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Conformational search of antisense nucleotides. Part 2

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Abstract

In this study is presented the phosphorotioate nucleotide compound (**1**) possessing an unusual moiety with a chiral P and a Se atoms double bonded. It required a force field parameterization for carrying out standard molecular mechanics calculations. A new set of MMFF parameters was developed and applied for the complete conformational search of the diastereoisomers, using both Monte Carlo and molecular dynamics, with the purpose to validate them by the comparison between calculated structural properties and NMR measurements.

Keywords: Antisense nucleotides; Force field parameterization; Molecular modeling

1. Introduction

Compound **1** belongs to a new class of nucleotides that when incorporated into antisense constructs, offer resistance from nuclease degradation. Since oligo-nucleoside alkylphosphonates specifically inhibit the expression of oncogenes and viral genes when targeted against specific mRNA or precursor mRNA [1], our derivative **1,** being structurally related to such molecules, is expected to possess similar biological activity. The main chemical features of compound **1** (Fig. 1) is represented by the presence of P linked to S and Se atoms, constituting a very uncommon moiety in nucleic acid chemistry. The available molecular mechanics force fields do not include suitable parameters for these atom types, such as Se. Thus, as already reported in our previous communication [2], MMFF [3] force field, implemented in MacroModel program [4], has been adapted for the parameterization. Consequently, accurate experimental data, such as X-ray crystallography and NMR measurements together with ab initio calculations, represent the starting point for the improvement of molecular mechanics force fields.

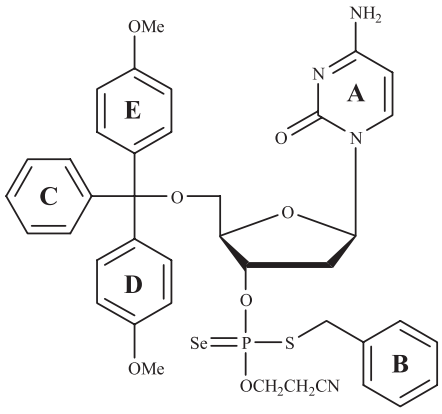

Fig. 1. Compound **1** (**1a** Sp; 1b Rp).

For the present work experimental and ab initio data were collected for the minimal chemical system **2** defining the peculiar group of compound **1** (Fig. 2). A search for Se-based phosphorotio-derivatives into the Cambridge Structural Database gave no answer for the chemical fragment **2** [1].

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[1] The only CSD structure with $SePSO_2$ moiety found is the *bis* (5,5-dimethyl-2-thioxo-1,3,2-dioxa-phosphorinan-2-yl) diselenide, that contains the phosphorus atom included into a 6 member ring and the sulfur atom, instead of the selenium, with sp^2 hybridization.

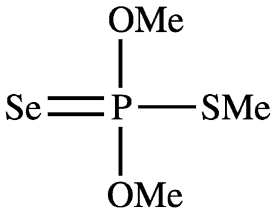

Fig. 2. Minimal chemical fragment compound **2.**

2. Experimental

From our previous work [2], due the different topology in compound **1,** only the Van der Waals radius of Se can be used. Following the approach reported in a recent paper [5] about ab initio evaluation of small O-containing P compounds, quantum mechanic calculations [6] have been carried out working with fragment **2.**

The information obtained in these simulations has been used for two purposes: the definition of the partial charge distribution of the atoms involved in the phosphoro-derivative group, and the determination of the unknown torsion energy profiles of the generic molecular system Se=P–Y–C, where Y can be an O or S atom.

All the ab initio calculations were performed with LACVP** basis set [7], B3LYP method and DFT theory as implemented in Jaguar program [6]. The hardware used for these simulations is Linux Intel biprocessor 1.0 MHz with 1 Gb of RAM. The partial charge distribution after the ab initio geometry optimization is reported in Fig. 3.

The torsional energy profiles have been found using the same ab initio basis set and theory, driving the two dihedral angles defining the unknown barriers in the generic molecular system Se=P–Y–C. The grid step has been fixed to 30° from 0 to 180° for symmetry reasons. The energetic profiles obtained in these calculations are reported in Fig. 4.

The implementation of ab initio data into the MMFF force field followed several steps, the first of them was the introduction of the new atom type as allowed by the MacroModel package. In this case, Se atom was not present in the original database. Some initial properties were specified in order to recognize the new atom type. Van der Waals radius has been derived from the periodic table and used for adding a new Se parameter to the GB/SA implicit model of solvation [8].

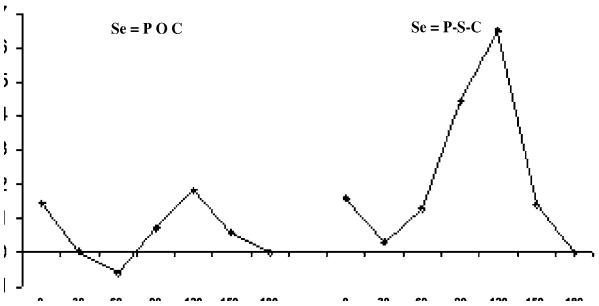

Fig. 4. LACVP** torsional energy profiles in kcal/mol in fragment **2.**

Table 1
Stretching and bending parameters implemented in MMFF

Bond stretching/bending	Equilibrium distance in Å/angle in degrees	Force constant (kcal/mol)
P=Se	2.121	3.744
P–S	2.133	5.243
P–O[a]	1.620	5.243
Se=P–S	117.34	1.219
Se=P–O[a]	114.98	1.219

[a] Average values between two stretching/bending.

The second implementation step consisted in the inclusion of stretching and bending parameters, related to P atom bonds and angles. Basing on the fragment **2** and on ab initio calculations performed by taking into account the literature data [5], the equilibrium bond distances and angles have been fixed as reported in the Table 1.

All other stretching and bending parameters involved in the phosphorotio-derivative substructure have been considered as in the original MMFF force field.

The third step was the implementation of the atom charges obtained from ab initio calculations. In MacroModel that distribution is derived from each bond dipole moment, the MMFF values are in agreement with the obtained ab initio data, so only Se=*P* dipole moment μ was added according to the equation:

$$\mu = L_0 \times Q/0.2082$$

where L_0 is the natural length of the bond (in Å) fixed in the force field and Q is the ab initio separation of charge [9]. Torsional energy parameters were included in the MMFF force field according to the equation implemented in Macro-

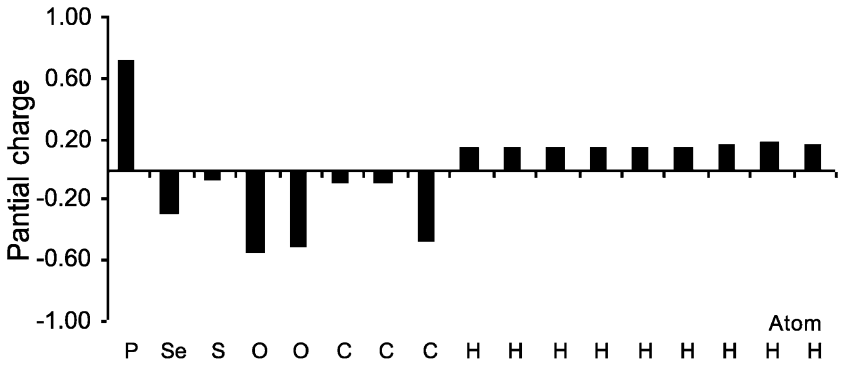

Fig. 3. LACVP** Mulliken charge distribution for chemical fragment compound **2.**

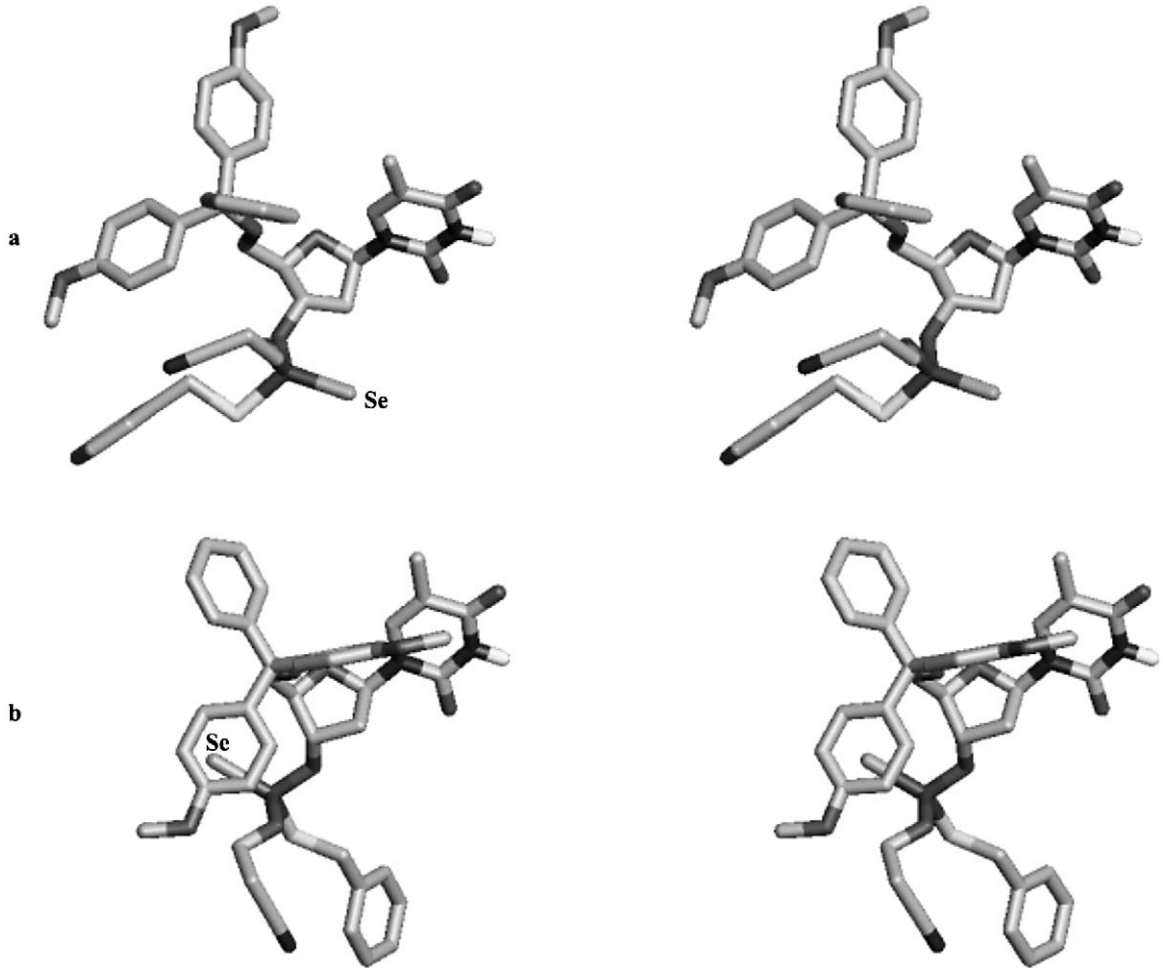

Fig. 5. Stereo view of **1a** (a) and **1b** (b) global minimum energy conformers.

Table 2
New torsional potentials parameterized in MMFF

Torsional definition				V_1	V_2	V_3
Se	P	O	C	–0.7608	–0.0133	2.0050
Se	P	S	C	–2.2600	3.8660	3.5960

Model that is able to reproduce almost any possible profile varying the values of the coefficients V_1, V_2 and V_3 [2]. The energy torsional profile obtained by the ab initio drive, extended to the whole spectrum from 0 to 360°, has been used to derive the three V variables best fitting the barrier. In Table 2 are reported the suitable torsional potential values.

3. Results and discussion

After the implementation of the new parameters, the capability of our modified MMFF to predict the conformational properties of both stereoisomers (**1a** Sp and **1b** Rp) of compound **1** was tested carrying out an extensive conformational search. The modified force field was applied both for Monte Carlo (MC) and molecular dynamics (MD) simulations. In the first case carrying out 10,000 iterations using GB/SA $CHCl_3$ as implicit model of solvation [7]. An energy multi-minimization step of the MC generated structures was performed with 5000 Polack Ribiere Conjugate Gradients in the same solvent conditions (Fig. 5).

Duplicated structures were identified and removed considering conformers within an energy window of 1 kcal/mol and similarity of 0.25 Å. For the MD simulation, after a preliminary 500 ps run, an extended 5000 ps simulation in GB/SA $CHCl_3$ was carried out at 300 K, sampling one structure every 5 ps. The collected 1000 conformations have been submitted to the same multi-minimization process as reported above. After this operation, the two MC and MD ensembles were directly comparable. Unfortunately, no crystallographic data were available for compound **1.** So, in order to evaluate theoretical results, the attention was focused on the C4–C3–O–P torsion. In detail, the torsional value for each conformation obtained by MC and MD was measured and coupled with a Boltzmann population analysis. The goal was to compare the theoretical torsion measurements with the difference (ΔJ) between ^3J(C4, P) and ^3J(C2, P) vicinal

[2] The torsional energy equation is computed using the following harmonic potential:
$E_{tor} = V_1/2(1 + \cos(\phi)) + V_2/2(1 - \cos(2\,\phi)) + V_3/2(1 + \cos(3\,\phi))$.

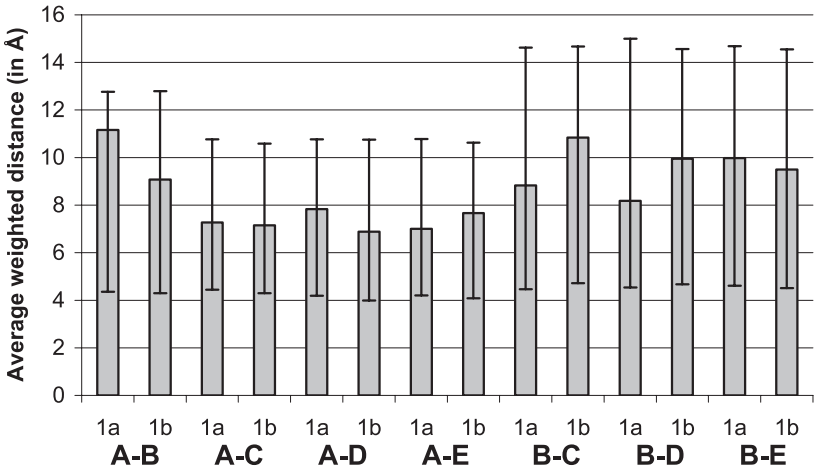

Fig. 6. Ar–Ar rings average distances with maximum and minimum values computed with MC ensembles.

Table 3
C4–C3–O–P torsion domain Boltzmann population analysis computed using MC and MD ensembles

Torsion domain	MD (%)		MC (%)	
	1a	**1b**	**1a**	**1b**
G+	0.00	0.01	0.00	0.00
T	48.02	0.94	88.33	38.82
G–	51.98	99.05	11.67	61.18

couplings constant. According to some authors ΔJ can be used to identify the torsion C4–C3–O–P domain [10]. Experimentally [11] was found that **1a** shows larger ΔJ values (*trans* domain preference) than **1b** (G– domain preference). In Table 3 the results of the simulations are reported.

According to the NMR data, computational results indicated for both stereoisomers showed none G+ conformational domain probability. Moreover, both simulations showed for compound **1b** the C4–C3–O–P torsion preferable in G– domain with minor *trans* component. Regarding the compound **1a,** MC and MD ensembles indicated, in agreement with experimental data, a consistent shift of the G– domain preference toward the *trans* one. In conclusion MC and MD analysis achieved similar results regarding the torsional domain comparable to the experimental data and, remarkably, convergence in the identification of the same global energy minima for **1a** and **1b** compounds (Fig. 6).

In order to investigate the different conformational properties showed by compounds **1a** and **1b** and to compare with the NMR observations [10], the stacking interactions among the aromatic rings were analyzed computing the average weighted distance (AWD) [3] between the centroids of each aromatic moieties (Fig. 6). Every AWD was founded over than 6 Å, so none of such values indicated a strong stacking interaction. To improve the structural analysis, basing on the C4–C3–O–P torsion domain, the conformations coming from MC and MD were clustered separately. On each cluster all possible stacking distances using a 5 Å cutoff were calculated and a Boltzmann population analysis on each conformation satisfying the geometrical threshold was applied. This procedure permitted to amplify the results showed in Fig. 6 indicating in A–B the only possible stacking interaction and highlighting a relationship between the A–B ring distance and the C4–C3–O–P torsion domain both in MD and in MC simulations (Table 4).

According to the NMR data [11], the *trans* domain is more represented in **1a** (Table 3) but the probability of stacking stabilization cannot explain the reason of the **1a** and **1b** diversity.

4. Conclusions

In conclusion, using ab initio and crystallographic data, our MMFF force field [2] was improved including parameters for the generic molecular system Se=P–Y–C, where Y can be an O or S atom. The new force field was validated successfully to study the antisense nucleotides **1a** and **1b** reproducing the available NMR C4–C3–O–P torsion domain data. Other analogs are now under study using both spectroscopic methods and molecular modeling techniques and will be discussed in a future communication.

Table 4
Boltzmann probability of A–B stacking interaction contribute to C4–C3–O–P torsion domain definition after MC and MD simulations

Ensemble	**1a**			**1b**		
	G+	*trans*	G–	G+	*trans*	G–
MD	0.00	0.00	0.00	0.00	0.94	0.00
MC	0.00	1.97	0.13	0.00	7.35	0.00

[3] Following equation describes the calculation of the average weighted distance (AWD):

$$AWD = \sum_{1}^{n} p_{(i)} d_{(i)}$$

where n is the total number of conformers, p the Boltzmann probability at 300 K, d the distance between the centroids and i the current conformer.

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