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Synthesis of $[(MeCyt)_2H]I$—structure and stability of a dimeric threefold hydrogen-bonded 1-methylcytosinium 1-methylcytosine cation†

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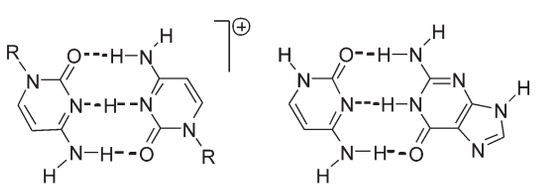
Received 30th April 2004, Accepted 18th June 2004
First published as an Advance Article on the web 10th August 2004

Reaction of trimethylsilyl-protected cytosine with methyl iodide afforded *N*1-methylated product. Subsequent treatment with ethanol resulted in cleavage of the protection group forming $[(MeCyt)_2H]I$ (**4**). Identity of **4** was confirmed by microanalysis, mass spectrometry, ^1H and ^{13}C NMR spectroscopy and by single-crystal X-ray diffraction analysis. Crystals of **4** consist of dimeric $[(MeCyt)_2H]^+$ cations and I^- anions. These ions are arranged in the crystal such that there is a strong base stacking (mean stacking distance 3,467 Å) and, furthermore, π interactions between I^- and cytosine rings (mean distance 3,737 Å). The dimeric $[(MeCyt)_2H]^+$ cations are centrosymmetric having three strong hydrogen bonds, namely two terminal N4–H···O′ ones (N4···O′ 2.815(4) Å) and a central N3–H···N3′ (N3···N3′ 2.813(4) Å) one. Quantum chemical calculations on the DFT level of theory show that the gas phase structure of the dimeric cation exhibits two different terminal N–H···O hydrogen bonds, a stronger (N4···O′ 2.722 Å) and a weaker one (N4′···O 2.960 Å). The central N3–H···N3′ hydrogen bond (N3···N3′ 2.852 Å) was characterized to have an unsymmetrically located proton and a typical double minimum potential with a very low activation barrier. The interaction energy between $[(MeCyt)H]^+$ and MeCyt yielding $[(MeCyt)_2H]^+$ was calculated to be −42.4 kcal mol^{-1} (ZPE and BSSE corrected). Comparison with the interaction energy (calculated on the same level of the theory) between cytosine and guanine yielding the triply hydrogen-bonded Watson–Crick dimer (−24.2 kcal mol^{-1}) revealed a much higher stability of the hydrogen bonds in $[(MeCyt)_2H]^+$.

Introduction

Hydrogen bonding between naturally occurring nucleic acid bases of the purine type (adenine, guanine) and the pyrimidine type (cytosine, thymine, uracil) is of great biological importance for secondary structures of DNA and RNA.[1–3] Hetero-base pair associations of the Watson–Crick and Hoogsten type (adenine–thymine, adenine–uracil, guanine–cytosine) are frequently found and very stable.[4] All of them (including the "reversed" base pairs) involve two hydrogen bonds except for Watson–Crick guanine–cytosine pairing which forms three hydrogen bonds. Although nucleic acid bases are abundantly functionalized with hydrogen donor and acceptor sites most homo-base pairs associations involve only two hydrogen bonds. An exception is the three-bonded cytosinium cytosine dimer $[(Cyt)_2H]^+$ (**I**) which is similar to the Watson–Crick base pairing between cytosine and guanine (**III**) in DNA and RNA.



R = H (**I**), R = Me (**II**) **III**

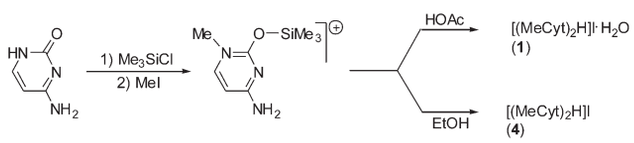
1-Methylcytosine (MeCyt) is the most simple model compound to mimic the hydrogen pattern of cytosine in nucleosides and nucleotides. Its protonated dimer $[(MeCyt)_2H]^+$ (**II**) exhibits an analogous structure as that of the parent compound **I**. In principle, X-ray crystallographic investigations gave insight into the structure of hydrogen bonding in **II**, although that will be affected in any case by cation–anion interactions and by base-stacking. But in structures investigated so far, the intra-dimer threefold hydrogen bonds are additionally affected by water molecules present in the lattice. Thus, the solid-state structures of $[(MeCyt)_2H]I{\cdot}H_2O$ (**1**),[5] $[(MeCyt)_2H]_2(SiF_6){\cdot}2H_2O$ (**2**),[6] and $[(MeCyt)_2H][Au(CN)_4]{\cdot}2H_2O$ (**3**)[7] show unsymmetrically triply hydrogen-bonded patterns where the solvent water molecules act as hydrogen acceptors for those H atoms of exocyclic amino groups of methylcytosine that are not involved in the intra-dimer hydrogen bonds.

Here we report on the synthesis and single-crystal X-ray structural diffraction analysis of $[(MeCyt)_2H]I$ (**4**) crystallizing without solvent molecules. Furthermore, quantum chemical calculations of the cation $[(MeCyt)_2H]^+$ are presented giving insight into its gas phase structure and the stability of the threefold hydrogen bond pattern.

Results and discussion

Synthesis and spectroscopy

According to Kistenmacher[5] reaction of trimethylsilyl-protected cytosine with methyl iodide resulted in *N*1-methylation. Cleavage of the protection group (O–$SiMe_3$) with acetic acid (6 M) gave 1-methylcytosine hemihydroiodide hemihydrate, $[(MeCyt)_2H]I{\cdot}H_2O$ (**1**) (Scheme 1). We found that deprotection with ethanol proceeded more moderately and, furthermore, a solvate free product, $[(MeCyt)_2H]I$ (**4**), was obtained in 49% yield (Scheme 1).



Scheme 1

The identity of **4** was confirmed by microanalysis, mass spectrometry, ^1H and ^{13}C NMR spectroscopy and by single-crystal X-ray diffraction analysis. In ^1H NMR spectra carbon bound protons were found as averaged resonances of protonated and non-protonated methylcytosine. N–H protons were not found in D_2O due to line broadening. In $[D_6]$DMSO two separated broad signals at 7.74 and 8.22 ppm were observed for four N–H protons. At 50 °C coalescence of these signals was observed. ^{13}C NMR spectra have shown one set of averaged signals for protonated and non-protonated methylcytosine. In the mass spectrum of **4** (70 eV ionisation energy)

† Electronic supplementary information (ESI) available: Cartesian coordinates of atom positions of calculated structures **5a**, **5b**, **6**, **7**, MeCyt, $(MeCyt)H^+$, Gua and Cyt. See http://www.rsc.org/suppdata/ob/b4/b407542k/

DOI: 10.1039/b407542k

the mass peak of MeCyt (M^+) (*m*/*z* 125) was found to be the base peak being flanked by M + H (*m*/*z* 126, 20%) and M − H (*m*/*z* 124, 12%) peaks. Fragmentation of MeCyt is similar to that of Cyt.[8]

Structure

$[(MeCyt)_2H]I$ (**4**) crystallized in the centrosymmetric space group $P2_1/n$. The crystal consists of dimeric cations $[(MeCyt)_2H]^+$ and iodide anions. The crystal packing is shown in Fig. 1. The dimeric cations are packed like a "staircase" with a typical base stacking distance of 3.467 Å (Fig. la). Between these infinite strands of cations the iodide anions are embedded such that there may be a stabilization *via* π interaction; the distance between iodide and mean plane of MeCyt is 3.737 Å.

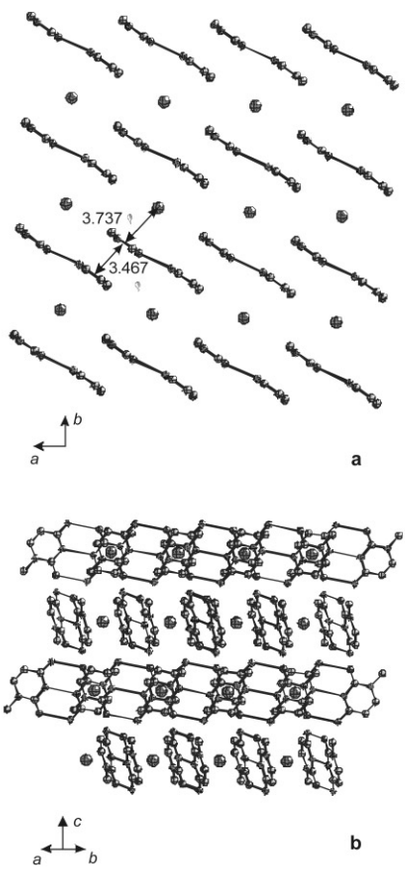


Fig. 1 Crystal structure of $[(MeCyt)_2H]I$ (**4**) showing the packing of $[(MeCyt)_2H]^+$ and I^-. The view direction is approximately along *c* (a) and perpendicular to *c* along *a* + *b* (b).

The threefold hydrogen-bonded cation $[(MeCyt)_2H]^+$ is shown in Fig. 2, selected bond lengths and angles are given in Table 1. Structural parameters of the hydrogen bonds are given in Table 2. The dimeric cation exhibits crystallographically imposed inversion symmetry. Consequently, the terminal N4–H···O′ hydrogen bonds are identical. The hydrogen atom of the central N3–H···N3′ hydrogen bond is disordered over two equally occupied positions. Thus, the central hydrogen bond is unsymmetrical despite the centrosymmetrical configuration that is generally favored in crystal structures of homo-base pairs.[4]

Interestingly, the dimer is not planar. Either of the two methylcytosine rings is planar in good approximation (greatest deviation of the mean plane for non-hydrogen atoms is 0.055(5) Å for C1) but they are parallel shifted by about 0.57 Å, see Fig. 2b. Looking at the crystal packing (Fig. la), it can be speculated that it is due to an optimized energy gain of base stacking and π interactions between I^- and cytosine rings. Preserving this structural motif but without parallel shift of the cytosine rings would result in equal base stacking and cytosine/I^- distances being up to 0.57 Å longer or shorter than the found one.

Table 1 Selected bond lengths (in Å) and angles (in deg) of $[(MeCyt)_2H]^+$ in **4**

C2–N1	1.384(4)	C4–N4	1.313(5)
C2–O	1.229(4)	C4–C5	1.418(5)
C2–N3	1.369(4)	C5–C6	1.336(5)
C4–N3	1.347(4)	C6–N1	1.367(5)
O–C2–N1	120.7(3)	C4–N3–C2	122.9(3)
O–C2–N3	122.3(3)	N4–C4–N3	118.9(3)

Table 2 Structural parameters of hydrogen bonds in $[(MeCyt)_2H]^+$ (atom distances in Å, angles in deg) in the structure experimentally found (**4**), in the calculated equilibrium structures (**5a**/**5b**), and in the transition state (**6**) for the isomerisation **5a** ⇌ **5b**

	Experimental (**4**)	**5a**	**5b**	**6**
N4···O′	2.815(4)	2.722	2.960	2.738
N4–H	0.90	1.048	1.020	1.031
H···O′	1.92	1.675	1.946	1.712
N4–H···O′	174	178.2	173.1	172.8
N3···N3′	2.813(4)	2.852	2.852	2.648
N3–H	0.84	1.060	1.792[a]	1.324
H···N3′	1.98	1.792	1.060[b]	1.324
N3–H···N3′	176	179.9	179.9[c]	179.9
N4′···O	2.815(4)	2.960	2.722	2.738
N4′–H′	0.90	1.020	1.048	1.031
H′···O	1.92	1.946	1.675	1.712
N4′–H′···O	174	173.1	178.2	172.8

[a] N3···H. [b] N3′–H. [c] N3···H–N3′.

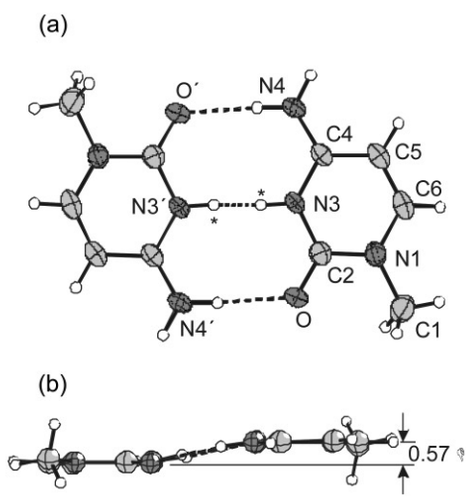


Fig. 2 (a) Structure of the dimeric cation $[(MeCyt)_2H]^+$ in **4**. The displacement ellipsoids are drawn at the 30% probability level, H atoms are drawn as circles of arbitrary size. The two equally occupied positions of the disordered proton are marked by asterisks. (b) Side view of the dimeric cation $[(MeCyt)_2H]^+$.

Quantum chemical calculations

To get insight into the gas phase structure of $[(MeCyt)_2H]^+$ and the stability of the threefold hydrogen bond, quantum chemical calculations on the DFT level of theory were performed using the hybrid functional B3LYP and the basis sets 6-31G* and 6-31G** for structure optimizations and energy calculations, respectively (see Experimental). Geometry optimization without any symmetry restriction led to the calculated equilibrium structure **5a** which is shown in Fig. 3a. Except for the hydrogen atoms of methyl groups the molecule is planar (greatest deviation of non-hydrogen atoms from mean plane: <0.001 Å). The geometry of hydrogen bonds is given in Table 2. All three hydrogen bonds are unsymmetrical and nearly linear (N–H···X 173.1–179.9°, X = N, O). Not unexpectedly, in the central N3–H···N3′ hydrogen bond the N3–C bonds of the protonated nitrogen atom N3 are slightly longer than those of the nitrogen atom N3′ that acts as hydrogen acceptor (1.363 *vs.* 1.344 Å; 1.386 *vs.* 1.361 Å). The angle C2–N3–C4 at protonated N3 is greater by 4.4° than that of non-protonated N3′ (125.1 *vs.* 120.7°).

The two terminal N4–H···O hydrogen bonds differ in N···O distance by 0.238 Å. The one which has the proton on the same side as the proton of the central N3–H···N3′ bond exhibits the shorter N4···O distance. The stronger N4–H···O hydrogen bond gives rise to a longer C2=O (1.243 *vs.* 1.222 Å) and a shorter C4–N4 bond (1.320 *vs.* 1.340 Å). Furthermore, the angles N3–C2–N1 (−3.4°; given is the difference between the angle in the protonated and the non-protonated half of the molecule) and O–C2–N1 (+3.9°) as well as N3–C4–C5 (−3.9°) and N4–C4–C5 (+3.3°) are sensitive to protonation at N3. All these values show the same trend as found in the comparison of the structures of the protonated and non-protonated 1-methylcytosine ([MeCytH]ClO_4 *vs.* MeCyt).[9,10]

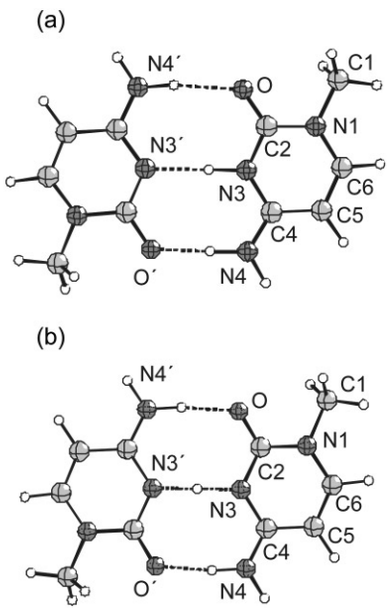


Fig. 3 Calculated structure of the dimeric cation $[(MeCyt)_2H]^+$: (a) ground state **5a**, (b) transition state **6**.

By means of a scan of the potential energy surface for the N3–H coordinate of the central N3–H···N3′ hydrogen bond, another equilibrium structure (**5b**) was found in which the proton of the central hydrogen bond has moved from N3 to N3′. Simultaneously, the longer terminal N4–H···O hydrogen bond was shortened and *vice versa.* Thus, as expected, the molecular structure **5b** is the same as **5a** (Table 2). In the same way the transition state **6** of the reaction **5a** ⇌ **5b** has been found. Its structure is very close to C_{2h} symmetry. The central N3···H···N3′ hydrogen bond is symmetrical and the terminal N4–H···O hydrogen bonds are identical. The structural parameters are in between the two corresponding hydrogen bonds of the equilibrium structures **5a**/**5b**.

The activation energy for the reaction **5a** ⇌ **5b** was found to be very low. The numerical values (3.7 and 1.1 kcal mol^{-1} without and with ZPE corrections, respectively) must not be overestimated in view of the accuracy of the level of theory used. After calculation the equilibrium structures of the monomeric cation $(MeCyt)H^+$ and the nonprotonated nucleobase MeCyt on the same level of theory, the energetics of the reaction

$$(MeCyt)H^+ + MeCyt \rightarrow [(MeCyt)_2H]^+$$

has been calculated to get insight into the stabilisation of $[(Mecyt)_2H]^+$ (**5a**/**b**) through hydrogen bonding. The interaction energy (corrected for the basis set superposition error, BSSE)[11] was shown to be −43.9 kcal mol^{-1} and −42.4 kcal mol^{-1} when the zero point vibrational energies were taken into account. For comparison, on the same level of theory the Watson–Crick cytosine–guanine base pair **7** (having an analogous triple of hydrogen bonds as $[(MeCyt)_2H]^+$) has been calculated. The optimized structure is shown in Fig. 4.

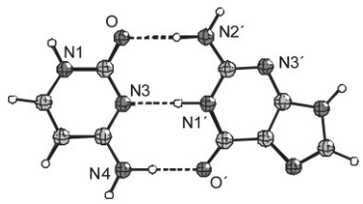


Fig. 4 Calculated equilibrium structure of the Watson–Crick cytosine–guanine dimer **7**. Geometrical parameters of hydrogen bonds (distances in Å, angles in deg): N2′–H′···O: N2′···O 2.937, N2′–H′ 1.023, O···H′ 1.914, N2′–H′···O 178.5; N1′–H′···N3: N1′···N3 2.950, N1′–H′ 1.033, N3···H′ 1.918, N1′–H′···N3 177.3; N4–H···O′: N4···O′ 2.818, N4–H 1.037, O′···H 1.781, N4–H···O′ 179.2.

The BSSE corrected interaction energies for the reaction

$$Cyt + Gua \rightarrow Cyt–Gua$$

without and with zero-point vibrational energies were calculated to be −25.6 and −24.2 kcal mol^{-1}, respectively. Analogous values for the cytosine–guanine dimer were obtained from quantum-chemical calculations using DFT methods (−26.5 kcal mol^{-1}) and the second order Møller–Plesset perturbation method (−25.8 kcal mol^{-1}), respectively.[12] Furthermore, reliability of all these calculations of the Cyt–Gua dimer is strengthened by other quantum-chemical calculations[13] and by experimental data from field-ionization mass spectrometric investigations.[14]

Thus, calculations performed within that work can be regarded to be reliable. Comparison between the two triply hydrogen-bonded systems discussed before exhibited a much higher interaction energy for the $[(MeCyt)_2H]^+$ cation (**5a**/**b**) over the neutral Watson–Crick Cyt–Gua dimer (**7**) (ΔE = 18.2 kcal mol^{-1}, ZPE and BSSE corrected). This markedly different stability is not caused by methylation in **5a**/**b**: Comparison (RI-MP2 level using the TZVPP basis set) between cytosine–guanine and 1-methylcytosine 9-methylguanine base pairs of Watson–Crick type showed that methyl substitution does not influence interaction energies by more than 0.2 kcal mol^{-1}.[15] Qualitatively, the difference in interaction energies in **5a**/**b** and **7** can be understood in terms of the central N–H···N hydrogen bond: Comparison of acidities of protonated cytosine (pK_a = 4.6)[16] and of the N1–H site of guanine (pK_a = 9.2–9.6)[17] shows that the first one is the much stronger hydrogen donor. On the other hand, due to the overall positive charge the cytosine hydrogen acceptor site in **5a**/**b** is expected to be weaker than that in **7**. Obviously, the first factor is decisive for the higher stability of hydrogen bonds in $[(MeCyt)_2H]^+$. In contrast to that highly stable triply hydrogen-bonded cation, the most stable neutral cytosine dimer $(Cyt)_2$ is held together by two hydrogen bonds and exhibits an interaction energy of −16.6 kcal mol^{-1} only.[18]

Experimental

Starting materials were commercially available from ACROS (cytosine) and Lancaster ($HN(SiMe_3)_2$, Me_3SiCl, MeI). NMR spectra were measured on a Varian Unity 500 spectrometer using solvent signals as internal references. The mass spectra were recorded on an AMD 402 Intectra. The microanalysis was measured on a Leco CHNS 932.

Synthesis of $[(MeCyt)_2H]I$ (4)

In an argon atmosphere a mixture of cytosine (1.00 g, 9.0 mmol) and Me_3SiCl (1,09 g, 10.0 mmol) in hexamethyldisilazane (20 mL) was refluxed for 3 hours. After adding methyl iodide (23 g, 0.16 mol) at room temperature, the mixture was heated at 60 °C for 2 h and then refluxed for a further 5 h. At room temperature, solvent was removed *in vacuo* and ethanol (20 mL) was added dropwise with stirring. Then, solvent was removed *in vacuo* and the residue was recrystallized from ethanol. Yield: 0.84 g, 49%. T_{dec} 233–235 °C. Found: C, 31.7; H, 4.0; N, 22.4. Calc. for $C_{10}H_{15}IN_6O_2$: C, 31.8; H, 4.0; N, 22.2%. δ_H (500 MHz, D_2O) 3.29 (6H, s, C*H*$_3$), 5.92 (2H, d, $^3J_{H,H}$ = 7.51 Hz, *H-5*), 7.57 (2H, d, $^3J_{H,H}$ = 7.51 Hz, *H-6*).

δ_H (500 MHz, [D_6]DMSO) 3.27 (6H, s, CH_3), 5.82 (2H, d, $^3J_{H,H}$ = 7.3 Hz, *H*-5), 7.74 (2H, br s, N*H*), 7.78 (2H, d, $^3J_{H,H}$ = 7.3 Hz, *H*-6), 8.22 (2H, br s, N*H*). δ_C (125 MHz, D_2O) 37.2 (s, *C*H_3), 94.6 (s, *C*5), 149.1 (s, *C*6), 154.3 (s, *C*2), 163.1 (s, *C*4). δ_C (125 MHz, [D_6]DMSO) 36.5 (s, *C*H_3), 92.9 (s, *C*5), 148.6 (s, *C*6), 152.3 (s, *C*2), 162.6 (s, *C*4). MS (70 eV) *m*/*z* 128 (89%, HI), 127 (39, I), 126 (20, M + H), 125 (100) (M^+), 124 (12, M − H), 110 (13, M − CH_3), 96 (24, M − HCO), 83 (35, M − NCO), 81 (24, M − CH_3 − HCO).

Crystallographic studies

Single crystals of **4** suitable for X-ray diffraction measurements were obtained by recrystallization from ethanol. Intensity data were collected on a STOE-STAD14 four-circle diffractometer with Mo-Kα radiation (0.71073 Å, graphite monochromator). An empirical absorption correction was applied using ψ-scans (T_{min}/T_{max} 0.886/0.995). The structure was solved by direct methods with SHELXS-86[19] and refined using full-matrix least-squares routines against F^2 with SHELXL-97.[20] Non-hydrogen atoms were refined with anisotropic and hydrogen atoms with isotropic displacement parameters. H atoms were found in the difference Fourier map and refined freely, except aromatic protons, which were refined according to the "riding model".

Crystal data and structure refinement. $C_{10}H_{15}IN_6O_2$, M_r = 378.18, monoclinic, a = 7.2200(8), b = 8.722(1), c = 11.662(1) Å, β = 98.27(1)°, U = 726.8(2) Å^3, T = 293(2) K, space group $P2_1/n$ (no. 14), Z = 2, ρ_{calc} = 1.728 g cm^{-3}, μ(Mo-Kα) = 2.212 mm^{-1}, 2560 reflections measured, 1280 unique (R_{int} = 0.0324) which were used in all calculations. The final $wR(F^2)$ was 0.0620 (all data).

CCDC reference number 239325. See http://www.rsc.org/suppdata/ob/b4/b407542k/ for crystallographic data in .cif or other electronic format.

Computational details

All DFT calculations were carried out by the Gaussian98 program package[21] using the hybrid functional B3LYP.[22] All systems have been fully optimized without any symmetry restrictions using the 6-31G(d) basis set of atomic orbitals. The resulting geometries were characterized as equilibrium structures and transition states, respectively, by the analysis of the force constants of normal vibrations. To get more reliable energies, single point calculations of all structures (including computation of force constants and the resulting vibrational frequencies) were carried out using the 6-31G(d,p) basis set of atomic orbitals. All interaction energies were corrected for basis set superposition errors (BSSE) that were estimated with counterpoise type calculations.[23]

Acknowledgements

Financial support from the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* is gratefully acknowledged.

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