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Conformational Search of Antisense Nucleotides

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Abstract—A preliminary MMFF implementation of selenium atom parameters necessary to model the nucleoside **1** is reported. X-ray structures of two compounds **1** and **2** have been used as references. Ab initio methods have been adopted for checking torsional energy profile and charge distribution. Monte Carlo calculations and energy minimization in solvation complete the conformational search. © 2001 Elsevier Science Ltd. All rights reserved.

Recently, the two diastereomerically pure compounds **1** and **2** were synthesized and structures determined by X-ray crystallography.[1] Respectively, they are a selenoester and a thioloester both in the Sp configuration. They belong to a new class of nucleotides that when incorporated into antisense constructs, offer resistance from nuclease degradation. Since oligo(nucleoside methanephosphonate)s specifically inhibit the expression of oncogenes and viral genes when targeted against specific mRNA or precursor mRNA,[2] our derivatives **1** and **2**, being structurally related to such compounds, are expected to possess similar biological activity. In order to study such potential activity and their conformational properties in solution, we have taken into account the molecular mechanics (MM) techniques.

For our MM calculations, we have adopted the MacroModel package[3] because the large availability of well referenced force fields that are supported.[4]

The first step of our work has been concentrated on the evaluation of each force field as implemented in version 6.0 of the MacroModel package. With respect to compound **1**, none of the available force fields is able to compute the energy, since the selenium atom is not implemented. Some difficulties were also encountered for compound **2**. Actually this molecule contains the unusual methanphosphothioate moiety whose specific MM parameters are implemented only in the original MMFF force field. In order to verify the appropriate quality of this force field for the conformational description of compound **2**, a deeper analysis using its crystallographic conformations[1] has been carried out together with a subsequent comparison of the crucial torsional barrier S–P–O–C3′ with respect to that obtained by an ab initio profile. The two solid-state conformations of **2a** and **2b**, shown in Figure 1, reveal significant differences only in the phosphoderivative side chain.

In order to systematically analyze the effect of the force field on the energy minimization, we have taken into account three dihedral angles named, and respectively

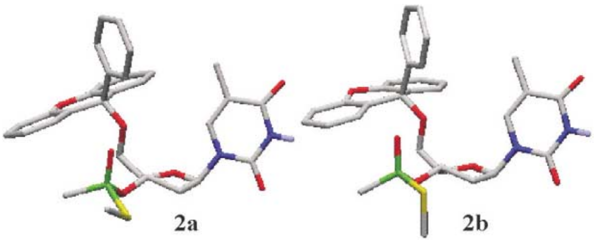

Figure 1. **2a** and **2b** X-ray conformations of compound **2**.[1]

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defined in agreement with the standard IUPAC nomenclature for nucleotides.[5] Also the sugar pucker ring conformation has been considered measuring the angle, defined as C5′–C4′–C3′–O3′.

In order to standardize the comparison of the energy minimization effects with respect to the crystallographic conformations of **1** and **2**, we have considered a common substructure including the sugar and the phosphoderivative moieties. The deviation in the atomic coordinates, expressed as root mean square of the substructure (RMSs), has been evaluated after MMFF minimization in vacuo and in GB/SA chloroform[6] and reported with the four defined torsional angles in Table 1.

The experimental torsional data shown revealed that the main difference between the **2a** and **2b** conformations is due to the dihedral angle. The conformational domains for the other three considered torsions are consistent. Actually, falls in the field of *gauche*− domain and in the *trans* one. More interestingly is that in both solid states a C3′-*endo* (N-type) sugar conformation is revealed. The MMFF minimization exhibits only a few differences in the selected torsional angles with respect to the X-ray structures. The larger RMS deviations in the **2b** conformation seem to be related to the ζ dihedral angle, which assumes in vacuo as well as in GB/SA chloroform a value lower than 20° with respect to the crystal structure. Interestingly, after MMFF minimization, the ζ torsional angles in both conformations converge to an average value of about 80°. Basically, this means that the real difference between the two conformations is due to the α dihedral angle. In order to compare the torsional barrier of the S–P bond, using a CPU intensive ab initio method, we have performed two drive computations, on the *S*-methyl (Sp) methanephosphothioate **5** substructure shown in Figure 2, instead of the entire molecule **2**.

The first energy profile estimation has been carried out following a recently published approach adopted for simple phosphoderivatives.[7] Thus, the first drive calculation has been carried out at restricted Hartree–Fock (RHF) level of theory with the standard STO-3G* basis set as implemented in the Spartan package,[8] while the second one has been carried out using the original MMFF force field. Both estimated profiles are reported in Figure 3.

There is a good level of correlation between the two estimated energy profiles. In both cases the *gauche*− domain is the most favored one, followed by the *gauche*+ rotamer, which is the second favored local minimum, and finally by the *trans* rotamer. The correlation resulted in acceptable relative energies of both barriers.

It is interesting to highlight that in the **2a** and **2b** crystal conformations the α dihedral angles are located respectively in the *gauche*− and *trans* domains, corresponding to the two energy minima of both MMFF and ab initio drive profiles observed.

Another important consideration arises from the analysis of the MM torsional constants used in the original MMFF force field. The MM energy profile for α is driven by a very simple set of constants ($V1=0$, $V2=0$ and $V3=0.358$) repeated three times for the C–S–P–X, where X is oxygen, carbon or oxygen double bonded to phosphorus. This set of variables introduces a simple harmonic trend that generates three isoenergetic global minima centered respectively in *gauche*+, *trans* and *gauche*−. Combining the torsional energy with other components, such as van der Waals and electrostatic terms, MMFF is able to reproduce the ab initio profile, indicating that the difference between the three domains concerning the α torsion in the original force field is basically under total control of non-bonded forces.

The second step of our work has been dedicated to the parameterization of the selenium atom into the MMFF force field in order to perform energy evaluations of compound **1**. Our work started with the introduction of the new atom type and all the specific parameters for it. According to a previous parameterization of Se in the MM2 force field, the van der Waals radius has been set

Table 1. Torsional angles in degrees and the substructure RMS deviations in Å the atomic coordinates of **2** in solid state and after MMFF energy minimization in vacuo and in GB/SA $CHCl_3$

	α	ζ	ε	δ	RMSs
X-ray conf. **2a**	−53.31	−84.02	−152.83	80.83	
MMFF (vacuo)	−49.81	−83.25	−146.82	80.04	0.133
MMFF ($CHCl_3$)	−54.28	−80.37	−146.23	79.79	0.153
X-ray conf. **2b**	−174.23	−102.70	−146.71	80.88	
MMFF (vacuo)	167.14	−81.24	−149.49	79.77	0.310
MMFF ($CHCl_3$)	173.45	−76.54	−145.40	79.72	0.337

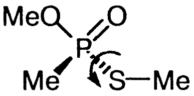

Figure 2. The structure of compound **5** and the torsional angle corresponding to α.

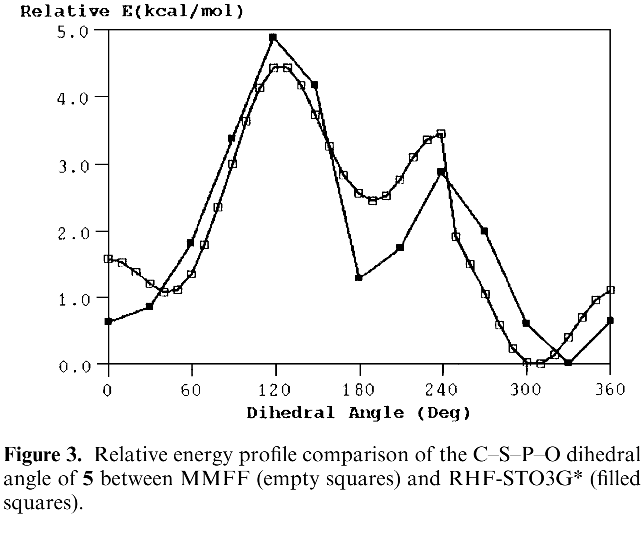

Figure 3. Relative energy profile comparison of the C–S–P–O dihedral angle of **5** between MMFF (empty squares) and RHF-STO3G* (filled squares).

to 2.25 Å.[9] This value has also been used in the GB/SA chloroform solvation treatment.

In order to realistically compute the electrostatic term, the charge distribution has been theoretically evaluated working on the two representative molecular fragments **3** and **4**. A comparison between the original MMFF force field and the RHF 3-21G* CHELPG charge distributions computed for compound **4** has revealed a good correlation. Then we compared the ab initio charge set distribution between **3** and **4**, obtaining meaningful differences only on the selenium and phosphorus formal charges (see Table 5). In MMFF, the new set of charges for compound **4** have been implemented adding an appropriate Se–P bond dipole moment.[10]

The missing selenium stretching and bending parameters obtained directly from the crystallographic structure of **1**[1] are reported in Table 2. The force constants applied to the equilibrium bond distances and angles have been extrapolated from compound **2** MMFF parameters.

The MMFF missing torsional parameters are related to two bonds: Se–P and C–Se. They have been introduced using the same kind of implementation found for the S–P bond energy profile, that is, applying a simple harmonic profile able to create three wells corresponding to the main rotamers *gauche*−, *gauche*+ and *trans*, leaving the discrimination under control of the non-bonded terms. The selenium based torsional set of parameters are reported in Table 3.

The new set of parameters allowed us to perform the energy minimization of **1** starting from its crystallographic coordinates. In vacuo and in GB/SA $CHCl_3$, we have obtained a substructure with RMS of about 0.1 Å. The corresponding four torsional values and RMS values for **1** are reported in Table 4.

The superimposition of the X-ray structure of **1** with the geometries obtained by MMFF minimization with and without solvent, shown in Figure 4, revealed very little deviation.

The third step has been dedicated to the complete conformational search of the two compounds **1** and **2** using the new force-field parameters and adopting the Monte Carlo algorithm.[11] All the rotatable bonds of **1** and **2**, including those pertinent to the sugar ring, have been randomized to generate 5000 conformations in vacuo. Then the energy minima conformers found within 12 kcal/mol above the global minimum have been submitted to full energy minimization in GB/SA $CHCl_3$. The convergence in the Monte Carlo search has been judged satisfactory as revealed by the average number of duplicates higher than six for both molecules.

The energy-minimized crystal structures of both compounds **1** and **2** have been localized with respect to all the other conformers obtained in an exhaustive Monte Carlo search. The conformation corresponding to the minimized X-ray structure of **1**, as obtained by the simulation in vacuo, possesses a relative energy of 4.07 kcal/mol above the global minimum, while the same conformer in GB/SA $CHCl_3$ is definitively more populated with a relative energy of 0.91 kcal/mol.

A similar effect can be also observed in the comparison between the two simulations of **2**. The minimized conformations **2a** and **2b** in vacuo respectively possess relative energies of 5.06 and 3.81 kcal/mol, in GB/SA $CHCl_3$ they fall to 3.80 and 2.67 kcal/mol.

Table 2. Stretching and bending selenium-based parameters added to the MMFF force field

Bond stretching/bending	Equilibrium distance in Å/angle (°)	Force constant (kcal/mol)
P–Se	2.224	3.744
C–Se	1.931	3.744
C–Se–P	97.7	1.219
Se–P=O	111.7	1.219
Se–P–C	107.3	1.219
Se–P–O	107.1	1.219

Table 3. Torsional selenium-based parameters added to the MMFF force field

Torsional definition	V1 and V2	V3
C–Se–P–O	0.000	0.358
C–Se–P=O	0.000	0.358
C–Se–P–C	0.000	0.358
Se–P–O–C	0.000	1.500

Table 4. Torsional angles in degrees and the substructure RMS deviation in Å of **1** in solid state and after MMFF energy minimization in vacuo and in GB/SA chloroform

	α	ζ	ε	δ	RMSs
Conf. (X-ray)	102.65	−77.76	−88.54	143.58	
MMFF (vacuo)	104.90	−82.89	−90.74	144.90	0.111
MMFF ($CHCl_3$)	108.63	−80.50	−91.75	145.93	0.104

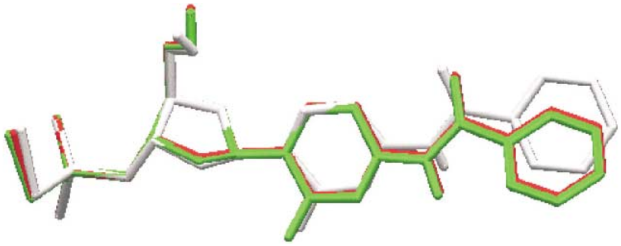

Figure 4. Polytube superimposition of crystallographic structure of **1** (white) with the MMFF energy minimized conformations in vacuo (red) and in GB/SA $CHCl_3$ (green).

Table 5. Comparison between the atomic charge distribution of compound **3** and **4** calculated with the RHF/3-21 G* ab initio method and with MMFF original (**4**) and after dipole moment implementation (**3**)[a]

Numbering scheme in models **3** and **4**	Atom description	Original MMFF compound **4**	MMFF compound **3** (μ Se–P = 1.15 D)	RHF/3-21 G* compound **4**	RHF/3-21 G* compound **3**
1	P	1.219	1.112	1.275	1.150
2	X (Se or S)	−0.478	−0.370	−0.457	−0.360
3	Osp2(P)	−0.700	−0.700	−0.767	−0.754
4	CP	0.000	0.000	−0.444	−0.298
20	H (CP)	0.000	0.000	0.089	0.044
27	H (CP)	0.000	0.000	0.16	0.12
28	H (CP)	0.000	0.000	0.16	0.13
5	C2′	0.280	0.280	0.528	0.543
13	H (C2′)	0.000	0.000	−0.035	−0.038
14	H C2′)	0.000	0.000	−0.015	−0.018
6	C3′	0.000	0.000	−0.529	−0.547
23	H (C3′)	0.000	0.000	0.154	0.159
26	H (C3′)	0.000	0.000	0.13	0.13
7	C4′	0.280	0.280	0.454	0.425
8	Osp3 (P)	−0.551	−0.551	−0.653	−0.637
9	C5′	0.280	0.280	0.501	0.546
16	H (C5′)	0.000	0.000	−0.047	−0.058
10	O (sugar)	−0.560	−0.560	−0.680	−0.693
11	C6′	0.280	0.280	0.325	0.319
18	H (C6′)	0.000	0.000	0.010	0.007
22	H (C6′)	0.000	0.000	−0.065	−0.062
12	O6′	−0.680	−0.680	−0.800	−0.801
21	C(X)	0.230	0.230	0.158	0.132
15	H (CX)	0.000	0.000	0.061	0.072
17	H (CX)	0.000	0.000	0.010	0.009
19	H (CX)	0.000	0.000	0.011	0.006
24	H (O6′)	0.400	0.400	0.492	0.492
25	H (C4′)	0.000	0.000	−0.02	−0.02

[a] The charge distribution correlation between the original MMFF and the 3–21 G* CHELPG set of charges for the compound **4** (X = S) is very good ($y = 1.1372x$, $r^2 = 0.8725$). The introduction of the dipole moment of 1.15 D allows a better correlation between the parameterised MMFF and the 3–21 G* set of charged for the compound **3** ($y = 1.1427x$, $r^2 = 0.8777$). The correlation is much better if we take into account that the MMFF put 0 in any aliphatic hydrogen. Especially in the methyl moieties the charge differences between ab initio and molecular mechanics charge estimations are lower than those included in the correlation equations and r^2.

For both molecules the solvating effect is ultimately important in the description of a chemical environment closer to the experimental conditions. The large difference in the relative energies of the X-ray minimized conformers of **1** and **2** can be explained in terms of the sugar puckering. Data in Tables 1 and 2 reveal two different conformations of the five-membered ring conserved after energy minimization both in vacuo and in GB/SA $CHCl_3$. In the lowest energy conformers obtained by the Monte Carlo search and after minimization in the solvent the average δ dihedral angle is about −35°, indicating the predominant C2′-*endo* (S-type) sugar conformation.

In conclusion, after a systematic analysis of six well known MM force fields, we have validated MMFF for the modeling of the antisense *S*-methyl derivative **2** using as experimental source the two X-ray conformations and, as theoretical reference, the barrier profile C–S–P–O and the charge distribution obtained with ab initio methods. The introduction of specific selenium parameters into the same force fields has allowed us to perform, for the first time, a conformational search of the new interesting compound **1**, which as an isostere of **2** can represent a lead molecule of a new class of potentially active antisense nucleotides. Other similar selenium-based derivatives are now under study using spectroscopic methods and molecular modeling and will be discussed in a future communication.

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References and Notes

1. Woźniak, L. A.; Wieczorek, M.; Pyzowski, J.; Majzner, W.; Stec, W. J. *J. Org. Chem.* **1998**, *63*, 5395.
2. Woźniak, L. A. *Rev. Heteroatom. Chem.* **1999**, *19*, 173.
3. (a) MM2: Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. (b) MM3: Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551. (c) AMBER: Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Chio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765. (d) Weiner, S. J.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230. (e) OPLS: Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657. (f) AMBER*: McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743. (g) MMFF: Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
4. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
5. Sundaralingam, M.; Pullman, B.; Saenger, W.; Sasisekharan, V.; Wilson, H. R. In *Conformations of Biological Molecules*

and Polymers; Bergman, E. D., Pullman, B., Eds.; Academic: New York, 1973; pp 815–820.

6. The GB/SA chloroform solvation treatment has been included to take better into account the solution environment of the crystallization process. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

7. Stewart, E. L.; Nevins, N.; Allinger, N. L.; Bowen, J. P. *J. Org. Chem.* **1997**, *62*, 5198.

8. Spartan, ver. 5.0. Wavefunction: Irvine, CA, 1997.

9. Allinger, N. L.; Allinger, J. A.; Yan, L. Q. *J. Mol. Struct. (THEOCHEM)* **1989**, *201*, 363.

10. The dipole moment has been determined iteratively and set equal to 1.15 D when able to reproduce the ab initio separation of charge between P and Se atoms.

11. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.