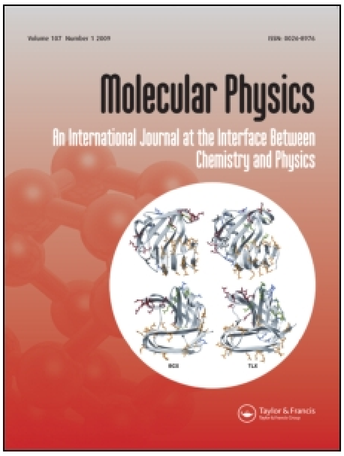

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INVITED ARTICLE

Conformers of gaseous threonine†

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Following an extensive search on the potential energy surfaces (PES) of the natural amino acid L-threonine (Thr) and its allotropic form L-allo-threonine (aThr), 56 and 61 conformers of Thr and aThr, respectively, have been located with the help of density functional theory (DFT). Accurate structures, relative energies, rotational as well as quartic and sextic centrifugal distortion constants, dipole moments, ^{14}N nuclear quadrupole coupling constants, anharmonic vibrational frequencies and double-harmonic infrared intensities have been determined from *ab initio* electronic structure calculations for the five most stable Thr and aThr conformers. The global minimum, **Thr-I**, has a cyclic triple H-bond motif with strong OH $\cdots$ N, C=O $\cdots$ HO, and a weaker NH $\cdots$ OH H-bond, where the latter two involves the side chain OH, and an energetically unfavourable trans-COOH arrangement. The best relative energies of the conformers, accurate within ±1 kJ mol^{-1}, have been determined through the first-principles composite focal-point analysis (FPA) approach. There are four and three conformers of Thr and aThr, respectively, within a relative energy of 5 kJ mol^{-1}. Similarly to other amino acids investigated, lower levels of electronic structure theory, especially the Hartree–Fock level, are unable to determine the correct relative energies of the conformers. The rotational, the quartic and sextic centrifugal distortion, and the ^{14}N nuclear quadrupole coupling constants as well as the anharmonic vibrational fundamentals and double-harmonic infrared intensities, all determined using DFT, should aid identification and characterization of the conformers of threonine and allo-threonine by rotational and vibrational spectroscopies, respectively.

Keywords: neutral L-threonine; neutral L-allo-threonine; amino acids; *ab initio* calculations; conformational analysis; structure elucidation; focal-point analysis

1. Introduction

During recent decades, determination and characterization of the structures of biomolecules, in particular those of proteins and peptides, have been in the focal point of experimental and theoretical research. Since amino acids (AA) are the building blocks of these biopolymers, the structural investigation of AAs, extending from solids to the gas phase, also received considerable attention. Experimental and theoretical structural investigations related to AAs, executed prior to 1999, have been reviewed in [1]. During the last decade a considerable number of novel experimental and first-principles computational investigations have been performed on different aspects of the structures of amino acids [2–4], peptides [5,6], and their various complexes [7]. The review of all these studies is beyond the scope of the present study and thus only a small number of high-level computational studies originating from our own group are mentioned.

There is only a limited number of natural amino acids and the relatively small size of these molecules allows the application of highly sophisticated experimental and computational techniques during studies related to their equilibrium and dynamical structures usually investigated through their rotational-vibrational spectra. Consequently, we have a detailed understanding of the basics of the structural characteristics of AAs. AAs are chiral, with the sole exception of glycine. In solution and in the solid state, AAs are found in different zwitterionic forms. In the gas phase, however, AAs exist in their neutral form. Furthermore, as AAs are very flexible molecules, all of them exhibit a large number of low-energy conformers and a complex, highly structured potential energy surface (PES). Due partially to the low volatility of AAs and their tendency to decomposition when heated, most of these conformers are not amenable to experimental scrutiny but they can be subjected to detailed quantum chemical investigations. Characterization of the complex PESs of amino acids should precede and supplement related experimental structural studies.

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†This paper is dedicated to Professor Fritz Schaefer on the occasion of his 65th birthday.

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Chiral AAs can play a role in chirally selective organocatalysis, the most important amino acid in this respect appears to be proline (Pro) [8]. As to other applications of AAs in chemistry, one may mention functionalization of fullerenes, carbon nanotubes and semiconductor quantum dots with amino acids [9]. Search for signs of life in the universe is also based principally on structural and spectroscopic characterization of AAs [10]. As of today, it seems that none of the AAs have been found outside of our own planet. Threonine, being an essential amino acid is involved in many studies with biochemical importance, for which the present conformational results could prove useful. As examples, we mention the studies of metallic complexes of threonine [11–13] and the possible use of Thr and its derivatives in organocatalysis [14].

The number of local minima on the respective PESs and the structural properties of the related conformers, including accurate relative energy estimates, are available for a number of amino acids [1]. The most accurate relative energies, mostly obtained within the focal-point analysis (FPA) approach [15,16], are available for the amino acids glycine (Gly) [4,17–19], alanine (Ala) [18,20], and proline (Pro) [1–3]. The present computational study expands the list of structurally particularly well characterized amino acids by establishing all of the lower-energy and almost all of the higher-energy conformers of L-threonine (see Figure 1), one of the two amino acids, the other one being isoleucine, exhibiting two chirality centres. This feature of threonine was one of the reasons for performing this study. The two diastereomers need to be examined separately during conformational and spectroscopic studies as for all practical purposes they appear as two distinct molecules. Obviously, both L-threonine and L-allo-threonine have an enantiomer pair, D-threonine and D-allo-threonine, respectively. In what follows, L-threonine and L-allo-threonine will be abbreviated as Thr and aThr, respectively. In the biosphere only Thr is abundant. Detailed structural characterization of the lowest-energy conformers of these diastereomers was another important aim of the present study. The present study yielded, as primary information, equilibrium structures, relative energies, and a large number of spectroscopic molecular parameters related to the vibrational and rotational spectra of the most important conformers of the two forms of threonine, Thr and aThr. The computational results obtained should help identification of at least some of the conformers of threonine by means of rotational and vibrational spectroscopies.

2. Computational details

Most of the atom-centred Gaussian basis sets selected for the electronic structure computations of this study contain both polarization and diffuse functions, as they are both needed for the determination of accurate structures and relative energies for H-bonded systems [21]. The subcompact 3-21G basis [22] lacks these functions and thus it has been used only for prescreening the conformers at the Hartree–Fock [23] level of electronic structure theory. The 6-31G* [24] and 6-311++G** [25] bases, used in this study extensively for geometry optimizations and subsequent force field determinations, contain 260 and 369 contracted Gaussian functions (CGFs), respectively, for threonine. The correlation-consistent, polarized-valence (aug)-cc-p(C)VnZ, $n = 2$ (D), 3 (T), 4 (Q) basis sets of Dunning and co-workers [26,27] have been employed extensively for single-point energy calculations within the FPA approach. (Note that only the augmented (aug) basis set contains diffuse functions, while tight functions, necessary for calculation of core correlation [28] and relativistic effects [29] are only part of core-polarized (C) basis sets.) For Thr, the aug-cc-pVDZ, cc-pVTZ, cc-pCVTZ, aug-cc-pVTZ, and aug-cc-pVQZ basis sets contain 265, 366, 470, 575, and 1054 CGFs, respectively. Only the pure spherical harmonics have been employed in the cc basis sets used in this study.

Electronic wave functions have been determined in this study by the single-configuration, self-consistent-field, restricted Hartree–Fock (RHF) method [23,30,31], by second-order Møller–Plesset perturbation theory, i.e. MP2 [32], by coupled cluster (CC) methods [33] including all single and double excitations (CCSD) [34] and in cases, additionally, a perturbative correction for contributions from connected triple excitations [CCSD(T)] [35], and by a hybrid density functional theory (DFT) approach usually abbreviated as B3LYP [36]. The T_1 diagnostic values of coupled cluster theory [37] are around 0.013 for the different conformers of Thr, suggesting that they can adequately be described by single-reference-based electron correlation methods. The eight lowest 1s-like core orbitals were kept frozen in all post-Hartree–Fock treatments unless otherwise noted.

Rotational barriers corresponding to the methyl group were computed via restricted optimizations [38–40], fixing the methyl torsional coordinate, at the MP2/cc-pVDZ level.

The electronic structure program packages Gaussian03 [41] and MOLPRO [42] have been used extensively during this study. The package MAB-ACESII [43] was employed for the methyl rotational barrier computations.

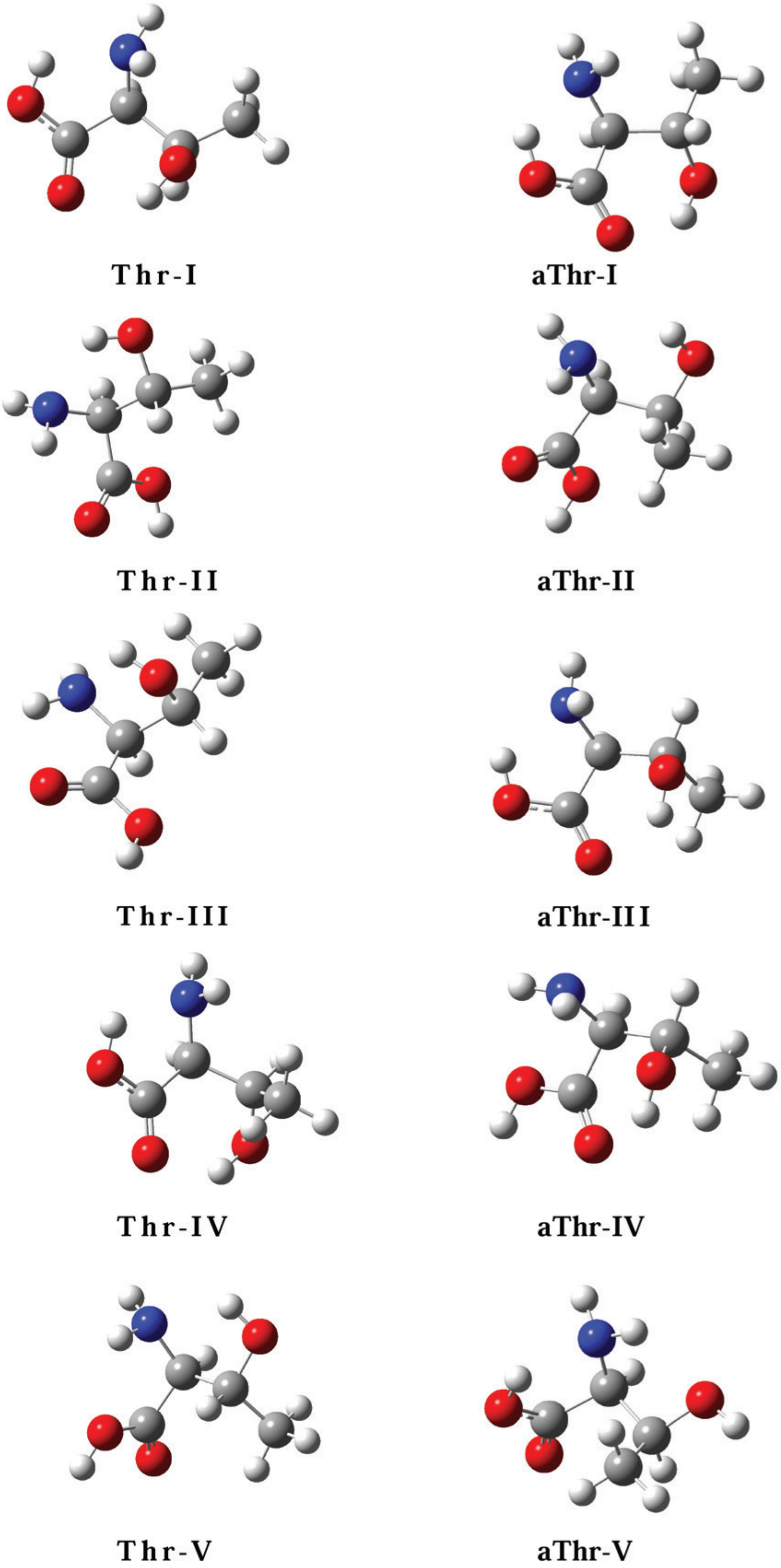

Figure 1. Pictorial representation of the five lowest-energy conformers of L-threonine (Thr) and L-allo-threonine (aThr).

2.1. *Geometry optimizations*

Similarly to a scheme first employed for glycine [17], the conformers of Thr and aThr determined in this study are numbered by Roman numerals (see Figure 1) and reflect the energy order of the conformers determined at the B3LYP/6-311++G** level.

Initial structures for the geometry optimizations of the conformers of Thr and aThr were generated by replacing the appropriate H atom of the global minimum of serine with a CH_3 group followed by an optimization at the HF/3-21G level. Then, five of the six torsional angles characterizing threonine, CCOH (carboxyl), CCC=O, CCNH, CCC-O, and CCOH (side chain), were modified systematically by 60°. This resulted in $6^5 = 7776$ initial structures for one diastereomer. Initial geometry optimizations on the 7776 structures were performed for each diastereomer at the inexpensive RHF/3-21G level. This simple level of electronic structure theory was chosen for the prescreening of the conformers as it was widely assumed [1] that the RHF/3-21G PESs of amino acids and peptides are more structured than their counterparts determined at more sophisticated, and thus computationally more demanding levels of electronic structure theory. Further optimizations were executed at the more reliable B3LYP/6-31G*, B3LYP/6-311++G**, and MP2/aug-cc-pVTZ levels. Initial structures for the latter optimizations were the local minima found on the RHF/3-21G PES of Thr and aThr.

Relative energies of the lowest-energy conformers of Thr and aThr, obtained by geometry optimizations at the HF/3-21G, DFT(B3LYP)/6-31G*, DFT(B3LYP)/6-311++G**, and MP2/aug-cc-pVTZ levels, are given in Table 1. Cartesian coordinates of the optimized structures and energies of all the conformers found in this study are provided as Supplementary Material.

2.2. *Focal-point analysis (FPA)*

In order to obtain accurate estimates of the relative energies of the conformers of Thr and aThr with corresponding uncertainties, the FPA approach [15,16] was utilized for both diastereomers. The five lowest-energy structures of Thr and aThr (Figure 1), obtained at the B3LYP/6-311++G** level, were included in this investigation.

Extrapolation of the energies to the complete basis set (CBS) limit at the RHF and MP2 levels was performed, as part of the FPA approach, using the formulas $E_n = E_{\mathrm{CBS}} + A(n+1)\exp(-9\sqrt{n})$ [44] and $\varepsilon_n = \varepsilon_{\mathrm{CBS}} + Bn^{-3}$ [45], respectively. In these formulas n is the cardinal number of the augmented, correlation-consistent basis sets aug-cc-pVnZ ($n \in \{3, 4\}$), A and B are parameters, E_n is the RHF energy, and ε_n is the MP2 correlation energy increment, and CBS refers to the complete basis set limit. Energy corrections were treated additively, taking advantage of the fact that the contribution of higher-level electron correlation to the requested relative energies does not change significantly with the size of the basis set. CCSD and CCSD(T) correlation energy increments were calculated using the cc-pVTZ basis set.

From the auxiliary corrections needed to be determined within the FPA approach, the core correction was obtained at the MP2/cc-pCVTZ level as the difference between explicit frozen-core (fc) and all-electron (ae) energy computations. The relativistic contributions to the relative energies were estimated within the Douglas–Kroll (DK) formalism [46], as implemented in MOLPRO, at the MP2(ae)/

Table 1. Relative energies (kJ mol^{-1}) of the most stable L-threonine and L-allo-threonine conformers obtained from geometry optimizations performed at the MP2/aug-cc-pVTZ, DFT(B3LYP)/6-311++G**, DFT(B3LYP)/6-31G*, and RHF/3-21G levels[a].

Conformer	MP2/ aug-cc-pVTZ	DFT (B3LYP)/ 6-311++G**	DFT (B3LYP)/ 6-31G*	RHF/ 3-21G
Thr-I	0.00	0.00	0.00	0.00
Thr-II	2.54	1.34	3.18	4.24
Thr-III	4.29	4.33	7.15	−0.96
Thr-IV	4.41	4.80	1.38	3.54
Thr-V	6.77	5.34	9.07	7.69
Thr-VI		7.30		2.68
Thr-VII		7.86		9.95
Thr-VIII		8.06		11.16
Thr-IX		10.82		24.21
Thr-X		11.34		−0.71
Thr-XI		11.47		4.49
Thr-XII		11.91		14.07
aThr-I	0.00	0.00	0.00	0.00
aThr-II	−1.13	0.64	5.48	−0.30
aThr-III	3.18	4.13	4.27	1.39
aThr-IV	5.79	6.43	8.77	1.87
aThr-V	3.50	7.08	13.65	12.53
aThr-VI		7.23		13.02
aThr-VII		7.30		8.32
aThr-VIII		7.42		14.56
aThr-IX		8.11		7.36
aThr-X		8.21		5.62
aThr-XI		10.01		7.81
aThr-XII		10.10		19.49
aThr-XIII		10.13		0.34

Note: [a]The relative energies of conformer **aThr-I** with respect to conformer **Thr-I** at the MP2, B3LYP/6-311++G**, B3LYP/6-31G*, and RHF levels are 3.67, 2.20, –1.06, and 2.37 kJ mol^{-1}, respectively.

cc-pCVTZ level. After deemed to be unimportant even at the level of precision of this study, the diagonal Born–Oppenheimer corrections (DBOC) [47] were not computed and thus were completely neglected. Zero-point vibrational energies (ZPVE) were obtained as harmonic and anharmonic correction values at the B3LYP/6-311++G** and B3LYP/6-31G* levels, respectively.

The resulting composite single-point relative energies are shown in Tables 2 and 3 for Thr and aThr, respectively.

2.3. *Spectroscopic parameters*

For all local minima, quadratic force constants were computed at the B3LYP/6-311++G** level using the B3LYP/6-311++G** optimized structures, thus avoiding the nonzero-force dilemma [48]. For the five lowest-energy conformers of Thr and aThr quartic force fields in normal coordinate space have been determined at the B3LYP/6-31G* level, again at the fully optimized B3LYP/6-31G* structure. The related harmonic and anharmonic vibrational fundamentals of Thr and aThr are reported in Tables 4 and 5, respectively. No scaling of the force fields or of the resulting vibrational wavenumbers has been attempted. The anharmonic vibrational fundamentals were obtained through second-order vibrational perturbation theory (VPT2) [49].

The optimized structures determine the equilibrium rotational constants, while the quadratic and cubic force fields yield the equilibrium quartic and sextic centrifugal distortion constants in the *A*-reduced representation. The anharmonic force fields determined also allow the calculation of vibrationally averaged ground-state rotational constants. For the five lowest-energy Thr and aThr conformers these spectrosopic constants are reported in Tables 6 and 7, respectively.

The ^{14}N nuclear quadruple coupling constants, resulting from the non-spherical distribution of the nuclear charge, have been determined at the B3LYP/6-311++G** level.

3. Conformational analysis

3.1. *Preliminary tests*

The method described in Section 2.1 for studying the conformational space of Thr and aThr systematically was tested on the neutral amino acids glycine (Gly) and L-alanine (Ala), where the number of conformers are well established as a result of previous high-level first-principles studies [17–20].

At the RHF/3-21G level, only 6 of the 8 and 11 of the 13 conformers were found for Gly and Ala, respectively. The RHF/3-21G optimizations failed to yield the high-energy conformers **Gly-VIIp** and

Table 2. FPA relative energies and energy increments (δ), all in kJ mol^{-1}, for the 5 lowest-energy conformers of L-threonine[a].

Energy term	Basis	Thr-I	Thr-II	Thr-III	Thr-IV	Thr-V
RHF	aug-cc-pVDZ	0.00	−3.14	−2.01	7.52	0.42
	aug-cc-pVTZ	0.00	−3.01	−2.11	7.80	0.72
	aug-cc-pVQZ	0.00	−2.99	−2.20	7.80	0.77
	CBS	0.00	−2.99	−2.21	7.79	0.78
δ[MP2]	aug-cc-pVDZ	0.00	4.60	6.25	−2.48	5.05
	aug-cc-pVTZ	0.00	5.36	6.47	−3.43	5.90
	aug-cc-pVQZ	0.00	5.36	6.43	−3.47	5.94
	CBS	0.00	5.36	6.39	−3.49	5.98
δ[CCSD]	cc-pVTZ	0.00	−0.74	−1.38	0.66	−0.93
δ[CCSD(T)]	cc-pVTZ	0.00	0.93	1.35	−1.08	1.19
MP2(ae) – MP2(fc)	cc-pCVTZ	0.00	0.08	0.00	0.04	0.08
Relativistic	cc-pCVTZ	0.00	−0.05	0.00	0.03	−0.04
ZPVE(harm)		0.00	−0.41	−1.05	0.68	−0.72
ZPVE(anharm) – ZPVE(harm)		0.00	0.14	0.20	−0.21	0.44
FPA relative energies		0.00	2.32	3.30	4.42	6.77

Notes: [a]All the FPA energy computations were performed employing the fully optimized B3LYP/6-311++G** structures as references. Energy increments (δ) refer to the previous level of theory, MP2 to RHF, CCSD to MP2, and CCSD(T) to CCSD. The abbreviations (fc) and (ae) show that the correlated-level electronic structure computations were done with the frozen core approximation or with all electrons, respectively. The ZPVE(harm)s (harmonic zero-point vibrational energies) correspond to B3LYP/6-311++G** harmonic frequencies. The ZPVE(anharm)s (anharmonic zero-point vibrational energies) were obtained at the B3LYP/6-31G* level based on quartic normal coordinate force fields and second-order vibrational perturbation theory (VPT2).

Table 3. FPA relative energies and energy increments (δ), all in kJ mol^{-1}, for the five lowest-energy conformers of L-allo-threonine[a].

Energy term	Basis	**aThr-I**	**aThr-II**	**aThr-III**	**aThr-IV**	**aThr-V**
RHF	aug-cc-pVDZ	0.00	−8.53	2.19	−3.63	4.70
	aug-cc-pVTZ	0.00	−8.55	2.22	−3.17	4.66
	aug-cc-pVQZ	0.00	−8.42	2.40	−2.98	4.88
	CBS	0.00	−8.41	2.43	−2.95	4.91
δ[MP2]	aug-cc-pVDZ	0.00	6.46	0.47	7.56	−1.77
	aug-cc-pVTZ	0.00	7.94	1.18	9.11	−0.66
	aug-cc-pVQZ	0.00	8.25	1.41	9.39	−0.29
	CBS	0.00	8.48	1.57	9.60	−0.01
δ[CCSD]	cc-pVTZ	0.00	−1.54	−0.68	−2.32	0.26
δ[CCSD(T)]	cc-pVTZ	0.00	2.14	0.53	1.89	0.89
MP2(ae) –MP2(fc)	cc-pCVTZ	0.00	−0.01	0.04	0.08	−0.04
Relativistic	cc-pCVTZ	0.00	−0.05	−0.03	−0.06	−0.02
ZPVE(harm)		0.00	−1.40	−0.53	−0.85	−0.91
ZPVE(anharm) – ZPVE(harm)		0.00	−0.01	0.09	−0.02	0.13
FPA relative energies		0.00	−0.80	3.42	5.37	5.21

Note: [a]See footnote a to Table 2. The relative energy of conformer **aThr-I** with respect to conformer **Thr-I** is 3.07 kJ mol^{-1}.

Gly-VIIIn for Gly. The most stable conformers {**Gly-Ip, Gly-IIn**} [4] were obtained {28, 49} times out of a total of 216 initial structures. Using the same conformational search but this time at the RHF/6-31G** level, all Gly conformers were found. These observations show the possible dangers of using RHF/3-21G optimizations for prescreening the possible conformers of amino acids (and most likely peptides). It is comforting, nevertheless, that RHF/3-21G optimizations always missed only the highest-energy conformers.

The discrepancies obtained for the amino acids Gly and Ala based on the initial prescreening of the conformers at the RHF/3-21G level led us to an extended study of the role of initial geometry optimizations. Taking the Thr conformers determined at the B3LYP/6-311++G** level, preliminary optimizations at the RHF/3-21G, RHF/3-21G**, and RHF/3-21+G** levels were done, continued by B3LYP/6-311++G** level optimizations. It was found that the final B3LYP/6-311++G** structures were often different from the initial B3LYP/6-311++G** structures when using the different basis sets for the preoptimizations at the RHF level. Thus, the conformers found on the PES corresponding to the final optimization level depend somewhat on the level of the initial optimizations. Since the conformers found by only one of the methods were of high relative energy (above 15 kJ mol^{-1} in all cases), it is safely concluded that this effect is due to the fine structure of the high-energy regions of the different PESs. While this observation is certainly somewhat disturbing, it seems to be not relevant for the low-energy conformers, of highest relevance for practical applications.

3.2. *Initial optimizations*

Using the inexpensive RHF/3-21G method, 68 and 66 conformers were found for L-threonine and L-allo-threonine, respectively. When B3LYP/6-311++G** geometry optimizations were performed, using the RHF/3-21G conformers as initial structures, some of the higher-energy RHF/3-21G conformers disappeared, thus finally 56 and 61 conformers were found this way for Thr and aThr, respectively. Of the 7776 initial structures for each diastereomer, {202, 455, 328, 78, 146} and {220, 422, 534, 336,131} ended up as {**Thr-I, Thr-II, Thr-III, Thr-IV,Thr-V**} and {**aThr-I, aThr-II, aThr-III, aThr-IV, aThr-V**}, respectively. This clearly shows that these lowest-energy conformers have substantial catchment regions. The smallest region corresponds to **Thr-XV** with only 12 initial structures ending up at that conformer.

3.3. *Comparison with the study of Zhang and Lin [50]*

The recent investigation of Zhang and Lin [50] is similar to our work in many respect. An important distinction is that in that paper only the L-threonine diastereomer was considered. For L-Thr, Zhang and Lin found 71 conformers at the B3LYP/6-311++G** level, while, as stated above, we found only 56 conformers. This result is the more curious as their

Table 4. Harmonic vibrational fundamentals (ω), their VPT2 anharmonic corrections ($\Delta\nu$), and double-harmonic vibrational intensities (I) of the five lowest energy conformers of L-threonine[a].

	Thr-I			**Thr-II**			**Thr-III**			**Thr-IV**			**Thr-V**		
Number	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I
1	3819	−177	46.8	3742	−186	64.9	3762	−188	84.1	3705	−195	131.3	3758	−187	78.7
2	3595	−181	15.3	3708	−199	91.8	3713	−196	60.9	3600	−182	16.1	3718	−198	84.5
3	3495	−108	11.1	3582	−181	12.1	3612	−183	21.9	3518	−129	1.1	3592	−181	11.8
4	3455	−262	277.5	3497	−191	6.2	3516	−142	18.8	3467	−259	264.4	3511	−105	4.8
5	3114	−142	18.4	3115	−142	18.9	3109	−148	23.5	3114	−143	15.6	3113	−143	18.3
6	3098	−141	28.9	3109	−143	24.4	3095	−135	35.2	3086	−113	26.6	3107	−143	24.9
7	3055	−131	9.9	3085	−139	8.3	3078	−130	2.0	3055	−138	5.1	3092	−141	5.7
8	3041	−119	7.7	3040	−133	13.2	3023	−160	23.0	3034	−144	29.1	3038	−134	11.9
9	3033	−132	13.5	2959	−106	38.3	3000	−137	19.8	3021	−165	21.4	2964	−109	39.5
10	1824	−31	324.4	1800	−31	296.8	1808	−33	304.2	1808	−31	287.4	1811	−29	321.0
11	1651	−50	32.9	1662	−31	39.5	1642	−58	77.4	1658	−48	38.1	1659	−57	36.8
12	1503	−42	4.4	1502	−36	3.2	1501	−73	4.8	1502	−51	9.7	1502	−39	4.0
13	1484	−9	6.7	1484	−20	6.0	1489	−39	6.0	1491	−48	5.5	1483	−19	3.6
14	1430	−34	6.3	1441	−46	54.3	1431	−30	55.5	1445	−39	16.5	1439	−47	44.4
15	1418	−40	427.2	1425	−32	4.4	1416	−44	23.4	1419	−45	232.8	1409	−32	29.7
16	1409	−34	9.6	1404	−34	23.6	1401	−17	5.6	1409	−33	215.9	1400	−36	0.9
17	1387	−36	4.9	1375	−5	20.3	1370	−34	11.6	1394	−39	21.4	1377	−5	3.6
18	1359	−35	8.0	1339	−19	31.8	1337	−4	24.0	1371	−33	41.7	1333	−14	53.6
19	1280	−32	57.0	1311	−11	81.1	1302	−30	3.1	1311	−30	18.0	1309	−26	59.6
20	1276	−37	0.4	1265	−39	7.5	1258	−31	21.0	1270	−23	2.5	1289	−29	14.8
21	1209	−40	21.5	1226	−33	20.0	1221	−35	49.2	1220	−46	23.6	1229	−18	17.5
22	1197	−37	9.7	1158	−34	75.1	1162	−33	112.9	1192	−36	9.6	1163	−37	213.1
23	1151	−29	24.6	1136	−31	167.6	1136	−32	154.5	1140	−32	8.9	1138	−31	43.8
24	1084	−24	14.4	1113	−40	54.7	1104	−28	27.3	1106	−31	50.5	1110	−37	7.7
25	1052	−18	55.9	1074	−29	12.9	1076	−30	52.8	1090	−31	79.6	1072	−31	14.8
26	1017	−24	11.5	1052	−21	35.6	1026	−22	25.2	1045	−21	5.2	1050	−19	31.7
27	984	−35	88.3	958	−41	46.6	937	−28	0.7	968	−32	23.1	932	−41	16.9
28	909	−24	54.3	925	−22	35.3	892	−45	77.0	903	−41	84.1	921	−23	88.3
29	878	−47	103.5	872	−29	38.8	869	−24	23.9	872	−40	83.5	868	−20	36.4
30	854	−26	51.0	854	−14	98.7	830	−27	75.8	837	−52	61.4	831	−21	92.2
31	822	−18	13.7	742	−19	37.1	728	−14	28.6	799	−18	26.7	752	−16	24.0
32	751	−15	6.7	666	−16	17.3	659	−3	34.0	754	−10	3.1	643	−20	58.8
33	650	−11	8.9	610	−41	108.9	630	−31	58.1	609	−29	18.0	595	−46	56.3
34	559	−12	9.3	601	−18	125.4	575	−41	163.9	575	−19	117.5	586	−19	160.0
35	532	−23	3.9	543	−11	1.0	545	−12	22.0	549	−11	3.8	548	−11	6.9
36	453	−50	7.9	469	−1	8.8	476	−10	28.5	491	−5	7.3	467	0	10.6
37	403	−11	88.0	385	−6	21.8	450	−3	4.6	456	−6	3.1	396	−8	6.7
38	368	−17	34.8	366	−3	6.3	349	−4	3.3	377	−5	7.8	365	−3	9.4
39	356	−7	24.7	307	−7	4.1	301	−3	0.5	337	−11	18.2	309	−8	8.3
40	310	−2	3.7	290	−21	7.4	270	−8	6.0	298	−29	13.0	291	−2	2.3
41	251	−2	12.6	267	−3	3.2	240	−12	0.9	251	−16	3.5	260	−1	1.5
42	225	−19	1.2	230	5	0.5	215	−23	11.8	245	−12	3.6	221	−4	0.6
43	179	−1	5.4	171	−1	1.0	181	−1	0.5	203	−11	8.0	162	−1	0.7
44	86	−3	3.1	84	0	5.3	81	2	1.2	111	−9	3.0	86	−2	3.5
45	51	−4	5.7	47	−2	0.9	69	7	2.2	67	−9	1.2	41	−3	1.6

Note: [a]Harmonic frequencies and intensities were calculated at the B3LYP/6-311++G** level, while anharmonic corrections correspond to the B3LYP/6-31G* level of theory. Frequencies and intensities are given in cm^{-1} and in $\mathrm{km\,mol}^{-1}$, respectively.

grid for the initial optimization was somewhat less systematic, it was based partially on chemical intuition, and they included much less (only 1296) initial points than the grid corresponding to the present study contains (7776). The difference is due to the different theoretical levels used for the initial optimizations, and in this case show that the RHF/3-21G PES is less structured (68 conformers found) than the PES obtained at the B3LYP/6-311++G** level (71 conformers found). Overall, Zhang and Lin obtained 16 conformers which were not found and missed one conformer which was found in the present study.

Table 5. Harmonic vibrational fundamentals (ω), their VPT2 anharmonic corrections ($\Delta\nu$), and double-harmonic vibrational intensities (I) of the five lowest energy conformers of L-allo-threonine[a].

	aThr-I			**aThr-II**			**aThr-III**			**aThr-IV**			**aThr-V**		
Number	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I	ω	$\Delta\nu$	I
1	3690	−197	191.6	3751	−186	70.1	3789	−182	38.5	3752	−190	56.6	3837	−175	35.5
2	3596	−180	12.5	3718	−196	67.3	3586	−182	14.0	3740	−186	85.5	3574	−182	19.3
3	3506	−125	1.6	3589	−182	13.4	3493	−103	9.8	3595	−182	12.3	3507	−100	1.4
4	3464	−263	268.4	3512	−188	6.8	3455	−265	279.7	3505	−131	4.9	3453	−251	273.3
5	3114	−142	16.0	3111	−142	20.3	3128	−141	7.0	3110	−142	20.6	3112	−143	15.5
6	3101	−141	27.5	3096	−137	36.5	3095	−137	25.1	3094	−128	32.1	3092	−137	22.6
7	3041	−140	7.3	3076	−138	9.1	3043	−106	19.6	3066	−133	7.3	3049	−132	20.6
8	3034	−117	15.4	3068	−130	1.6	3035	−116	24.0	3059	−136	13.2	3037	−128	5.6
9	2937	−85	50.2	3029	−101	13.8	3032	−128	14.0	3028	−151	13.8	3031	−121	15.2
10	1808	−21	307.4	1804	−33	299.0	1821	−28	305.2	1780	−34	279.2	1834	−30	338.2
11	1660	−45	32.5	1672	−8	40.7	1653	−45	35.2	1643	−80	39.6	1668	−33	45.6
12	1501	−37	5.5	1503	−100	5.0	1507	−69	3.5	1505	−68	5.9	1501	−42	10.3
13	1488	−6	4.0	1489	17	5.1	1490	11	13.0	1493	−40	10.4	1497	−43	3.5
14	1446	−54	11.0	1429	−30	45.2	1429	−46	174.6	1444	−39	31.2	1426	−58	128.5
15	1416	−52	435.3	1425	−35	26.8	1415	−41	158.8	1412	−14	14.9	1417	−20	232.0
16	1405	−30	61.4	1412	−19	13.8	1408	−30	107.8	1395	−54	19.3	1405	−46	30.6
17	1385	−38	12.3	1377	−40	7.6	1400	−36	42.5	1394	−41	31.4	1384	−33	5.3
18	1369	−5	0.3	1330	−28	36.2	1352	−34	14.5	1343	−32	12.7	1364	−39	14.6
19	1315	−1	76.0	1295	−27	16.1	1316	−34	7.6	1317	−34	9.3	1317	−41	1.5
20	1269	−32	4.4	1263	−33	9.6	1267	−39	4.5	1285	−35	2.9	1260	−34	11.6
21	1216	−42	36.9	1215	−36	31.0	1204	−41	27.4	1212	−34	10.7	1228	−36	14.4
22	1189	−28	4.8	1173	−35	14.3	1194	−32	19.2	1147	−37	243.0	1192	−34	25.8
23	1152	−31	15.1	1130	−34	250.5	1141	−30	33.6	1143	−30	129.4	1125	−30	34.9
24	1111	−31	26.0	1111	−28	98.6	1091	−27	34.9	1090	−26	11.2	1109	−28	79.0
25	1084	−32	57.0	1072	−29	18.5	1074	−25	8.9	1080	−34	19.9	1063	−27	4.7
26	1056	−30	10.9	1027	−28	17.7	1035	−21	42.3	1045	−22	32.6	1021	−29	37.4
27	975	−28	26.3	958	−39	30.9	973	−36	106.9	943	−23	7.8	964	−34	109.8
28	919	−34	78.2	921	−20	48.2	915	−26	46.6	901	−42	32.5	930	−26	42.1
29	884	−29	63.6	850	−41	131.1	880	−45	110.3	829	−52	136.2	883	−56	72.8
30	847	−62	18.9	828	−18	4.7	860	−23	38.2	816	−23	25.2	867	−25	25.9
31	840	−14	102.8	723	−21	19.4	813	−17	8.7	771	−17	34.4	829	−15	8.9
32	736	−12	5.8	650	−13	15.8	754	−14	8.2	709	−9	9.6	731	−10	17.0
33	631	−20	126.3	644	−8	78.3	607	−9	0.6	629	−30	99.3	648	−13	16.6
34	591	−9	3.9	570	−64	20.4	552	−34	11.0	569	−49	207.7	623	−10	8.5
35	571	−8	5.7	549	−21	152.0	524	−27	11.6	531	−9	19.6	495	−7	0.6
36	485	−1	15.6	457	−5	25.9	476	−15	20.1	498	−5	6.9	424	−5	11.7
37	406	−10	1.6	414	−2	4.0	437	−9	102.2	391	−2	0.9	362	−10	12.1
38	357	−10	10.2	362	−5	7.2	366	−18	31.0	358	−6	26.1	325	−32	5.6
39	347	−32	25.7	302	−10	5.1	349	−6	9.7	324	−3	14.7	306	−7	38.7
40	331	−7	3.7	255	−19	1.7	331	−3	8.0	269	−4	3.7	280	3	73.4
41	302	−13	10.6	249	−2	3.2	241	−1	3.0	241	−7	0.2	272	1	25.4
42	235	−18	6.2	228	−18	8.6	229	−1	5.1	230	−6	3.6	248	−11	0.3
43	208	−7	6.7	166	0	1.9	199	0	5.5	187	−5	3.6	174	−1	10.5
44	94	−2	0.9	82	−1	6.0	86	−2	4.1	120	−6	3.5	86	−7	3.9
45	71	−8	2.0	62	1	0.9	47	−4	5.1	45	−5	0.6	80	−4	2.2

Note: [a]See footnote ([a]) to Table 4.

Since the dependence of the results of the conformational search on the method of initial optimization seems to be only relevant for higher-energy conformers, it is important to stress that all conformers of L-Thr up to a relative energy of 19 kJ mol^{-1} agree in the two studies.

4. Structures

The MP2 and DFT(B3LYP) levels of electronic structure theory yield equilibrium structures which may suffer from different problems. It is the intramolecular basis set superposition error (BSSE) that can be quite significant at the MP2 level, especially when

Table 6. Equilibrium rotational constants (A_e, B_e, C_e), their vibrational corrections ($\Delta A, \Delta B, \Delta C$) leading to ground-state rotational constants A_0, B_0, and C_0, A-reduced quartic ($\Delta_J, \Delta_K, \Delta_{JK}, \delta_J, \delta_K$) and sextic ($\Phi_J, \Phi_K, \Phi_{JK}, \Phi_{KJ}, \phi_j, \phi_k, \phi_{jk}$) centrifugal distortion constants, ^{14}N nuclear quadrupole coupling tensor elements ($\chi_{aa}, \chi_{bb}, \chi_{cc}$), and total dipole moments (μ_{total}) of the five lowest-energy conformers of L-threonine[a].

	Thr-I	**Thr-II**	**Thr-III**	**Thr-IV**	**Thr-V**
A_e	3201.77	2865.57	3128.28	2679.22	2882.14
ΔA	−32.91	−24.61	−38.07	−27.82	−25.09
B_e	1498.69	1587.52	1476.30	1749.67	1549.45
ΔB	−25.80	−13.55	−14.75	−17.45	−17.18
C_e	1256.81	1194.30	1294.91	1354.93	1222.85
ΔC	−8.64	−10.49	−9.29	−10.67	−6.24
Δ_J	0.258	0.270	0.172	0.211	0.300
Δ_K	−0.017	−0.142	−0.118	0.192	−0.526
Δ_{JK}	0.404	0.269	0.723	0.006	0.697
δ_J	0.065	0.052	0.020	0.035	0.069
δ_K	0.155	0.620	−1.217	−0.028	−0.727
Φ_J	−0.15	−0.10	0.08	−0.02	−0.15
Φ_K	20.45	13.57	−1.49	−0.20	61.88
Φ_{JK}	6.61	4.77	−4.70	0.10	26.28
Φ_{KJ}	−27.18	−18.09	5.05	−0.47	−87.56
ϕ_j	−0.08	−0.04	0.02	0.00	−0.04
ϕ_k	70.70	27.48	−59.03	1.89	138.08
ϕ_{jk}	−0.36	−1.23	2.03	−0.06	−1.54
χ_{aa}	−4.08	−4.83	−0.94	−4.51	−4.80
χ_{bb}	2.12	2.92	3.24	2.66	2.84
χ_{cc}	1.96	1.90	−2.30	1.85	1.95
μ_{total}	4.37	2.36	3.02	5.14	3.00

Note: [a]The equilibrium rotational and quartic centrifugal distortion constants, given in MHz and kHz, respectively, correspond to the parent isotopologue, and were determined at the B3LYP/6-311++G** level. Vibrational corrections and sextic centrifugal distortion constants, given in MHz and mHz, respectively, were determined from anharmonic force fields obtained at the B3LYP/6-31G* level. The nuclear quadrupole coupling tensor elements given correspond to the ^{14}N nucleus (in MHz). Dipole moments are given in debye.

smaller basis sets are used, hindering the determination of truly accurate equilibrium structures. By design, this is much less of a problem at the DFT levels. The usual functionals of DFT theory, like B3LYP, suffer, however, from the inability to describe dispersion effects. Therefore, it is comforting to note that the RHF, DFT(B3LYP), and MP2 levels produce the same minima for the five lowest-energy conformers of Thr and aThr. This means that the structures predicted for the lowest-energy conformers are as dependable as can be expected at these levels of electronic structure theory.

It is of fundamental interest to investigate which intramolecular factors (electronic effects including exchange, electrostatic, and hyperconjugative interactions, steric repulsion, H-bonding capabilities, and dispersive interactions) lead to stabilization of the many possible conformers of Thr and aThr. Most of these effects are operative in stabilizing the configurations resulting from rotations around the appropriate single bonds of these molecules.

Threonine is a representative of the three hydroxyamino acids whereby the OH group in the side chain is capable of strong intramolecular H-bonds. The subsequent increase in the number of low-energy conformations has been proved in this study. The presence of NH, carboxylic OH, and side-chain OH H-bond donors and N, C=O, carboxylic OH, and side-chain OH H-bond acceptors allow for the existence of the following types of H-bonds: (a) bifurcated H-bonds between NH_2 and C=O, similar to that found in the most stable conformers of glycine [4,17] and alanine [18,20]; (b) simple H-bonds, like N–H to side-chain OH, carboxylic OH, and C=O; (c) carboxylic OH to C=O, side-chain OH, and NH_2; and (d) side-chain OH to carbonyl oxygen, nitrogen, and carboxylic OH.

The structure of the global minimum, **Thr-I**, is stabilized by strong OH $\cdots$ N, C=O $\cdots$ HO and weaker NH $\cdots$ O H-bonds, whereby the C=O $\cdots$ HO, NH_2, and the side-chain OH functional groups are interconnected via the three intramolecular H-bonds, while the –COOH group is in an energetically unfavorable

Table 7. Equilibrium (A_e, B_e, C_e) rotational constants, their vibrational corrections ($\Delta A, \Delta B, \Delta C$) leading to ground-state rotational constants A_0, B_0, and C_0, A-reduced quartic ($\Delta_J, \Delta_K, \Delta_{JK}, \delta_J, \delta_K$) and sextic ($\Phi_J, \Phi_K, \Phi_{JK}, \Phi_{KJ}, \phi_j, \phi_k, \phi_{jk}$) centrifugal distortion constants, ^{14}N nuclear quadrupole coupling constants ($\chi_{aa}, \chi_{bb}, \chi_{cc}$), and total dipole moments (μ_{total}) of the five lowest-energy conformers of L-allo-threonine[a].

	aThr-I	**aThr-II**	**aThr-III**	**aThr-IV**	**aThr-V**
A_e	3076.01	3019.18	2773.73	2738.22	3059.09
ΔA	−25.24	−29.23	−29.65	−24.31	−28.66
B_e	1639.65	1464.42	1702.83	1627.34	1483.53
ΔB	−15.83	−9.65	−15.32	−17.21	−14.60
C_e	1117.74	1316.22	1341.31	1417.04	1336.00
ΔC	−9.71	−16.16	−13.67	−15.98	−14.96
Δ_J	0.102	0.278	0.250	0.277	0.244
Δ_K	0.248	0.084	0.157	0.909	0.055
Δ_{JK}	0.027	0.170	0.438	0.226	0.190
δ_J	0.032	0.048	0.060	0.016	0.030
δ_K	0.170	2.004	−1.337	−0.661	1.386
Φ_J	0.00	−0.13	0.03	−0.11	0.01
Φ_K	−0.37	28.67	1.20	7.30	18.87
Φ_{JK}	−0.06	11.43	−0.45	4.46	6.76
Φ_{KJ}	0.10	−40.04	−1.50	−16.01	−25.65
ϕ_j	0.00	0.01	0.04	0.03	0.04
ϕ_k	0.00	117.36	−1.27	20.51	89.59
ϕ_{jk}	0.00	−5.55	1.60	−6.23	−1.09
χ_{aa}	−4.64	−5.02	−4.65	−0.34	−0.21
χ_{bb}	2.67	3.02	2.75	0.67	2.86
χ_{cc}	1.97	2.00	1.90	−0.33	−2.65
μ_{total}	5.31	1.85	4.41	2.75	4.57

Note: [a]See footnote ([a]) to Table 6.

trans conformation. In the case of **Thr-II**, although the –COOH group is now in the favourable cis conformation, this substantial energy gain, on the order of 20 kJ mol^{-1}, is not able to compensate for the energy difference between the H-bonds in **Thr-I** and **Thr-II**. **Thr-II** exhibits weaker NH ⋯ O=C and OH ⋯ N H-bonds. The H-bond arrangements in the most stable conformers of Thr are completely different from those for glycine and α-alanine, where the global minima have a cis –COOH arrangement with a bifurcated (and thus less strong) NH ⋯ O=C H-bonds, while the second lowest energy conformers have a trans –COOH arrangement and strong OH ⋯ N H-bonds. This shows, and this conclusion is similar to that obtained in a MW study on the conformers of cysteine [51], that the presence of a polar side chain has a major effect on the conformational preferences of amino acids.

In the case of L-allo-threonine, the global minimum, **aThr-I**, is stabilized, very similarly to **Thr-I**, by strong OH ⋯ O=C and OH ⋯ N H-bonds, while the –COOH group is again in the less favourable trans conformation. As to **aThr-II**, the –COOH group is in a cis conformation, while OH ⋯ N and NH ⋯ O=C H-bonds can be found.

A number of different secondary structural effects characterize the other conformers. Among them one can find the OH ⋯ NH ⋯ O=C structural motif in **Thr-III** whereby the same NH group is included in a H-bond chain. In **Thr-IV**, the stability is due to the OH ⋯ O=C and OH ⋯ N bonds. While **aThr-III** has the same H-bonds as **Thr-I**, the change in chirality causes the methyl group to be in a highly unfavourable position. Furthermore, **aThr-IV** is stabilized by a sequential NH ⋯ OH ⋯ O=C H-bond arrangement involving NH ⋯ OH and OH ⋯ O=C H-bond, including the same side-chain OH group.

In all five low-energy Thr and aThr conformers, the methyl group is found in a staggered position with respect to the β-carbon atom. One can compute straightforwardly the pure (vibrationless) internal rotation barrier of the CH$_3$ group in Thr and aThr. This was done in the present study at the MP2(fc)/cc-pVDZ level. The rotation barriers determined for the methyl group in Thr and aThr are between 1100 and 1300 cm^{-1}. Furthermore, note that for the three lowest-energy conformers of alanine [20], the methyl rotation barrier is also about 1200 cm^{-1}, computed, as part of this study, at the same level of theory. The characteristic increase of the rotation barrier in these natural amino acids with respect to ethane, where the barrier is 1140 cm^{-1} at this level [16], originates most probably from increased hyperconjugation, due to the presence of CC and CX ($X = O, N$) bonds. Note also that the internal rotation barrier of the methyl group can be substantially smaller than the barriers reported; for example, it is only about 400 cm^{-1} in acetaldehyde [40] and much smaller in toluene. Observation of transitions between the energy levels under the slightly different barriers, compounded by different reduced masses due to the structural differences of the conformers, should help identification of the low-energy conformers of Thr and Ala, as well.

Note, finally, that the only threonine conformer considered by Lakard [52] in a study of the vibrational fundamentals of hydroxyamino acids at the harmonic level, though the figure included in that paper is not particularly instructive, is definitely not the global minimum and it is not even among the five most stable conformers.

5. Relative energies

As observed repeatedly for amino acids, the introductory Hartree–Fock level of electronic structure theory, independently of the basis set used, is unable to yield the correct relative energies of the conformers of Thr and aThr (see Tables 2 and 3). At the extrapolated RHF/CBS level, **Thr-II** and **Thr-III** both have lower energies than **Thr-I**, while **Thr-IV** has a very high relative energy. These RHF/CBS results clearly show the inadequacy of this level of theory in predicting relative conformational energies for this class of compounds due to its inadequacy in describing fine electrostatic and H-bonding effects. The MP2 level of theory stabilizes **Thr-IV** substantially, while it destabilizes all the other conformers considered. For Thr, the explicit MP2/aug-cc-pVQZ and the extrapolated MP2/CBS relative energies deviate at most by 0.04 kJ mol^{-1}, an excellent agreement indeed, contributing greatly to the overall accuracy of the FPA relative conformational energies. For aThr, the extrapolation contributions are larger by almost an order of magnitude but they are still surprisingly small on an absolute scale; consequently, the error due to MP2 extrapolation remains a small factor of the overall estimated precision of the FPA relative energies. Compared with δ[MP2], the δ[CCSD] and δ[CCSD(T)] energy increments are relatively small, they can affect relative energies by at most 2 kJ mol^{-1}. Overall, it seems safe to attribute about 0.7 kJ mol^{-1} accuracy to the CCSD(T)/CBS limit FPA relative energies. Based on the CCSD – RHF and CCSD(T) – CCSD increments, the relative energy corrections due to electron correlation effects beyond CCSD(T) are estimated to be considerably less than 0.5 kJ mol^{-1}, thus these missing corrections should not affect significantly the CCSD(T)/CBS limit FPA relative energies and their uncertainty estimates.

The DFT(B3LYP) level performs reasonably well, the FPA relative energies, including the ZPVE corrections, are quite similar to the B3LYP/6-311++G** relative energies. This shows again the utility of the DFT approach for conformational energy studies.

Interestingly, but again in line with our study on the conformers of Pro [2], the relative energies are barely affected by consideration of the core–core and core–valence correlation and the relativistic effects. Even the largest change is smaller than 0.1 kJ mol^{-1}. This is comforting and makes accurate relative energy predictions for the conformers of the building blocks of biopolymers considerably less expensive.

ZPVE corrections are substantial and can affect relative energies on the order of 2 kJ mol^{-1}. It is also of general interest to compare harmonic and anharmonic ZPVE corrections to the relative energies. The anharmonic contributions to relative energies of the Thr conformers are on the order of 0.5 kJ mol^{-1}. This is significant but not substantial given the other sources of error in the present computations contributing to the final FPA relative energy estimates.

The above statements nearly stand for aThr, as well. The RHF level of theory gives relative energies with considerable error, underestimating the relative energies of **aThr-II** and **aThr-IV**. The MP2 increment compensates most of this error by destabilizing these conformers. Compared to MP2, δ[CCSD] and δ[CCSD(T)] energy corrections are fairly small yet can affect relative energies by over 2 kJ mol^{-1}. Core correlation and relativistic effects are on the order of 0.01 kJ mol^{-1}, while ZPVE corrections can reach nearly 1.5 kJ mol^{-1}.

In the case of aThr, the B3LYP/6-311++G** level gives a different relative energy order than the FPA approach. In the cases of **aThr-I** and **aThr-II**, this is due to the ZPVE corrections, which shows the importance of including ZPVE for conformational studies. It is interesting to find that the FPA relative energies give the same relative energy order as the MP2/aug-cc-pVTZ level for the optimized structures.

Since the FPA energy difference between **aThr-I** and **aThr-II** is only 0.8 kJ mol^{-1} and the assumed uncertainty of the present FPA relative energies is ±1 kJ mol^{-1}, even the present extensive computations are insufficient to determine the lowest-energy conformer of aThr. Similarly, the small FPA energy difference between **aThr-IV** and **aThr-V** does not allow an unambiguous determination of their energy order.

In order to estimate the uncertainty on the FPA energies resulting from the choice of reference structures, MP2/aug-cc-pVTZ//B3LYP/6-311++G** relative energies were compared with MP2/aug-cc-pVTZ// MP2/aug-cc-pVTZ energies. The average effect is 0.1 and 0.3 kJ mol^{-1} for Thr and aThr, respectively, suggesting that the DFT reference structures chosen are appropriate for the purposes of the present study.

6. Vibrational fundamentals

Many vibrational fundamentals are found to be characteristic for some of the conformers of Thr and aThr (*cf.* Tables 4 and 5). One of the most useful fundamentals characterizing the conformers is the intense streching vibrational motion of the carboxyl group, ω_{10}, at about 1800 cm^{-1}. The spread in ω_{10} is substantial, from 1780 (**aThr-IV**) to 1834 cm^{-1} (**aThr-V**). The anharmonic corrections alter this picture only slightly as all the VPT2 corrections are between 28 and 34 cm^{-1}. When the C=O group is not involved in H-bonding, e.g. for **aThr-V**, the fundamental is at a high wavenumber, while a strong H-bond between C=O and the side-chain OH results in a substantially lower wavenumber, as for **aThr-IV**. The exact wavenumber of this fundamental should also depend on the cis *vs* trans arrangement of the carboxylic group, though this effect cannot be seen clearly from the data of Tables 4 and 5.

Other simple nuclear motions, such as OH stretching (ω_1, ω_2) and NH stretching (ω_3, ω_4), should also be discussed. The OH stretching motion characterizes conformers by intensity ratios (I_1/I_2) varying from 0.7 to 15.3, and by frequency differences (ω_1–ω_2) varying between 12 and 263 cm^{-1}, with anharmonic corrections altering only slightly these differences. Correlation between the H-bond structure and the OH band origins cannot be so easily used for identification purposes as ω_{10}. Nearly the same stands for the NH stretching motion. The NH stretches also characterize conformers by their relative intensities (I_3/I_4) and frequency differences (ω_3–ω_4). Interestingly, in most cases the ω_3–ω_4 frequency differences are considerably modified by the anharmonic corrections.

Various other fundamentals, including ω_{14}, ω_{15}, ω_{18}, ω_{21}, ω_{22}, ω_{24}, and ω_{26}, can also be found where the intensities or the wavenumbers are characteristic of the different conformers. Nevertheless, in these cases the use of this information to identify conformers based on the availability of the infrared spectrum, for example in a matrix isolation study, is not straightforward due to the presence of nearby fundamentals of similar energies.

7. Rotational spectra

There are three possible routes to identify conformers via rotational spectroscopy if results from related electronic structure computations are available. The most obvious route is based on the comparison of measured effective rotational constants with equilibrium or vibrationally averaged computed rotational constants. The second route uses the information contained in computed dipole moments and selection rules and thus allows interpretation of measured relative intensities of rotational lines. In the case of amino acids a third route is also open, namely one can use computed ^{14}N nuclear quadruple coupling constants for the identification of a conformer.

At the B3LYP/6-311++G** level, the rotational constants corresponding to the optimized equilibrium structures are accurate enough to be useful to deduce the presence of most of the conformers when evaluating experimental data of microwave and millimetre-wave spectroscopic studies. Furthermore, some of the conformers (e.g. **Thr-I** and **Thr-IV**, as well as **aThr-I**) have substantial dipole moments thus helping the observation of the related rotational transitions. Nevertheless, given the limited accuracy of the rotational constants obtained at the B3LYP and MP2 levels, in certain cases it might be quite difficult to distinguish the conformers of Thr and aThr based on this information alone. This holds, for example, for **Thr-II** and **Thr-V**. Unfortunately, in this case the ^{14}N nuclear quadruple coupling constants are also rather similar, hindering further the unequivoval identification of these conformers. In contrast, while the rotational constants of **Thr-I** and **Thr-III** are rather similar, in this case the ^{14}N nuclear quadruple coupling constants allow for an easy distinction between the measured spectroscopic data of the two conformers.

The relatively accurate rotational and quartic centrifugal distortion (QCD) constants determined should facilitate the observation of the low-energy conformers of L-Thr and L-aThr, especially **Thr-I** and **aThr-I**. The sextic centrifugal distortion constants are so small (they are given in mHz units in Tables 6 and 7) that it is unlikely that they could be determined experimentally with any reasonable certainty. Their smallness also means that the experimental spectrum could straightforwardly be fitted with just the effective rotational and quartic centrifugal distortion constants.

8. Summary

Following an extensive search utilizing $6^5 = 7776$ initial structures for each diastereomer, 68 and 66 conformers (local minima on their related PESs) were found for L-threonine and L-allo-threonine, respectively, at the RHF/3-21G level of electronic structure theory. After geometry optimizations at the B3LYP/6-311++G** level, 56 and 61 conformers remained for L-threonine and L-allo-threonine, respectively, within an energy range of 51 and 52 kJ mol^{-1}, respectively. The PESs of the Thr and aThr molecules clearly depend on the level of theory used for their determination. Lacking high-level definitive computational studies, only conformers obtained at several medium theoretical levels should be considered as ones whose likely existence is confirmed by theory. Fortunately, the results obtained show that the conformers found in the lower-energy regions of the PESs have the same bonding arrangements at the HF, DFT(B3LYP), and MP2 levels of theory, thus these conformers should in fact exist.

Following the FPA approach, relative energy predictions with an accuracy of ± 1 kJ mol^{-1} were determined for the five lowest-energy conformers of both diastereomers of Thr. The computations clearly predict the global minimum for Thr, called **Thr-I**. However, for aThr the relative energies of two conformers, **aThr-I** and **aThr-II** are the same within the uncertainty of the FPA approach. In contrast to the global minimum of Gly and Ala, **Thr-I** contains an energetically unfavorable trans carboxylic group, and it is stabilized by strong OH $\cdots$ N, C=O $\cdots$ HO and weaker NH $\cdots$ O H-bonds, whereby the C=O $\cdots$ HO, NH_2, and the side-chain OH functional groups are interconnected via three intramolecular H-bonds. **aThr-I** is stabilized, very similarly to **Thr-I**, by strong OH $\cdots$ O=C and OH $\cdots$ N H-bonds, while the COOH group, to allow for these bonds, is again in the less favorable trans conformation. **Thr-II** and **aThr-II** also have similar structures, with energetically favorable cis carboxylic groups, but weaker H-bonds, NH $\cdots$ O=C and OH $\cdots$ N for both **Thr-II** and **aThr-II**. The structural differences between the most stable conformers of Gly and Ala and those of Thr and aThr are clearly due to the presence of the polar side chain. The side chain's significant effect on conformer stability was already observed for cysteine [51]. In the notation of Alonso *et al.*, the presence of OH and SH polar side chains favor type-**III** conformers over type-**I** conformers known as global minima for Gly and Ala. As expected, the methyl group is in a staggered conformation for all the low-energy conformers of Thr and aThr, with rotational barriers varying between 1100 and 1300 cm^{-1}.

Spectroscopic parameters (harmonic and anharmonic frequencies, double-harmonic vibrational intensities, rotational constants with corresponding vibrational corrections, A-reduced quartic and sextic centrifugal distortion constants, dipole moments and nuclear quadrupole coupling constants) were calculated for the conformers of Thr and aThr. Several of these parameters show the possibility for the experimental distinction of the conformers in their measured spectra. Most important are the characteristic streching C=O (ω_{10}), NH (ω_3, ω_4) and OH (ω_1, ω_2) vibrational fundamentals if the vibrational spectra of the conformers were available. Assignment of the conformers based on pure rotational spectra can be based on rotational constant (equilibrium, A_e, B_e, and C_e, as well as vibrationally averaged A_0, B_0, and C_0 constants are provided), A-reduced quartic centrifugal distortion constants ($\Delta_J, \Delta_K, \Delta_{JK}, \delta_J$, and δ_K) and nuclear quadrupole coupling constants (χ_{aa}, χ_{bb}, and χ_{cc}) corresponding to the ^{14}N nucleus. It seems feasible that conformers of Thr (and similarly those of Ala) could be identified if transitions between the energy levels corresponding to internal rotation characterized by slightly different methyl rotation barriers became available.

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References

[1] A.G. Császár and A. Perczel, Progr. Biophys. Mol. Biol. **71**, 243 (1999).

[2] E. Czinki and A.G. Császár, Chem. Eur. J. **9**, 1008 (2003).

[3] W.D. Allen, E. Czinki, and A.G. Császár, Chem. Eur. J. **10**, 4512 (2004).

[4] V. Kasalová, W.D. Allen, H.F. Schaefer III, E. Czinki, and A.G. Császár, J. Comput. Chem. **28**, 1373 (2007).

[5] P. Hudáky, I. Jákli, A.G. Császár, and A. Perczel, J. Comput. Chem. **22**, 732 (2001).

[6] I. Jákli, A. Perczel, Ö. Farkas, A.G. Császár, C. Sosa, and I.G. Csizmadia, J. Comput. Chem. **21**, 626 (2000).

[7] F. Pollreisz, Á. Gömöry, G. Schlosser, K. Vékey, I. Solt, and A.G. Császár, Chem. Eur. J. **11**, 5908 (2005).

[8] (a) G. Guillea and D.T. Ramon, Tetrahedron-Asymmetry **17**, 1675 (2006); (b) B. List, Synlett **11**, 1675 (2001).

[9] (a) X. Michalat, F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, G. Sundaresan, S.S. Wu, S.S. Gambhir, and

S. Weiss, Science **307**, 538 (2005); (b) A. Mavrandonakis, S.C. Farantos, and G.E. Froudakis, J. Phys. Chem. B **110**, 6048 (2006).

[10] F. Combes, Q.R. Nguyeu, and G. Wlodarczak, Astron. Astrophys. **308**, 618 (1996).

[11] S. Daydé, D. Champmartin, P. Rubini, and G. Berthon, Inorg. Chim. Acta **339**, 513 (2002).

[12] Y. Inomata, T. Takeuchi, and T. Moriwaki, Spectrochim. Acta **40A**, 179 (1984).

[13] S. Boudesocque, Z. Damaj, L. Dupont, J.-B. Behr, and E. Guillon, J. Inorg. Biochem. **102**, 1514 (2008).

[14] (a) S.S.V. Ramasastry, H. Zhang, F. Tanaka, and C.F. Barbes III, J. Am. Chem. Soc. **129**, 288 (2007); (b) N. Utsumi, M. Imai, F. Tanaka, S.S.V. Ramasastry, and C.F. Barbes III, Organ. Lett. **17**, 3445 (2007).

[15] W.D. Allen, A.L.L. East, and A.G. Császár, in *Structures and Conformations of Non-Rigid Molecules*, edited by J. Laane, M. Dakkori, B. van der Veken, and H. Oberhammer (Dordrecht, Kluwer, 1993), p. 343.

[16] A.G. Császár, W.D. Allen, and H.F. Schaefer III, J. Chem. Phys. **108**, 9751 (1998).

[17] A.G. Császár, J. Am. Chem. Soc. **114**, 9568 (1992).

[18] C.H. Hu, M.Z. Shen, and H.F. Schaefer, J. Am. Chem. Soc. **115**, 2923 (1993).

[19] A.G. Császár, J. Mol. Struct. **346**, 141 (1995).

[20] A.G. Császár, J. Phys. Chem. **100**, 3541 (1996).

[21] M.J. Frisch, J.A. Pople, and J.E. Del Bene, J. Phys. Chem. **89**, 3664 (1985).

[22] J.S. Binkley, J.A. Pople, and W.J. Hehre, J. Am. Chem. Soc. **102**, 939 (1980).

[23] C.C.J. Roothaan, Rev. Mod. Phys. **23**, 69 (1951), Rev. Mod. Phys. **32**, 179 (1960).

[24] R. Ditchfield, W.J. Hehre, and J.A. Pople, J. Chem. Phys. **54**, 724 (1971).

[25] (a) R. Krishnan, J.S. Binkley, R. Seeger, and J.A. Pople, J. Chem. Phys. **72**, 650 (1980); (b) T. Clark, J. Chandrasekhar, G.W. Spitznagel, and P. v. R. Schleyer, J. Comp. Chem. **4**, 294 (1983); (c) M.J. Frisch, J.A. Pople, and J.S. Binkley, J. Chem. Phys. **80**, 3265 (1984).

[26] T.H. Dunning Jr, J. Chem. Phys. **90**, 1007 (1989).

[27] R.A. Kendall, T.H. Dunning Jr, and R.J. Harrison, J. Chem. Phys. **96**, 6796 (1992).

[28] A.G. Császár and W.D. Allen, J. Chem. Phys. **104**, 2746 (1996).

[29] G. Tarczay, A.G. Császár, W. Klopper, and H.M. Quiney, Mol. Phys. **99**, 1769 (2001).

[30] P. Pulay, Mol. Phys. **17**, 197 (1969).

[31] W.J. Hehre, L. Radom, P.v.R. Schleyer, and J.A. Pople, *Ab Initio Molecular Orbital Theory* (Wiley-Interscience, New York, 1986).

[32] (a) C. Møller and M.S. Plesset, Phys. Rev. **46**, 618 (1934); (b) R. Krishnan, M.J. Frisch, and J.A. Pople, J. Chem. Phys. **72**, 4244 (1980).

[33] (a) J. Čížek, J. Chem. Phys. **45**, 4256 (1966); (b) J. Čížek and J. Paldus, Physica Scripta **21**, 251 (1980).

[34] R.J. Bartlett, C.E. Dykstra, and J. Paldus, in *Advanced Theories and Computational Approaches to the Electronic Structure of Molecules*, edited by C.E. Dykstra (Reidel, Dordrecht, 1984), p. 127.

[35] K. Raghavachari, G.W. Trucks, J.A. Pople, and M. Head-Gordon, Chem. Phys. Lett. **157**, 479 (1989).

[36] (a) P.A.M. Dirac, Proc. Cambridge Philos. Soc. **26**, 376 (1930); (b) A.D. Becke, Phys. Rev. B **38**, 3098 (1988); (c) A.D. Becke, J. Chem. Phys. **98**, 5648 (1993); (d) C. Lee, W. Yang, and R.G. Parr, Phys. Rev. B **37**, 785 (1988); (e) S.H. Vosko, L. Wilk, and M. Nusair, Can. J. Phys. **58**, 1200 (1980).

[37] T.J. Lee and P.R. Taylor, Int. J. Quantum Chem. Symp. **23**, 199 (1989).

[38] V. Szalay, A.G. Császár, and M.L. Senent, J. Chem. Phys. **117**, 6489 (2002).

[39] W.D. Allen, A. Bődi, V. Szalay, and A.G. Császár, J. Chem. Phys. **124**, 224310 (2006).

[40] A.G. Császár, V. Szalay, and M.L. Senent, J. Chem. Phys. **120**, 1203 (2004).

[41] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komáromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, and J.A. Pople, *Gaussian 03, Revision C.02* (GaussianInc., Wallingford CT, 2004).

[42] MOLPRO is a package of *ab initio* programs written by H.-J. Werner, P.J. Knowles, R. Lindh, F.R. Manby, M. Schütz, P. Celani, T. Korona, G. Rauhut, R.D. Amos, A. Bernhardsson, A. Berning, D.L. Cooper, M.J.O. Deegan, A.J. Dobbyn, F. Eckert, C. Hampel, G. Hetzer, A.W. Lloyd, S.J. McNicholas, W. Meyer, M.E. Mura, A. Nicklaß, P. Palmieri, R. Pitzer, U. Schumann, H. Stoll, A.J. Stone, R. Tarroni, T. Thorsteinsson.

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Varner, J. Vázquez. The program contains the integral packages *MOLECULE* (J. Almlöf and P. R. Taylor), *PROPS* (P. R. Taylor), and *ABACUS* (T. Helgaker, H.J. Aa. Jensen, P. Jørgensen, and J. Olsen). For the current version, see http://http://www.aces2.de.

[44] (a) W. Klopper and W. Kutzelnigg, J. Mol. Struct. (Theochem) **135**, 339 (1986); (b) A. Karton and J.M.L. Martin, Theor. Chem. Acc. **115**, 330 (2006); (c) G. Tasi and A.G. Császár, Chem. Phys. Lett. **438**, 139 (2007).

[45] W.M. Klopper, K.L. Bak, P. Jørgensen, J. Olsen, and T. Helgaker, J. Phys. B **32**, 103 (1999).

[46] M. Douglas and N.M. Kroll, Ann. Phys. **82**, 89 (1974).

[47] N.C. Handy, Y. Yamaguchi, and H.F. Schaefer, J. Chem. Phys. **84**, 4481 (1986).

[48] W.D. Allen and A.G. Császár, J. Chem. Phys. **98**, 2983 (1993).

[49] (a) D.A. Clabo Jr, W.D. Allen, R.B. Remington, Y. Yamaguchi, and H.F. Schaefer, Chem. Phys. **123**, 187 (1988); (b) W.D. Allen, Y. Yamaguchi, A.G. Császár, D.A. Clabo Jr, R.B. Remington, and H.F. Schaefer, Chem. Phys. **145**, 427 (1990); (c) H.H. Nielsen, Rev. Mod. Phys. **23**, 90 (1951); (d) D. Papousek and M.R. Aliev, *Molecular Vibrational-Rotational Spectra* (Elsevier, Amsterdam, 1982); (e) J.K.G. Watson, in *Vibrational Spectra and Structure*, edited by J.R During (Elsevier, Amsterdam, 1997), Vol. 6, p. 1.

[50] M. Zhang and Z. Lin, J. Mol. Struct. (Theochem) **760**, 159 (2006).

[51] M.E. Sanz, S. Blanco, J.C. López, and J.L. Alonso, Angew. Chem. Int. Ed. **47**, 6216 (2008).

[52] B. Lakard, J. Mol. Struct. (Theochem) **681**, 183 (2004).