Solvent effect on the tautomerism of 4-aminopyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides

Nuria Campillo, Ibon Alkorta,*,† Juan Antonio Páez and Pilar Goya

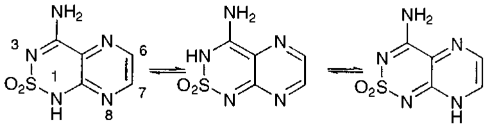
Instituto de Química Médica (C.S.I.C.), Juan de la Cierva, 3, 28006-Madrid, Spain

A detailed study of the tautomerism of 4-amino-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide has been carried out using different theoretical methods. Semiempirical, molecular orbital and density functional theory based *ab initio* methods have been used to study all the possible tautomers of this compound in the gas phase. Several continuum solvation models have been used to estimate the relative stability of the tautomers in water. Complexes with one and two molecules of water have been considered to take into account the specific solute–solvent interactions. Finally, these results have been compared with UV spectrophotometry data, giving good agreement between theoretical and experimental results in aqueous solution.

Introduction

The 4-aminopyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide system which was first synthesized by our group[1] has shown interesting biological activities as diuretic,[2] anthelmintic[3] and bronchodilator[4] agents.

This structure presents an interesting case of tautomerism with three possible tautomers as depicted in Scheme 1.



1-NH 3-NH 8-NH

Scheme 1

Two previous experimental studies[1,5] have dealt with the tautomeric equilibria of these compounds in the solid state and different solution media. The main conclusions obtained so far are that the 1-NH tautomer is the only one present in the solid state and in several organic solvents such as chloroform, dimethyl sulfoxide and methanol. However, in aqueous solution, a mixture of the 1-NH and 8-NH tautomers is present, the relative proportion of them depending on the substituents in positions 6 and 7 of the heterocyclic ring.

As far as theoretical approaches are concerned, the presence of the SO_2 chemical group in these compounds has seriously limited the application of quantum mechanical methods for their study. Two publications concerning theoretical calculations on related substances[6,7] have led to the following conclusions: first, the need to include polarization functions for an adequate description of the SO_2 group and second, that a good description of their electronic properties can be obtained with density functional theory (DFT) based methods.

In the present work, we wish to report a systematic study of the tautomerism of the parent compound 4-aminopyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, **1** (depicted in Scheme 1), both from a theoretical and an experimental point of view. Thus, the relative stability of the possible tautomers of this compound in the gas phase has been studied by semiempirical, molecular orbital and density functional theory based *ab initio* calculations. To reproduce the experimental tautomeric equilibrium in solution, the ability of several solvent continuum models, self-consistent reaction field (SCRF) and Poisson–Boltzmann methods has been tested. Complexes with one and two molecules of water have also been calculated to consider explicit solute–solvent interactions. Finally, experimental information about the tautomeric equilibrium in aqueous solution has been obtained from UV spectrophotometry and compared with the theoretical results.

Results and discussion

Theoretical calculations

The study of the tautomeric stability in the gas phase has been carried out using two semiempirical methods, AM1[8] and PM3,[9] one molecular orbital *ab initio* method, RHF/6-31G*[10] and one density functional theory based *ab initio* method, BLYP/6-31G*.[11] Even though the semiempirical methods did not provide good results for related molecules in previous studies, they were selected due to their speed compared to *ab initio* methods and to obtain initial geometries for the latter calculations.[7] The molecular *ab initio* calculations at the RHF/6-31G* level have been shown to provide excellent geometrical descriptions of analogous systems and similar results have been obtained in the electronic characteristics of these molecules from DFT calculations.[7]

The results of the gas phase calculations of the three possible tautomers of **1**, using semiempirical (AM1 and PM3), density functional theory (BLYP/6-31G*) and molecular orbital *ab initio* methods (RHF/6-31G*) are gathered in Table 1. In all the methods used, the most stable tautomer is the 1-NH, with a very large energy difference from the second most stable tautomer, the 8-NH. The relative energy differences lie between 40 and 65 kJ mol^{-1}, except in the case of the AM1 semiempirical method which clearly behaves differently from the rest of the methods used here. Finally, the least stable tautomer is the 3-NH. These results indicate that only tautomer 1-NH should be present in the gas phase and in apolar media due to the large energy difference from the rest of the tautomers. As mentioned before, this result is in agreement with the experimental results in the solid state and apolar solvents obtained for related substituted 4-aminopyrazino[2,3-*c*][1,2,6]thiadiazines.[1,5]

The dipole moments of the tautomers (Table 2) indicate that the present structures are highly polar, the most polar of them being the 8-NH tautomer followed by the 3-NH and finally the 1-NH with all the methods used.

The SCRF methods,[12] where a molecule is located in a cavity

---

† Fax: 9-1-564 48 53. E-Mail: ibon@pinar1.csic.es

Table 1 Energetic results in vacuum and with the different solvation models of the three tautomers of **1**

		1-NH		3-NH		8-NH	
	Method	Computed energy[a]	E_{rel}/kJ mol^{-1}	Computed energy[a]	E_{rel}/kJ mol^{-1}	Computed energy[a]	E_{rel}/kJ mol^{-1}
Vacuum	RHF/6-31G*	−1011.654 678	0.00	−1011.625 397	76.86	−1011.629 981	64.85
	BLYP/6-31G*	−1015.703 660	0.00	−1015.683 490	52.97	−1015.688 260	40.42
	AM1	6.88	0.00	19.99	54.89	29.03	92.68
	PM3	−22.94	0.00	−8.32	61.17	−12.15	45.15
Solvation models	SCRFRHF/6-31G*	−1011.667 487	0.00	−1011.644 048	61.50	−1011.656 229	29.54
	SCRFBLYP/6-31G*	−1015.711 554	0.00	−1015.696 500	39.54	−1015.708 186	8.83
	SM2/AM1	−22.76	0.00	−12.24	44.02	−6.48	68.12
	SM3/PM3	−55.72	0.00	−43.73	50.12	−47.60	33.93
	PB RHF/6-31G*[b]		0.00		74.39		41.17
	PB BLYP/6-31G*[b]		0.00		45.10		18.91
·H_2O	BLYP/6-31G*	−1092.111 793	0.00			−1092.103 742	21.13
	SCRFBLYP/6-31G*	−1092.121 114	0.00			−1092.117 291	10.04
·$2H_2O$	BLYP/6-31G*	−1168.523 339	0.00			−1168.519 125	11.05
	SCRFBLYP/6-31G*	−1168.531 381	0.00			−1168.529 644	4.56

[a] *Ab initio* total energies in hartrees and semiempirical $\Delta H^{\circ}_{\mathrm{F}}$ in kcal mol^{-1}. [b] Relative energy (kJ mol^{-1}) with respect to the 1-NH tautomer taking into account the solvation energy calculated with the Poisson–Boltzmann method with each set of charges plus the *ab initio* energy *in vacuo*.

Table 2 Calculated dipole moments (Debye) of the three tautomers of **1** *in vacuo*

	RHF/6-31G*	BLYP/6-31G*	AM1	PM3
1-NH	8.48	6.46	7.55	6.28
3-NH	10.49	8.51	9.00	7.87
8-NH	11.86	10.10	10.30	9.11

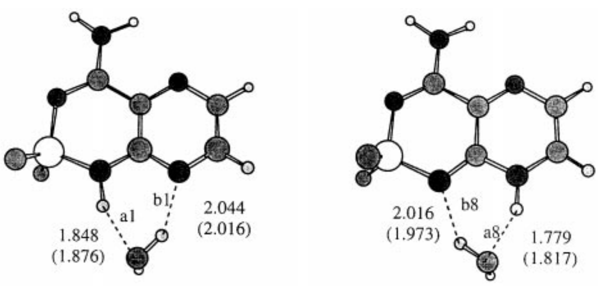


Fig. 1 Optimized geometries at the BLYP/6-31G* level of the complexes with one water molecule. In parentheses, the hydrogen bond distances of the optimized geometries with the SCRF model are indicated.

surrounded by a dielectric continuum that is polarized by the molecular electronic distribution, provide a simple solution to the complex process of solvation. Several cavity shapes have been used depending on the methods. Regarding the methods used here, the Onsager model[13] uses a spherical cavity while the SM2/AM1[14] and SM3/PM3[15] models represent the molecule as the union of the van der Waals atomic spheres. This method does not consider specific solute–solvent interactions; for this reason, in some cases, the inclusion of some solvent molecules can provide additional information.

Another method that has been successfully used to study the solvation energy corresponds to the resolution of the Poisson–Boltzmann equation by a finite difference method.[16] In this model, the electrostatic part of the solvation energy is calculated from the atomic charge distribution of the molecule in a very fast fashion making it adequate for the evaluation of this property in polar molecules and large macromolecules.[17–19]

The theoretical results in solution by inclusion of the continuum solvent models produce a relative stabilization of the 8-NH and 3-NH tautomers, as can be seen from the data obtained with the SCRF and Poisson–Boltzmann methods, gathered in Table 1. However, the most stable structure still corresponds to the 1-NH tautomer. The relative stabilization effect is larger in the 8-NH tautomer, between 35 and 31 kJ mol^{-1} in the *ab initio* methods and only 11 kJ mol^{-1} in the SM3/PM3 model, providing a relative energy difference of only 9 kJ mol^{-1} at the BLYP/6-31G* level. The effect in the case of the 3-NH tautomer is less important, around 14 kJ mol^{-1} for all the SCRF methods used here, and thus, the energy difference is large enough (over 39 kJ mol^{-1}) not to expect the detection of this tautomer in aqueous solution. The Poisson–Boltzmann model provides a stabilization of approximately 22 and 7 kJ mol^{-1} for the 8-NH and 3-NH tautomers, respectively. These results indicate similar behavior of the Poisson–Boltzmann model and the SCRF *ab initio* methods in contrast to the poor performance of the SCRF semiempirical methods in this kind of molecule.

The effect of solute–water interactions has been studied in the two more stable tautomers, 1-NH and 8-NH. The water molecule has been located interacting in the vicinity of the nitrogens involved in the tautomeric process of these two species (Fig. 1). This is the part of the molecule with the larger structural and electronic differences between these tautomers. Thus, the interactions with the solvent could be clearly different for both structures.

The calculated energy difference of the supramolecular systems *in vacuo* (Table 1) indicates that the 8-NH tautomer forms stronger hydrogen bonds (HB) with water than the 1-NH as the relative energy becomes only 21 kJ mol^{-1} as compared to 40 kJ mol^{-1} obtained for the two isolated tautomers (Table 1).

The calculation of the water–pyrazinothiadiazine system with the SCRF model, surprisingly, provides energy differences similar to those in the isolated systems in the same conditions. These results indicate that the simple SCRF model is able to account, in this case, for the highly different hydrogen bond interactions between **1** and water in the N1–N8 region. Geometrically, the shortest hydrogen bonds become longer and the opposite is observed for the longest ones to a very similar extent (Fig. 1).

A second water molecule has been located in the same region to allow a more flexible hydrogen bonding net between the solvent and the solute. The results (Table 1, Fig. 2) show, again, the preference of the 8-NH tautomer to form hydrogen bonded complexes. In this case, the relative energies of this tautomer are *in vacuo* 11 kJ mol^{-1} and in the SCRF model 5 kJ mol^{-1}. The obtained hydrogen bonds are in this case almost linear in all the cases as depicted in Fig. 2.

The Atoms in Molecules (AIM) methodology,[20] based on the

Table 3 Atomic charges (e) and electron density ρ (e au^{-3}) at the hydrogen bond critical point calculated with the AIM methodology

	Atomic charges		ρ at the HB critical points[a]				
Tautomer	1-H/8-H	N-8/N-1	a1/a8	b1/b8	c1/c8	d1/d8	e1/e8
1-NH	0.43	−1.05	0.037	0.026	0.051	0.046	0.036
8-NH	0.50	−1.50	0.043	0.027	0.055	0.051	0.034

[a] The hydrogen bonds are labelled in Figs. 1 and 2.

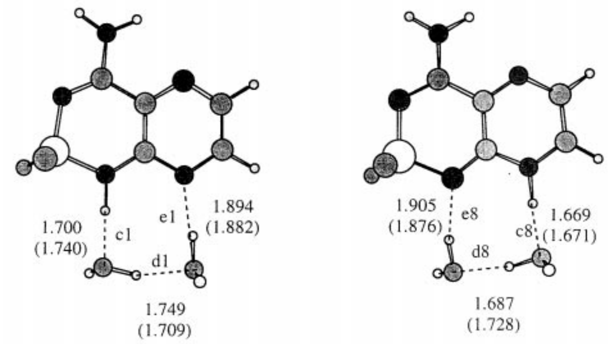


Fig. 2 Optimized geometries at the BLYP/6-31G* level of the complexes with two water molecules. In parentheses, the hydrogen bond distances of the optimized geometries with the SCRF model are indicated.

analysis of the electron density of the system to be studied, has been shown to be a powerful tool to characterize atomic properties and molecular interactions, as hydrogen bonds (HB).[21,22] In this case, the AIM methodology has been used to obtain the atomic charges of the two atoms of the pyrazinothiadiazine involved in the HB (N-8 and 1-H for the 1-NH tautomer and N-1 and 8-H for the corresponding 8-NH). In addition, the electron density (ρ) at the HB critical points and their corresponding Laplacians have been evaluated to characterize these interactions (Table 3).

Analysis of the calculated atomic charges indicates that the 8-NH tautomer has more positive charge on the hydrogen, indicating that it should be a better hydrogen bond acceptor. In addition, the more negative charge of the nitrogen involved in the HB in the 8-NH tautomer reinforces its ability to form HB when compared to the 1-NH species.

Regarding the electron density at the HB critical points, they show typical values for this kind of interaction. The positive value of the Laplacian indicates that they correspond to closed-shell interactions of the HB type. The larger values of ρ in the complexes with the 8-NH tautomer indicate, again, that they are stronger than in the complexes with the 1-NH tautomer.[23,24] A comparison of the complexes with one and two water molecules shows that the ones with two water molecules have larger values of ρ and shorter HB distances which indicates stronger interactions.

Experimental studies

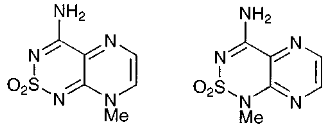
The low solubility of these compounds in aqueous solution has limited the application of spectroscopic techniques other than UV, which only requires a very low concentration, in the study of tautomeric equilibria. In addition to 4-amino-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, **1** (Scheme 1), the corresponding blocked tautomers are necessary to obtain a qualitative estimation of the tautomeric equilibrium (Scheme 2). The 8-methyl derivative, **2**, has already been described in the literature[1] and the 1-methyl derivative, **3**, was synthesized specially for this study from **1** and methyl iodide by phase transfer catalysis as indicated in the Experimental section.

The presence of tautomers 1-NH or 8-NH in the neutral species can be deduced from the characteristic 350 and 400 nm UV absorption bands, respectively (Table 4). From comparison of the UV spectra of the neutral form of **1** with the N-8 methyl, **2**, and N-1 methyl, **3**, derivatives it can be concluded that compound **1** exists in aqueous solution as a mixture of the two tautomeric forms 1-NH and 8-NH.

Table 4 UV spectral data in water of compounds **1**, **2** and **3**

Compound	λ_{max}/nm			log ε		
1[a]	246	336	401	3.95	3.66	3.47
2[a]	258	313	401	3.98	3.18	3.76
3	248	346		3.98	3.58	

[a] Taken from ref. 1.



2 **3**

Scheme 2

An approximate method to evaluate the relative concentration of the tautomers considers that the extinction coefficients, ε, of the blocked and free tautomers are the same. Comparison of the data available for related compounds such as the isopterines[25] indicates that the difference between the ε values in these cases is a maximum of 7%. Applying this approach to the 4-aminopyrazinothiadiazines and using the 401 nm band characteristic of the 8-NH tautomer, a proportion of 51 ± 8% of this tautomer is calculated in aqueous solution. This result indicates that the 1-NH and 8-NH tautomers are almost equally stable in aqueous solution.

The experimental results are in acceptable agreement with the most complex model calculated here, SCRF of the pyrazinothiadiazines hydrogen bonded with two molecules of water, that indicates an energy difference between the 1-NH and the 8-NH tautomers of only 4.56 kJ mol^{-1}. Additional energetic terms such as the zero point energy or the solvation entropy and structural factors such as the inclusion of more explicit water molecules, not taken into account due to computational limitations, could further reduce this difference.

Conclusions

The following conclusions can be drawn from this study of the tautomerism of 4-amino-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide:

For the gas phase, the semiempirical, density functional theory and *ab initio* methods indicate that the most stable tautomer is the 1-NH.

The continuum solvation models used (SM2/AM1, SM3/PM3, Onsager/BLYP/6-31G*, Onsager/RHF/6-31G* and Poisson–Boltzmann) indicate the stabilization of the 8-NH tautomer in aqueous media. The results obtained by inclusion of one or two water molecules are similar to those of methods indicated before.

These results are in agreement with the experimental UV data which also show an increase in the ratio of the 8-NH tautomer *versus* the 1-NH tautomer in aqueous solution and in the absence of the 3-NH tautomer.

Methods

Experimental

Melting points: Reichert–Jung Thermovar micro melting point apparatus, uncorrected values. NMR: Varian XL-300 and Varian Unity-500 (300 and 125 MHz, for ^{1}H and ^{13}C, respectively). The signals of solvents were used as references. The UV spectra were recorded on a Perkin-Elmer Lambda 5 Spectrophotometer. Elemental analyses were performed on a Heraeus CHN-rapid analyzer.

4-Amino-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, **1**, and 4-amino-8-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, **2**, were synthesized following the procedure described elsewhere.[1]

4-Amino-1-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, 3. A mixture of 4-amino-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (3.0 g, 15.0 mmol), water (70 ml), potassium carbonate (1.04 g, 7.5 mmol), dichloromethane (125 ml), tetrabutylammonium bromide (3.4 g) and methyl iodide (6 mmol) was stirred at room temperature for 24 h. The organic layer was separated, evaporated to dryness and recrystallized from methanol–water to give 1.84 g (57%). Mp 258–260 °C. δ_H(DMSO-d$_6$) 8.94 (br s, 2H, NH$_2$), 8.74 (d, 1H, CH), 8.44 (d, 1H, CH), 3.39 (s, 3H, CH$_3$). δ_C(DMSO-d$_6$) 158.4 (C-4), 148.5 (C-7), 149.2 (C-8a), 136.8 (C-6), 123.8 (C-4a), 28.2 (CH$_3$) (Found: C, 34.18; H, 3.44; N, 33.09; S, 15.02. $C_6H_7N_5O_2S$ requires C, 33.80; H, 3.31; N, 32.85; S, 15.04%).

Theoretical calculations

The semiempirical calculations in the gas phase were carried out with the MOPAC 6.0 program[26] and the AM1[8] and PM3[9] Hamiltonians. The PRECISE keyword was used to increase the convergence criteria and the EF (Eigenvalue Follower) optimization method to obtain the minimum structures.

The solvation effects were taken into account, at the semiempirical level, *via* the self-consistent reaction field (SCRF) models SM2/AM1[14] and SM3/PM3[15] implemented in the AMPAC program.[27]

The *ab initio* calculations were performed using the GAUSSIAN94 package.[28] The standard 6-31G* basis set[11] at the HF level and with the BLYP[12] functional were used.

The cavity size where the solute is located was calculated in the Onsager model,[13] from the 0.001 au electron density envelope scaled by a factor of 1.33 to obtain the solute volume. The corresponding molecular radii, which had been increased by 0.5 Å to account for the nearest approach of solvent molecules, were used.[29] The relative permittivity of liquid water ($\varepsilon = 78.5$) was used.

The systems were fully optimized, both in the gas phase and the simulated solvent environment, until the default convergence criteria of the program were satisfied.

In addition, an estimation of the stabilization due to the solvation process was calculated by resolution of the Poisson–Boltzmann equation[16] as implemented in the DelPhi program.[30] The atomic radius defined by Gavezzotti[31] was used to define the molecular cavity. The atomic charges used correspond to the Mulliken partition of the optimized *ab initio* geometries in the gas phase. The solvation stabilization was obtained as the energy difference of two calculations, one *in vacuo* ($\varepsilon = 1$) and the other simulating a water environment. For each case a previous calculation was carried out with a larger grid in order to obtain the electrostatic potential in the boundary regions.

The electron density at the bond critical points of the hydrogen bonded complexes of the pyrazinothiadiazine tautomers and water were calculated within the Atoms in Molecules (AIM) methodology[21] and the AIMPAC program package.[32]

Acknowledgements

Thanks are given to Professor Manuel Yañez for helpful comments. This work has been supported by the Spanish project SAF97-0044-C02.

References

1 P. Goya, J. A. Páez and W. Pfleiderer, *J. Heterocycl. Chem.*, 1984, **21**, 861.

2 P. Goya, J. A. Paez, I. Alkorta, E. Carrasco, M. Grau, F. Anton, S. Julia and M. Martinez-Ripoll, *J. Med. Chem.*, 1992, **35**, 3977.

3 A. Herrero, C. Ochoa, C. Perez, F. Rodriguez-Caabeiro, A. Jimenez, C. de Armas, A. Criado and M. Font, *Arzneim.-Forsch./Drug Res.*, 1993, **43**, 163; A. Herrero, C. Ochoa and M. Stud, *An. Real Acad. Farm.*, 1989, **55**, 451.

4 J. Cortijo, M. Marti-Cabrera, L. Berto, F. Anton, E. Carrasco, M. Grau and E. J. Morcillo, *Eur. J. Pharmacol.*, 1997, **333**, 69.

5 P. Goya, A. Herrero, M. L. Jimeno, C. Ochoa, J. A. Páez, F. H. Cano, C. Foces-Foces and M. Martínez-Ripoll, *Heterocycles*, 1988, **27**, 2201.

6 I. Alkorta, *Theor. Chim. Acta*, 1994, **89**, 1.

7 I. Alkorta, C. García-Gómez, J. A. Páez and P. Goya, *J. Phys. Org. Chem.*, 1996, **9**, 203.

8 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.

9 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209 and 221.

10 P. C. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213.

11 B. Delley, *J. Chem. Phys.*, 1991, **94**, 7245.

12 O. Tapia and O. Goscinki, *Mol. Phys.*, 1975, **29**, 1653.

13 L. Onsager, *J. Am. Chem. Soc.*, 1936, **58**, 1486.

14 C. J. Cramer and D. G. Truhlar, *Science*, 1992, **256**, 213.

15 C. J. Cramer and D. G. Truhlar, *J. Comput. Chem.*, 1992, **13**, 1089.

16 I. Klapper, R. Hagstrom, R. Fine, K. Sharp and B. Honig, *Proteins*, 1986, **1**, 47.

17 M. K. Gilson and B. Honig, *Proteins*, 1988, **4**, 7.

18 C. Lim, D. Bashford and M. Karplus, *J. Phys. Chem.*, 1991, **95**, 5610.

19 E. Rajasekaran, B. Jayaram and B. Honig, *J. Am. Chem. Soc.*, 1994, **116**, 8238.

20 R. F. W. Bader, in *Atoms in Molecules. A Quantum Theory*, Oxford University Press, New York, 1990.

21 M. T. Carroll and R. W. Bader, *Mol. Phys.*, 1988, **65**, 695.

22 I. Alkorta, I. Rozas and J. Elguero, *Chem. Soc. Rev.*, 1998, **27**, 155.

23 O. Mo, M. Yañez and J. Elguero, *J. Chem. Phys.*, 1992, **97**, 6628.

24 I. Alkorta and J. Elguero, *J. Phys. Chem.*, 1996, **100**, 19 367.

25 W. Pfleiderer and H. Fink, *Chem. Ber.*, 1963, **96**, 2950.

26 J. J. P. Stewart, Mopac 6.0, QCPE Program 455, Bloomington, IN, USA, 1990.

27 AMPAC (5.0), Semichem Inc., Schawnee, KS, USA.

28 GAUSSIAN94, M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J. R. Cheeseman, T. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, J. B. Foresman, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomperts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. P. Stewart, M. Head-Gordon, C. Gonzalez and J. A. Pople, Gaussian, Inc., Pittsburgh, PA, USA, 1995.

29 M. W. Wong, M. J. Frisch and K. B. Wiberg, *J. Am. Chem. Soc.*, 1991, **113**, 4776.

30 Delphi (2.5), Biosym Technologies, San Diego, CA, USA.

31 A. Gavezzotti, *J. Am. Chem. Soc.*, 1983, **105**, 5220.

32 F. W. Bieger-Koenig, R. F. W. Bader and T. H. J. Tang, *J. Comput. Chem.*, 1982, **3**, 317.

Paper 8/03576H
Received 13*th May* 1998
Accepted 10*th July* 1998