Cytosine–guanine base pairing in a hydrogen-bonded complex of stable open-shell molecules with $S = 1$ spins†

Hiroyuki Tanaka,[a] Daisuke Shiomi,*[ab] Tomoaki Ise,[ab] Kazunobu Sato[a] and Takeji Takui*[a]

Received 30th April 2007, Accepted 17th May 2007
First published as an Advance Article on the web 7th June 2007
DOI: 10.1039/b706521n

A bio-inspired motif of hydrogen-bonded nucleobases is introduced to stable organic biradical crystals. We have synthesized a hydrogen-bonded complex based on both a cytosine-substituted nitronyl nitroxide biradical (**1**) and a closed-shell alkyl-substituted guanine (**2**). From single-crystal X-ray structure analyses, the cytosine and guanine moieties are found to form a Watson–Crick pair with threefold hydrogen bonds. Magnetic susceptibility measurements reveal that the cytosine-substituted biradical **1** has a triplet ($S = 1$) ground state with a singlet–triplet energy gap of $2J/k_B = 27.0$ K. This complex is the first example of Watson–Crick type base pair possessing stable high-spin organic radical moieties, which is fully characterized by single-crystal X-ray structure analysis. A face-to-face overlapping is found between the planar nucleobases, while side-by-side hydrogen bonds are found between the Watson–Crick pairs. Thus, the introduction of the radical substituent into the nucleobase results in little disturbance to the molecular arrangement usually found in nucleobase molecules: the ground-state spin multiplicity of biradical **1** is maintained.

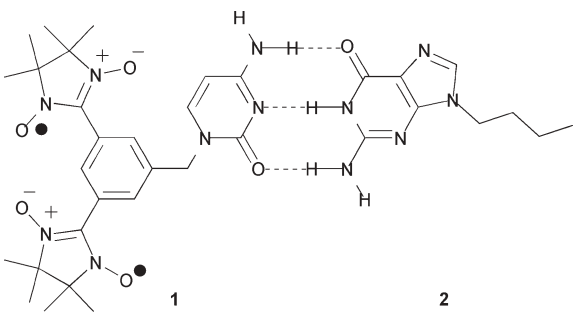
Introduction

The last few decades have witnessed rapid development of molecule-based magnetism.[1] Molecular arrangement in a crystalline solid is closely related to bulk magnetic properties of molecular assemblages such as ferromagnetism underlain by intermolecular magnetic interactions. Thus, an important issue in molecule-based magnetics is control of molecular packing, or relative arrangement of open-shell building block molecules in a crystalline solid state. Molecular packing can be partially controlled by giving the building block molecules specific noncovalent bondings. Among intermolecular noncovalent bondings, hydrogen bonding is promising in controlling the molecular arrangement in the crystalline solid. Hydrogen-donating and accepting molecules such as phenol, carboxylic acid, and pyridine derivatives have been introduced to the stable radical family of nitroxide and imino or nitronyl nitroxide.[2] Nucleobases (nucleic acid base) such as guanine, cytosine, adenine and thymine found in a DNA duplex can be further fascinating carriers of noncovalent bondings as they can form complementary hydrogen bonds with high selectivity and directionality, as compared with other hydrogen-donating and accepting molecular systems. Several examples have been found in which stable radicals of nitroxide are introduced to nucleobases in crystalline solid states.[3–6] Solution chemistry and ESR spectroscopy have been reported for spin-labeled nucleosides and nucleotides.[7,8] In this study, we have synthesized an open-shell substituted heteromolecular complex of cytosine (**1**) and guanine (**2**) derivatives (Scheme 1). The cytosine is substituted with a stable nitronyl nitroxide biradical with a spin $S = 1$ in the ground state. This is the first example of Watson–Crick type base pair possessing stable high-spin ($S > 1/2$) organic radical moieties,[9] which is fully characterized by single-crystal X-ray structure analysis and bulk magnetic susceptibility. The influence of the radical substituent on the molecular arrangement by the hydrogen bonding of the nucleobase moieties is examined.

Experimental

Synthesis

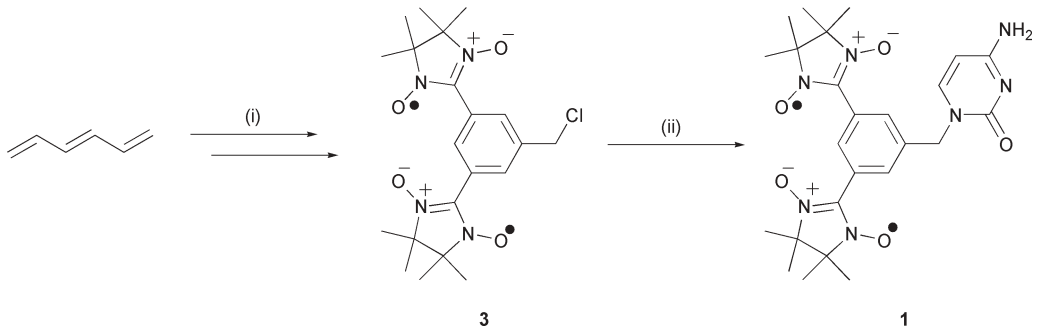
The cytosine-substituted biradical **1** was synthesized by a direct coupling of cytosine with a 5-chloromethylphenyl derivative of nitronyl nitroxide **3**,[10] as shown in Scheme 2.The precursor **3** has been prepared from hexatriene using literature procedures.[11,12] The precursor **3** (100 mg, 0.23 mmol) was added to a suspension of cytosine (38.8 mg, 0.35 mmol), K_2CO_3 (46.8 mg,



Scheme 1 Nucleobase pair substituted with nitronyl nitroxide.

[a]*Departments of Materials Science and Chemistry, Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka, Japan 558-8585. E-mail: shiomi@sci.osaka-cu.ac.jp; takui@sci.osaka-cu.ac.jp*
[b]*PRESTO, Japan Science and Technology Agency, Saitama, Japan 332-0012*

† Electronic supplementary information (ESI) available: ORTEP drawings and results of DFT molecular orbital calculations. See DOI: 10.1039/b706521n



Scheme 2 Synthesis of **1**. Steps: (i) procedures reported in ref. 10; (ii) cytosine, K_2CO_3 and KI in acetonitrile for 18 h.

0.34 mmol), and KI (53.6 mg, 0.32 mmol) in dry acetonitrile (6 ml). The reaction mixture was stirred at 80 °C for 18 h. After filtration, the reaction solution was concentrated and chromatographed on silica gel using acetone–methanol (7 : 1 in volume) as an eluent to yield a blue solid of **1** (66.3 mg, 58.2%). Elemental analysis found: C 57.52, H 6.57, N 17.60. Calcd. for $C_{25}H_{33}N_7O_5$ + CH_3OH: C 57.44, H 6.86, N 18.04%.

The closed-shell guanine derivative **2** was synthesized by the literature method.[13] Single crystals of the complex of **1** and **2** were obtained by recrystallization from a mixed solution of ethanol, chloroform and diethyl ether. Elemental analysis found: C 55.58, H 6.72, N 21.61. Calcd. for $C_{34}H_{46}N_{12}O_6$ + C_2H_5OH + H_2O: C 55.23, H 6.95, N 21.47%.

ESI-MS

Electrospray ionization mass spectrometry (ESI-MS) experiments were carried out on a JEOL JMS700T Tandem Mstation. An equal-volume mixture of methanol and chloroform was used as the solvent for electrospray. The spray voltage was 2.0 kV in the positive-ion mode.

Crystal structure analysis

Single crystal diffraction data of the complex of **1** and **2** were collected on a Rigaku Mercury CCD diffractometer with graphite monochromated Mo Kα radiation at 193 K. The crystal structure was solved by using direct methods (SHELXS97)[14] in a program package *CrystalStructure*.[15] Anisotropic thermal parameters analysis was applied for non-hydrogen atoms. Hydrogen atoms with isotropic thermal parameters were refined as riding models. From powder X-ray diffraction experiments, we have confirmed that the crystal structure of the polycrystalline sample used in magnetic susceptibility measurements is the same as that determined in the single crystal X-ray experiments.

Magnetic susceptibility measurements

The static paramagnetic susceptibility χ_p was measured for polycrystals on a SQUID magnetometer with an applied magnetic field of 0.1 T in the temperature range of 1.9–300 K. The magnetic responses were corrected with diamagnetic blank data of the sample holder obtained separately. The diamagnetic contribution of the sample itself was estimated from Pascal's constants.

Results and discussion

ESI-MS

Formation of a complex of **1** and **2** in solution was examined by ESI-MS. The positive ion mode spectrum of **1** + **2** in a 1 : 1 ratio in a mixed solution of methanol and chloroform (1 : 1) showed three doublet peaks at *m*/*z* = (208, 230), (512, 534), and (720, 742), as shown in Fig. 1. The doublets of *m*/*z* = (208, 230) and (512, 534) come from $\{(\mathbf{2} + H)^+, (\mathbf{2} + Na)^+\}$ and $\{(\mathbf{1} + H)^+, (\mathbf{1} + Na)^+\}$, respectively. The largest in intensity of the three doublets is *m*/*z* = (720, 742), as assigned to a one-to-one complex of **1** and **2** $\{(\mathbf{1} + \mathbf{2} + H)^+, (\mathbf{1} + \mathbf{2} + Na)^+\}$. This result is indicative of the formation of a stable 1 : 1 complex of **1** and **2** in solution. An additional peak is found at *m*/*z* 851, which is assignable to a tetramer of **2**, $(\mathbf{2}_4 + Na)^+$.[16,17]

Crystal structure

The crystalline solid of the complex of **1** and **2** was stable under aerated conditions at room temperature. The crystallographic data for the complex of **1** and **2** are listed in Table 1. The crystal contains one mole of ethanol as crystal solvent.[18] Fig. 2 depicts the asymmetric unit of the hydrogen-bonded complex of **1** : **2** in a 1 : 1 ratio with the atom numbering. CCDC reference numbers 609687. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b706521n

The cytosine and guanine moieties are found to form a Watson–Crick pair with threefold hydrogen bonds, (i) N7H–O6 = 2.867(5) Å, (ii) N6–HN8 = 2.947(5) Å and (iii) O5–HN9 = 2.942(4) Å. The hydrogen bond lengths fall within

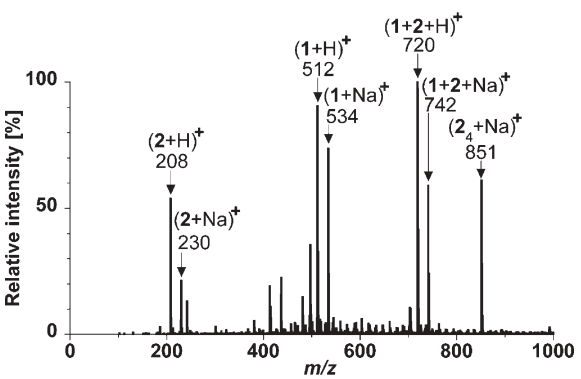


Fig. 1 Positive-ion ESI-MS of **1** + **2** in a 1 : 1 ratio in a mixed solution of methanol and chloroform (1 : 1).

Table 1 Crystallographic data for hydrogen-bonded complex (**1** + **2**)

Formula	$C_{36}H_{52}N_{12}O_7$ (**1** + **2** + EtOH)
Crystal system	Triclinic
Space group	*P*-1
a/Å	7.596(6)
b/Å	12.988(1)
c/Å	20.897(2)
α/°	100.891(1)
β/°	95.406(1)
γ/°	95.522(1)
V/Å^3	2002(3)
Z	2
ρ_{calc}/g cm^{-3}	1.269
T/K	143
Unique reflections used (All reflections)	7900
R ($I > 2\sigma(I)$)[a]	0.0999
R_{w2} (All reflections)[b]	0.2666
Goodness of fit	1.001

[a] $R = \Sigma||F_o|-|Fc||/\Sigma|F_o|$.

[b] $R_{w2} = \left[\sum w(F_o^2 - F_c^2)^2 \Big/ \sum w(F_o^2)^2\right]^{\frac{1}{2}}$, $w = 1/\left[0.0022F_o^2 + 1.0000(F_o^2)\right]/(4F_o^2)$

the typical values of Watson–Crick pairs of cytosine–guanine complexes, which were studied previously by X-ray single-crystal structure analyses.[19,20]

The Watson–Crick pairs are hydrogen-bonded to form a ribbon-shaped structure ((iv) N7H–N11 = 3.006(6) Å, (v) O5–HN9 = 2.905(5) Å) within the molecular plane of the hydrogen-bonded nucleobase moieties, as depicted in Fig. 3(a). The side-by-side hydrogen-bonding gives an alternating arrangement of the cytosine and guanine moieties (–C–G–C–G–) along the $a + b$ axis. This type of side-by-side hydrogen-bonding mode has been found in nonpolymeric cytosine–guanine complexes.[19] In oligonucleotide duplexes, hydrogen-bonded nucleobase moieties are stacked with the molecular planes facing each other.[20] The face-to-face overlapping of planar nucleobases is found in the neighboring cytosine and guanine moieties of the complex as well, resulting in the columnar structure in the direction nearly perpendicular to the hydrogen-bonding molecular plane, as shown in Fig. 3(b).[21] Thus, introduction of the radical substituent into the nucleobase results in little disturbance to the molecular arrangement usually found in nucleobase molecules or intermolecular hydrogen bonding motifs other than the Watson–Crick pairing.

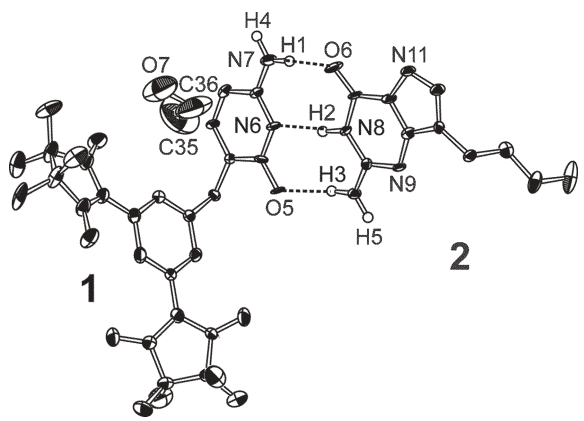


Fig. 2 ORTEP[25] view of the Watson–Crick pair (cytosine–guanine pair) with the thermal ellipsoids at the 50% probability level. The hydrogen atoms are omitted for clarity except those in the hydrogen bonding moiety as shown by the dotted lines.

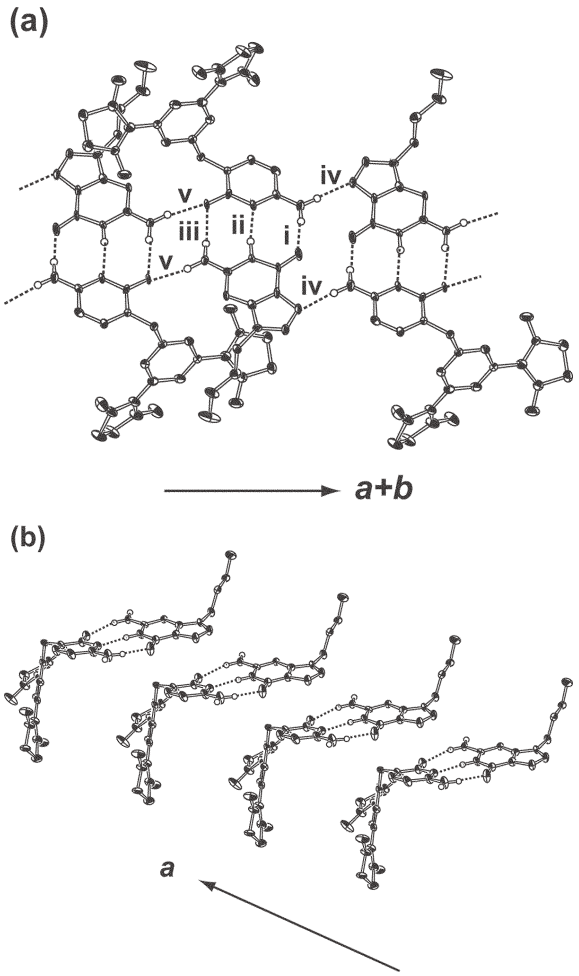


Fig. 3 (a) Hydrogen-bonded network along the $a + b$ axis. The intermolecular hydrogen bonds are labeled i through v. (b) Stacking of the complex running along the *a* axis. The methyl groups are omitted for clarity. The thermal ellipsoids are given at the 50% probability level.

The phenyl and the cytosine rings in **1** have a large dihedral angle of 100.8° around the methylene bridge. It is expected that the delocalization of unpaired electron spin in **1** is truncated at the methylene bridge. It was found from molecular orbital calculations that the cytosine moiety has little spin density.[22] Thus, the cytosine group in **1** should play a role primarily in determining the molecular packing structure instead of propagating intermolecular magnetic interactions.

Magnetic properties

The temperature dependence of paramagnetic susceptibility χ_p measured for randomly oriented polycrystals of the complex is shown in Fig. 4 in the $\chi_p T$ versus T plots.[23] The $\chi_p T$ value of 0.75 emu K mol^{-1} at 300 K corresponds to 2 moles of free

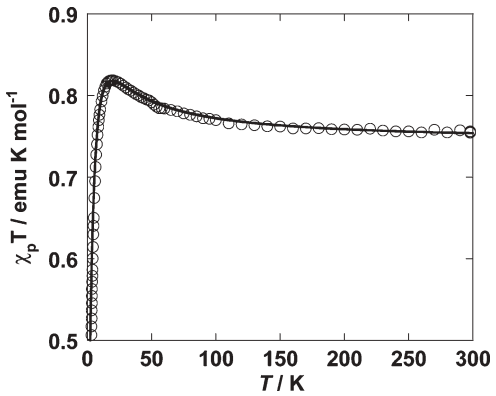


Fig. 4 Temperature dependence of paramagnetic susceptibility χ_p of the complex in the $\chi_p T$ *versus* T plots. The solid line is calculated from eqn (1).

$S = 1/2$ spins with the g-factor of 2.0, indicating that the purity of the polycrystals is satisfactory. The $\chi_p T$ value increases gradually as the temperature is lowered, and the value decreases rapidly below 20 K, indicating that both a strong ferromagnetic interaction and a weak antiferromagnetic one undergo in the crystal. The ferromagnetic interaction is naturally ascribed to the intramolecular exchange interaction in the biradical **1**, as found in precursor **3** with a similar structure,[10] while the weak antiferromagnetic interaction is attributed to intermolecular contacts. An estimate of the intra- and inter-molecular exchange interactions is acquired by assuming the singlet–triplet equilibrium,[24] given as

$$\chi_p = \frac{\alpha N_A g^2 \mu_B^2}{k_B (T-\theta)} \times \frac{2}{3+\exp(-2J/k_B T)} \qquad (1)$$

where $2J$ denotes the intramolecular exchange interaction, *i.e.*, the singlet–triplet energy gap of **1**. The intermolecular antiferromagnetic interaction is approximated by a mean-field signified by the Weiss constant θ. N_A, k_B, and α in eqn (1) are the Avogadro constant, Boltzmann constant and purity, respectively. The observed $\chi_p T$ is well reproduced by assuming $2J/k_B = 27.0 \pm 0.3$ K, $\theta = -2.3 \pm 0.1$ K, $g = 2.006$, and $\alpha = 0.99$. An intermolecular contact around the nitroxide oxygen atom with a large spin density is found at O2–C18 = 3.158(8) Å between two neighboring inversion-related molecules **1**, which is one of the possible contacts contributing to the intermolecular antiferromagnetic interactions of $\theta = -2.3$ K. The positive J value shows that the ground state of the biradical **1** is triplet ($S = 1$) in the complex.

Conclusions

We have synthesized the first example of a cytosine–guanine complex of the Watson–Crick type, which carries a stable biradical in the triplet ($S = 1$) ground state. The X-ray crystallographic analysis has shown that the complex possesses side-by-side hydrogen bonding and face-to-face stacking extending over the Watson–Crick pairs in the crystal. Introduction of the large radical substituent into the nucleobase results in little disturbance to the intermolecular hydrogen bonding motifs usually found in cytosine–guanine pairings in crystalline solid states. Thus, biradical **1** maintains the ground-state triplet in the crystal.

Acknowledgements

This work has been supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Sports, Culture, Science and Technology, Japan. Financial supported from PRESTO of Japan Science and Technology Agency is acknowledged. The authors thank Professor Nobuyuki Aikawa at Osaka City University for the help in the powder X-ray diffraction experiments.

References

1 For an overview of molecule-based magnetism, see: (*a*) *Magnetic Properties of Organic Materials*, ed. P. M. Lahti, Marcel Dekker, New York, 1999; (*b*) *Molecular Magnetism: New Magnetic Materials*, ed. K. Itoh and M. Kinoshita, Gordon & Breach, Amsterdam (Kodansha as copublisher, Tokyo), 2000; (*c*) Proceedings of the 9th International Conference on Molecule-Based Magnets, Tsukuba, Japan, 2004, ed. G. Christou, *Polyhedron* 2005, **24**, 2063–2912.
2 (*a*) J. Cirujeda, L. E. Ochando, J. M. Amigo, C. Rovira, J. Rius and J. Veciana, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 55–57; (*b*) J. Cirujeda, M. Mas, E. Molins, F. Lanfranc de Panthou, J. Laugien, J. G. Park, C. Paulsen, P. Rey, C. Rovira and J. Veciana, *J. Chem. Soc., Chem. Commun.*, 1995, 709–710; (*c*) J. Veciana, J. Cirujeda, C. Rovira, E. Molins and J. J. Novoa, *J. Phys. I*, 1996, **6**, 1967–1986; (*d*) M. M. Matsushita, A. Izuoka, T. Sugawara, T. Kobayashi, N. Wada, N. Takeda and M. Ishikawa, *J. Am. Chem. Soc.*, 1997, **119**, 4369–4379; (*e*) H. Nagashima, N. Yoshioka and H. Inoue, *Polyhedron*, 2001, **20**, 1151–1155; (*f*) T. Otsuka, T. Okuno, K. Awaga and T. Inabe, *J. Mater. Chem.*, 1998, **8**, 1157–1163; (*g*) P. M. Lahti, J. R. Ferrer, C. George, P. Oliete, M. Julier and F. Palacio, *Polyhedron*, 2001, **20**, 1465–1473.
3 R. Feher, D. B. Amabilino, K. Wurst and J. Veciana, *Mol. Cryst. Liq. Cryst.*, 1999, **334**, 333–346.
4 (*a*) P. Taylor, P. R. Serwinski and P. M. Lahti, *Chem. Commun.*, 2003, 1400–1401; (*b*) P. Taylor, P. M. Lahti, J. B. Carroll and V. M. Rotello, *Chem. Commun.*, 2005, 895–897.
5 D. Shiomi, M. Nozaki, T. Ise, K. Sato and T. Takui, *J. Phys. Chem. B*, 2004, **108**, 16606–16608.
6 K. Das, M. Pink, S. Rajca and A. Rajca, *J. Am. Chem. Soc.*, 2006, **128**, 5334–5335.
7 *Spin Labeling I, II*, ed. L. J. Berliner, Academic Press, New York, 1976, 1979.
8 For recent examples of stable nitroxides, see: (*a*) T. Kaneko, M. Aso, N. Koga and H. Suemune, *Org. Lett.*, 2005, **7**, 303–306; (*b*) A. Okamoto, T. Inasaki and I. Saito, *Tetrahedron Lett.*, 2005, **46**, 791–795.
9 A Watson–Crick type base pair functionalized with $S = 1/2$ radical moieties has been synthesized: T. Ise, D. Shiomi, K. Sato and T. Takui, *Chem. Commun.*, 2006, 4832–4834.
10 Synthesis and magnetic susceptibility measurements of **3** have been reported. The precursor **3** has an intramolecular ferromagnetic interaction of $2J/k_B = 29.4$ K: H. Tanaka, T. Ise, D. Shiomi, K. Sato and T. Takui, *J. Low Temp. Phys.*, 2006, **142**, 601–604.
11 P. S. Traas, H. J. Takken and H. Boelens, *Tetrahedron Lett.*, 1977, **18**, 2129–2132.
12 E. F. Ullman, J. H. Osiecki, D. G. B. Boocock and R. Darcy, *J. Am. Chem. Soc.*, 1972, **94**, 7049–7059.
13 N. Ramzaeva, E. Alksnis, Y. Goldberg and M. Lidaks, *Synth. Commun.*, 1989, **19**, 3121–3128.
14 G. M. Sheldrick, SHELXS97 and SHELXL97, University of Gottingen, Germany, 1997.
15 The crystal structure was solved by direct methods using a program package *CrystalStructure* (ver. 3.81) by Rigaku/Molecular Structure Corporation, The Woodlands, TX.
16 For example, see: (*a*) J. R. Williamson, M. K. Raghuraman and T. R. Cech, *Cell*, 1989, **59**, 871–880; (*b*) D. Sen and W. Gilbert,

Nature, 1990, **344**, 410–414; (*c*) C. C. Hardin, E. Henderson, T. Watson and J. K. Prosser, *Biochemistry*, 1991, **30**, 4460–4472; (*d*) T. Miura, J. M. Benevides and G. J. Thomas, Jr., *J. Mol. Biol.*, 1995, **248**, 233–238; (*e*) M. Vairamani and M. L. Gross, *J. Am. Chem. Soc.*, 2003, **125**, 42–43.

17 (*a*) G. N. Parkinson, M. P. H. Lee and S. Neidle, *Nature*, 2002, **417**, 876–880; (*b*) D. J. Patel, *Nature*, 2002, **417**, 807–808.

18 On the final difference Fourier map, density peaks other than those for ethanol were found, which were assigned to oxygen atoms of disordered water molecules, as found in the elemental analysis. The refinement of the disordered oxygen atoms was unsuccessful, resulting in divergence of the least-squares calculation.

19 (*a*) E. J. O'Brien, *Acta Crystallogr.*, 1967, **23**, 92–106; (*b*) S. Fujita, A. Takenaka and Y. Sasada, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1707–1712.

20 W. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, 1984, ch. 6 and 11.

21 The ORTEP drawings for nearest-neighbor molecules and tables of interatomic distances in CIF are provided in the ESI†.

22 DFT molecular orbitals and spin density distribution of **1** have been calculated using the molecular geometry of the X-ray crystal structure. The results of the spin density distributions are given in ESI†.

23 The diamagnetic contribution of -460×10^{-6} emu mol^{-1} was subtracted.

24 B. D. Bleany and K. Bowers, *Proc. R. Soc. London, Ser. A*, 1952, **214**, 451–465.

25 (*a*) C. K. Johnson, ORTEP-II: A FORTRAN Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, TN, USA, 1976; (*b*) N. Burnett and C. K. Johnson, ORTEP-III: Oak Ridge Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, Report ORNL-6895, Oak Ridge National Laboratory, Oak Ridge, TN, USA, 1996.