptide bond is usually described by the dihedral angle ω ($C_{\alpha1}$-C_1-N_2-$C_{\alpha2}$) (see Figure 1). However, it has been shown by *Feigel et al. 1993* [37] and *Fischer et al. 1994* [32] that a simple twisting of ω leads to incorrect results for the energy height of the activation barrier (see Table 1). The degrees of freedom are not only the torsion ω but also the out-of-plane deformation of the amide nitrogen (pyramidalisation). For this reason the virtual dihedral angles ζ ($C_{\alpha1}$-O_1-$C_{\delta2}$-$C_{\alpha2}$) and η (C_1- $C_{\alpha2}$-N_2- $C_{\alpha2}$) (see Figure 1) were used (see also [32]). The dihedral angle ζ describes the peptide bond and adopts a value of 0° for a *cis* and 180° for a *trans* conformation.

The pyramidalisation of the peptide bond nitrogen atom depends on the dihedral angle η which is 0° for a plane sp^2-nitrogen and 120° or -120°, respectively, for a pyramidal sp^3-nitrogen atom.

In this way all four possible transition states can be recorded. On the one hand, these four transition states result from two possible orientations of the lone pair of the nitrogen which is in periplanar (endo) or antiperiplanar (exo) position to the carbonyl group and on the other hand from two alternatives in the twisting of the peptide bond (syn or anti, respectively). Accordingly to *Fischer et al. 1994* [32] these transition states are named anti-endo ζ -80°, η 120°, anti-exo ζ -80°, η -120°, syn-endo ζ 80°, η 120° and syn-exo ζ 80°, η -120° (see Figure 2 and Tables 2 to 6).

All semiempirical calculations were performed using the eigenvector following method (keyword EF or TS, respectively) by setting the SCF cut off criterium to SCFCRT = 1.D-12 and that one of the gradient to GNORM = 0.1. A grid search was carried out for ζ in 20° increments from 180° to -180° and for η from 120° to -120° in steps of 10°.

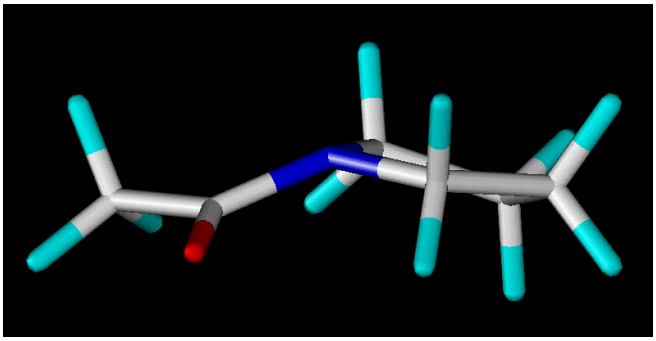


Figure 4a. *PM3 optimised conformation (pyramidal nitrogen) of N-acetyl-pyrrolidine without a force field correction of the peptide bond.*

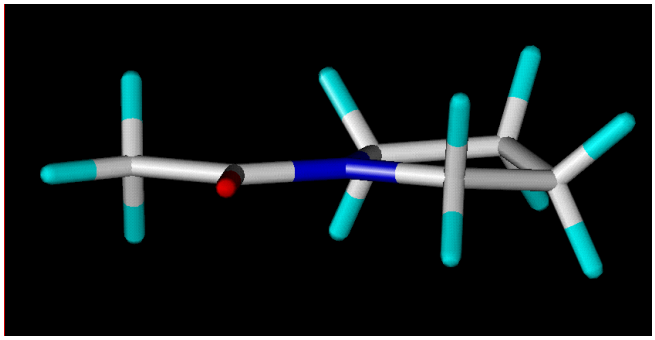


Figure 4b. *PM3 optimised conformation (planar nitrogen) of N-acetyl-pyrrolidine with a force field correction (MMOP/HTYP) of the peptide bond.*

Table 2. *Ground and transition states of acetamide calculated with AM1 and PM3*

variable	keywords	trans	cis	anti-endo	anti-exo	syn-endo	syn-exo
energy	**NOMM**	-50.7	-50.7	-41.1	-37.1	-37.1	-41.1
[kcal/mol]		-51.0	-51.0	-45.5	-42.9	-42.9	-45.5
	MMOK	-50.7	-50.7	-31.8	-27.6	-27.6	-31.8
		-49.3	-49.4	-25.3	-22.7	-22.7	-25.2
	MMOK/HTYP	-50.7	-50.7	-32.7	-28.6	-28.6	-32.7
		-49.3	-49.3	-31.3	-28.8	-28.8	-31.3
ζ [°]	**NOMM**	-179.9	-0.1	-86.0	-80.7	80.6	87.8
		-172.1	6.1	-85.9	-80.8	81.4	86.1
	MMOK	-179.9	-0.1	-86.0	-80.7	80.8	86.3
		-179.9	-0.1	-85.7	-80.9	80.6	86.7
	MMOK/HTYP	-179.9	-0.1	-86.1	-81.0	80.9	86.1
		-179.4	-0.1	-86.4	-81.1	81.1	86.4
η [°]	**NOMM**	179.9	179.9	116.8	-117.3	117.3	-117.3
		133.7	-134.3	120.4	-119.6	119.6	-120.5
	MMOK	179.6	179.9	116.6	-117.4	117.4	-116.7
		179.6	179.6	112.7	-111.1	111.3	-112.6
	MMOK/HTYP	179.9	179.9	117.1	-118.0	118.0	-122.7
		179.3	176.7	114.8	-113.4	113.4	-123.9
ω [°]	**NOMM**	179.9	-0.1	-122.6	-57.6	57.3	124.5
		165.1	31.4	-119.9	-59.5	60.2	120.0
	MMOK	179.9	-0.1	-122.7	-57.5	57.6	123.2
		179.9	-0.1	-124.6	-53.8	53.6	125.8
	MMOK/HTYP	-179.9	-0.1	-122.7	-58.2	58.2	122.7
		179.3	-2.0	-123.9	-55.9	55.9	123.9

For the investigation of the influence of the pyrrolidine ring conformation to peptide bond rotation a grid search for ζ and χ in 10° increments ranging from -40° to 40° was performed. The influence of the C-terminal amide group was estimated by varying ζ and ψ in 20° increments. For all grid searches the keywords EF and DMAX = 0.1 were used.

The determination of the transition states was performed by using the highest energy values for ζ where for η the lowest energy was obtained. These conformations were optimised in direction to a transition state (TS, DMAX = 0.1). In such case where no transition state could be calculated the NLLSQ minimiser was applied. The resulting transition states were proved by the eigen values (one negative) of the hessian matrix using FORCE.

Results and discussions

The investigations of the barriers for peptide bond rotations were carried out for the following compounds: acetamide, N-methylacetamide, N,N-dimethylacetamide, N-acetyl-pyrrolidine and N-acetyl-(S)-proline-methylamide.

For the determination of the transition state of rotation around a peptide bond it is insufficient to twist the dihedral angle ω [32, 37]. In this case the resulting barriers of rotation are too high (see Table 1). The reason for this is an incorrect consideration of the nitrogen inversion. Therefore, we calculated for all investigated compounds η-ζ maps to determine the correct transtition states. The conformational map for η-ζ rotation is shown for example for N-acetyl-(S)-proline-methylamide in Figure 3.

The comparison of the calculated activation barriers is listed in Table 1. These results are based on the modifica-

Table 3. *Ground and transition states of N-methyl-acetamide calculated with AM1 and PM3*

variable	keywords	trans	cis	anti-endo	anti-exo	syn-endo	syn-exo
energy	**NOMM**	-47.3	-47.1	-38.4	-35.0	-35.0	-38.4
[kcal/mol]		-51.4	-51.9	-46.9	-44.6	-44.6	-46.9
	MMOK	-47.3	-47.1	-33.3	-29.7	-29.7	-33.3
		-50.0	-50.7	-36.0	-33.7	-33.7	-36.0
	MMOK/HTYP	-47.3	-47.1	-29.3	-25.6	-25.6	-29.3
		-49.8	-50.7	-32.1	-29.9	-29.9	-32.2
ζ [°]	**NOMM**	-180.0	0.2	-92.4	-80.6	80.5	92.6
		-175.2	-12.9	-95.8	-79.2	79.0	96.1
	MMOK	180.0	-179.1	-93.1	-80.4	80.4	92.8
		178.7	2.9	-95.3	-79.9	79.8	95.4
	MMOK/HTYP	180.0	0.5	-93.2	-80.3	80.2	93.3
		-179.1	0.5	-95.5	-80.0	80.0	95.6
η [°]	**NOMM**	179.9	-178.8	124.1	-128.1	128.2	-124.3
		-136.1	138.2	123.7	-123.7	123.7	-123.7
	MMOK	-180.0	-175.9	121.1	-123.8	124.1	-121.2
		-153.6	-157.6	118.7	-118.2	118.2	-118.8
	MMOK/HTYP	-180.0	-177.3	119.2	-121.1	121.2	-119.2
		163.2	-174.2	117.1	-116.3	116.3	-117.1
ω [°]	**NOMM**	180.0	0.9	-118.2	-64.0	64.0	118.4
		-166.3	-33.7	-121.3	-60.3	60.0	121.6
	MMOK	180.0	2.9	-120.6	-61.9	62.0	119.8
		-170.7	14.4	-122.8	-58.2	58.0	122.9
	MMOK/HTYP	180.0	2.0	-121.1	-60.5	60.5	121.2
		174.1	3.6	-123.8	-57.2	57.2	123.8

tions within MOPAC which will be described in the following.

Since the amide bonds in N,N-dialkylamides are not recognised for a force field correction within the original MOPAC-program we have modified the source code of MOPAC slightly and introduced a new keyword MMOP. With this keyword the detection of peptide bonds without an N-H group is possible and a force field term for the twisting of such peptide bonds can be added. In general, a peptide bond is recognised by measuring the distances between the atoms of this bond (C_1, O_1, N_2, H). MMOP works in the same manner as MMOK except searching for two N-C bonds included in a peptide bond.

With this keyword the bug in the original MOPAC where in the case of two N-H bonds the additional force field term is applied twice has also been corrected. The results obtained with this modifications are summarised in Tables 2-6 for all investigated molecules. In each table the upper rows indicate AM1 and the other corresponding rows the PM3 results (highlighted in red).

In spite of these improvements of MOPAC the calculated barriers for the twisting of the peptide bond are in each case to small in comparison with experimentally measured values or results from ab initio calculations (see Table 7). The constants k of the force field term are stored in the array HTYPE[2] for AM1 and HTYPE[3] for PM3. It seems that

Table 4. *Ground and transition states of N,N-dimethylacetamide calculated with AM1 and PM3*

variable	keywords	trans	cis	anti-endo	anti-exo	syn-endo	syn-exo
energy	**NOMM**	-41.4	-41.3	-33.6	-30.7	-30.7	-33.6
[kcal/mol]		-52.3	-52.3	-49.0	-47.1	-47.1	-49.0
	MMOP	-41.3	-41.3	-28.2	-25.1	-25.1	-28.2
		-51.1	-51.1	-37.4	-35.4	-35.4	-37.4
	MMOP/HTYP	-41.3	-41.3	-24.0	20.8	-20.8	-24.0
		-50.9	-50.9	-33.3	-31.2	-31.2	-33.3
ζ [°]	**NOMM**	179.6	0.0	-85.4	-79.1	78.9	86.1
		-159.8	16.6	-85.9	-79.2	79.0	86.2
	MMOP	179.7	-0.4	-85.8	-78.9	78.8	85.8
		177.4	-2.0	-86.4	-79.0	79.0	86.3
	MMOP/HTYP	-179.1	-0.3	-86.0	-78.6	78.6	86.0
		-179.1	-1.3	-86.5	-78.9	78.8	86.5
η [°]	**NOMM**	-171.6	-171.4	129.7	-133.4	133.4	-129.2
		141.6	-141.6	129.6	-130.3	130.3	-129.4
	MMOP	-174.3	-178.1	126.7	-130.1	130.1	-126.5
		157.5	-157.9	125.5	-126.2	126.2	-125.5
	MMOP/HTYP	163.2	178.1	124.9	-128.0	128.0	-124.8
		163.2	162.9	124.2	-124.9	124.8	-124.2
ω [°]	**NOMM**	-176.7	5.0	-114.9	-67.1	66.9	115.6
		-179.2	39.1	-115.0	-65.4	65.2	115.4
	MMOP	-177.7	-1.5	-116.4	-65.4	65.3	116.5
		-172.0	-8.1	-117.4	-63.1	63.1	117.3
	MMOP/HTYP	174.1	-1.5	-117.4	-64.2	64.1	117.5
		174.1	-6.7	-118.2	-62.3	62.2	118.1

the parametrised value for N-methylacetamide of 14.0 kcal/mol is too low in comparison to experimental values (18.3 to 18.9 kcal/mol, see Table 7). To enlarge the activation barriers these constants were adjusted for better correlation with experimental values. As the result of systematic conformational studies the AM1 HTYPE[2] array was enlarged from 3.3191 to 5.9864 and that one for PM3 (HTYPE[3]) from 7.1853 to 9.8526. These new constants will be applied if the new keyword HTYP is used in the MOPAC calculations. The results of calculated barriers for rotation of a peptide bond are summarised in Table 7 and compared with the results obtained by using the original parameters (MMOK/MMOP without HTYP) as well as with experimental results.

The comparison of the calculated isomerisation energies with experimental results (Table 7) is not without its problems. The experimental values listed in Table 7 result from different methods and measurements in distinct solutions and at various temperatures. Depending on the methods used and applied evaluation differences in the isomerisation energies between 0.7 kcal/mol [40] and 2.8 kcal/mol [48] can be obtained. This is also indicated by the values listed in particular for N, N-dimethylacetamide. The free activation enthalpies for the isomerisation range from 15.6 kcal/mol to 21 kcal/mol.

Furthermore, the comparison between the experimental free enthalpies and the calculated heat of formation differ-

Table 5. *Ground and transition states of N-acetyl-pyrrolidine calculated with AM1 and PM3*

variable	keywords	trans	cis	anti-endo	anti-exo	syn-endo	syn-exo
energy	**NOMM**	-46.0	-46.0	-38.4	-35.0	-35.0	-38.4
[kcal/mol]		-57.3	-57.3	-53.5	-51.0	-51.0	-52.5
	MMOP	-46.0	-46.0	-33.2	-29.7	-29.7	-33.2
		-55.9	-55.9	-41.4	-39.9	-39.9	-41.4
	MMOP/HTYP	-46.0	-46.0	-29.1	-25.6	-25.6	-29.1
		-55.8	-55.8	-37.3	-35.9	-35.9	-37.3
ζ [°]	**NOMM**	177.0	-2.2	-86.1	-78.9	79.6	86.1
		-167.4	-7.4	-86.3	-78.4	77.7	86.4
	MMOP	172.6	-7.4	-86.3	-77.4	80.2	86.3
		178.5	-1.1	-86.3	-77.4	80.2	86.3
	MMOP/HTYP	179.9	0.1	-86.4	-77.5	79.6	86.4
		-179.9	-0.1	-86.9	-79.0	79.0	86.9
η [°]	**NOMM**	-162.1	160.1	127.9	-130.4	130.5	-128.1
		145.0	145.0	129.3	-128.3	128.2	-129.2
	MMOP	-175.0	175.0	126.0	-126.6	126.6	-126.2
		-165.0	164.4	125.2	-124.5	124.0	-125.4
	MMOP/HTYP	-179.4	179.9	124.6	-124.6	124.6	-124.8
		177.8	177.9	124.1	-123.5	123.5	-124.2
ω [°]	**NOMM**	-173.7	-14.4	-117.4	-63.4	64.1	117.3
		173.1	-28.9	-116.8	-61.2	60.5	117.1
	MMOP	176.1	-10.9	-118.4	-63.3	63.1	118.3
		-174.1	-11.2	-119.3	-60.3	60.3	119.2
	MMOP/HTYP	179.7	0.0	-119.1	-59.7	61.7	119.0
		179.0	-1.4	-119.9	-59.7	59.7	119.9
χ [°]	**NOMM**	-1.6	-1.7	-0.3	-0.6	-0.0	-0.1
		-19.0	19.2	-12.8	-1.3	1.5	1.3
	MMOP	-1.3	-1.0	-0.4	-3.3	-2.9	-0.2
		15.1	-16.5	-0.8	0.0	0.3	-1.3
	MMOP/HTYP	-0.1	-0.7	-0.3	-2.5	-2.1	-0.3
		16.1	-15.6	-0.5	0.0	0.2	-0.1

Table 6: *Ground and transition states of N-acetyl-(S)-proline-methylamide calculated with AM1 and PM3*

variable	keywords	trans	cis	anti-endo	anti-exo	syn-endo	syn-exo
energy [kcal/mol]	**NOMM**	-81.7	-79.9	-70.0	-69.6	-65.5	-72.2
		-93.3	-93.7	-86.0	-87.6	-82.6	-89.2
	MMOP	-81.5	-79.7	-64.5	-64.2	-59.6	-67.1
		-92.4	-92.3	-74.2	-76.6	-70.5	-77.8
	MMOP/HTYP	-81.4	-79.6	-60.0	-59.9	-55.1	-62.9
		-92.1	-91.8	-69.7	-72.4	-66.1	-73.5
ζ [°]	**NOMM**	178.2	2.1	-96.3	-71.6	82.0	88.3
		177.4	1.0	-97.3	-79.2	73.3	88.0
	MMOP	178.5	0.2	-94.1	-73.9	81.4	87.6
		178.9	0.1	-90.7	-79.3	79.3	87.5
	MMOP/HTYP	178.5	-0.3	-93.0	-74.8	81.0	87.5
		179.4	-0.2	-90.2	-79.6	80.3	87.6
η [°]	**NOMM**	-158.4	-154.9	139.6	-135.2	145.0	-128.5
		-146.3	-142.2	136.9	-126.3	141.4	-130.0
	MMOP	-164.8	-165.8	134.5	-131.2	140.8	-126.6
		-164.4	-152.2	131.0	-123.2	134.4	-126.4
	MMOP/HTYP	-167.1	-170.7	131.8	-128.7	137.4	-125.2
		-171.4	-156.9	129.3	-122.0	132.3	-125.2
ω [°]	**NOMM**	-170.7	17.3	-124.4	-56.4	71.9	119.4
		-166.0	24.2	-126.3	-62.5	59.3	118.2
	MMOP	-173.4	8.8	-124.1	-57.8	69.2	119.5
		-173.3	17.2	-121.2	-61.4	63.7	119.1
	MMOP/HTYP	-174.5	5.1	-124.0	-58.0	67.1	119.8
		-176.2	13.9	-121.4	-61.2	63.2	119.7
ϕ [°]	**NOMM**	-88.3	-88.0	-34.7	-97.1	-48.4	-107.4
		-99.1	-104.4	-38.0	-92.6	-46.8	-106.4
	MMOP	-84.9	-80.5	-30.5	-100.0	-45.5	-108.3
		-85.1	-98.1	-32.9	-95.4	-41.3	-109.1
	MMOP/HTYP	-82.9	-77.3	-29.4	-102.1	-43.4	-108.5
		-81.0	-94.6	-32.2	-92.8	-40.6	-109.0
ψ [°]	**NOMM**	63.6	-51.9	-47.9	25.5	-64.6	-46.8
		93.7	-55.9	-63.1	-44.7	-71.9	-59.7
	MMOP	64.0	-53.0	-44.6	28.8	-65.2	-46.5
		100.3	-56.5	-69.1	-45.1	-75.6	-59.6
	MMOP/HTYP	65.0	-52.5	-55.5	28.6	-67.3	-45.9
		100.4	-54.5	-69.5	-42.8	-74.6	-58.4
χ [°]	**NOMM**	-4.6	0.6	-5.4	0.9	-9.6	2.6
		-20.9	-17.9	-12.5	-7.9	-10.7	-20.1
	MMOP	-5.9	-3.6	-7.3	3.5	-10.4	3.8
		-18.5	-15.8	-11.9	-8.1	-9.6	-20.4
	MMOP/HTYP	-6.1	-4.1	-8.6	5.6	-9.8	5.0
		-18.0	-15.5	-10.9	-2.2	-9.0	-20.7

Table 7: *Barriers of rotation (in kcal/mol) for trans-cis isomerisations of the peptide bond*

Compound	NOMM	MMOK MMOP	HTYP	experimental $\Delta G^{\#}$ [kcal/mol]	Ref.
acetamide	9.6	18.9	18.0	16.7-17.3	[38]
	5.5	24.1	18.0		
N-methylacetamide	8.9	14.0	18.0	18.3-18.9	[39]
	4.5	14.0	17.6		
N, N-dimethylacetamide	7.8	13.1	17.3	18.2-18.6	[40]
				18.3 (366 K)	[41]
				17.4-20.3	[42]
				21.0 (401 K)	[43]
	3.3	13.7	17.6	15.6 (gas phase)	[44]
N-acetylpyrrolidine	7.6	12.8	16.9	16.4-17.1 [a] (303-343 K)	[45]
	3.8	14.5	18.5	16.4 [b] (358 K)	[46]
N-acetyl-(S)-proline-	9.4	14.4	18.5	18.7-20.7 [c] (333K)	[47]
methylamide	4.1	14.6	18.6	17.9 [d]	[32]

Enthalpies (differences in heats of formation) in kcal/mol (standard conditions 298 K, 1 atm)
[a] N-acetyl-4-methylpiperidine and N-acetylmorpholine,
[b] N-acetyl-4-methylpiperidine,
[c] N-acetylprolinemethylester,
*[d] ab inito 6-31G**

ences requires the estimation of the entropy contributions. The measured activation entropies of peptide bond isomerisations are positive for peptides but for simple amides negative activation entropies have also been reported [40]. The calculations of free enthalpy differences gave deviations smaller than 0.5 kcal/mol in comparison to the obtained enthalpy differences with both AM1 and PM3. Since obviously solvents do influence the isomerisation energies [38,39,42,43,47-52] which are not considered in these calculations we list the enthalpies of the barriers for peptide bond isomerisations only (see Tables 1 and 7).

Despite these problems, the agreement between the theoretical and experimental values of the peptide bond isomerisation energies is more satisfactoring than obtained with the original MOPAC force field correction constants.

The introduction of a force field term for peptide bonds preceding a proline residue leads not only to an improved description of the peptide bond rotation but also of the geometry of the nitrogen bonds inside the five membered ring (see Figure 4). Without a force field correction the pyrrolidine nitrogen atom adopts a pyramidal structure in particular with PM3 calculations which is indicated by the dihedral angle η 145° (PM3) in Tables 5 and 6 for *trans* and *cis* conformations (see Figure 4a). The introduction of MMOP causes a more realistic planar geometry ($\eta \approx 177°$) of such a peptide bond (comp. Figure 4a and 4b).

The puckering of the pyrrolidine ring in proline is a function of the dihedral angle χ. In comparison to the X-ray structure of acetyl-proline-methylamide (χ = -36.2°) [53] the obtained values of the dihedral angle χ by AM1 and PM3 calculations are too small (see Tables 5 and 6). Local minima for $\chi \approx 20°$ or -20° possess a relative energy 0,1 to 0,6 kcal/mol higher than for $\chi = 0°$. These minima could be found only with NLLSQ and not by using TS as a keyword. The determined two transition states of each χ-ζ map are conformationally and energetically identical with the transition states obtained by the η-ζ maps.

The fact that AM1 and PM3 calculate five membered rings too flat is known from the literature and is due to approaches within the semiempirical methods [54]. The energetical minimum of the H-H interaction is about 2.1 Å for both methods. Since the potential hypersurface of five membered rings is very flat and the distance of diaxial protons is shortened from

a twisted to a planar conformation from 2.5 Å to 2.3 Å the H-H repulsion is a minimum in a plane conformation.

These effects could not be corrected by the force field correction for proline peptide bonds.

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