ARTICLE

OBC
www.rsc.org/obc

Acyclic, achiral enamide nucleoside analogues. The importance of the C=C bond in the analogue for its ability to mimic natural nucleosides

Asger B. Petersen, Michael Å. Petersen, Ulla Henriksen, Steen Hammerum and Otto Dahl *
Department of Chemistry, University of Copenhagen, The H. C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen, Denmark. E-mail: dahlo@kiku.dk; Fax: +45 3532 0212; Tel: +45 3532 0176

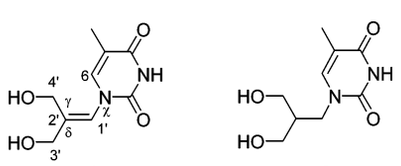
Received 30th June 2003, Accepted 15th August 2003
First published as an Advance Article on the web 4th September 2003

The conformations of an acyclic, achiral enamide thymidine analogue **1** have been studied by model building and geometry calculations, as well as by NMR NOE and UV experiments. The results indicate that there are no significant barriers to rotation around any of the σ bonds, in particular the N1–C1′ enamide bond, and that the analogue should be able to accommodate conformations that mimic the conformations of natural nucleosides in A- and B-type helices quite well. For comparison the saturated analogue **2** has been prepared and built into oligonucleotides. It is shown that incorporation of **2** in oligonucleotides results in a much larger depression of the melting temperature (ΔT_m −10 to −12.5 °C) than does incorporation of **1** (ΔT_m −5 to −6.5 °C).

Introduction

Nucleoside analogues are potential antiviral and anticancer agents,[1] and may improve the *in vivo* properties of antisense oligonucleotides which are promising highly selective drugs.[2] Many acyclic nucleoside analogues have shown high antiviral activity, but are usually poor monomer building blocks for antisense-directed purposes, because of their flexibility which results in reduced binding of the modified antisense oligonucleotide to a target mRNA. Thus, a reduction of 6–13 °C in the melting temperature (T_m, the temperature at which half of a duplex is dissociated) of DNA–RNA 17-mer duplexes has been found when the DNA oligonucleotide is modified with one acyclic nucleoside analogue in the middle of the sequence.[3]

We have prepared[4] and built into oligodeoxyribonucleotides[5] an acyclic, achiral enamide nucleoside analogue, 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine **1** (Chart 1). The analogue was designed to mimic natural nucleosides, *i.e.* to be able to exist in conformations which place the two hydroxy groups and thymine close to the positions of the 3′-hydroxy group, the 5′-hydroxy group, and thymine of thymidine located in both natural dsDNA (B-type double helices) and dsRNA (A-type double helices). The analogue was arrived at by model building, and the C=C double bond seemed important to obtain good overlap between the above-mentioned essential groups. However, oligonucleotides containing one or two **1** were found to bind to complementary DNA and RNA strands with a reduced T_m (2–6.5 °C per single introduced base modification).[5] The present paper describes our studies on the preferred conformations of **1** by NMR, UV, and geometry calculations. We address the following questions: Are conformations of **1** which fit into A- or B-type helices minima, or how much energy is required to reach these conformations from the minimum energy conformation(s)? Is conjugation in the enamide structure of **1** high enough to result in a substantial barrier to rotation around the N^1–$C^{1'}$ bond? We have also prepared the hitherto unknown[6] saturated analogue **2** and show here that oligonucleotides containing this analogue bind much less well to DNA and RNA than the oligonucleotides containing **1** in the same positions.



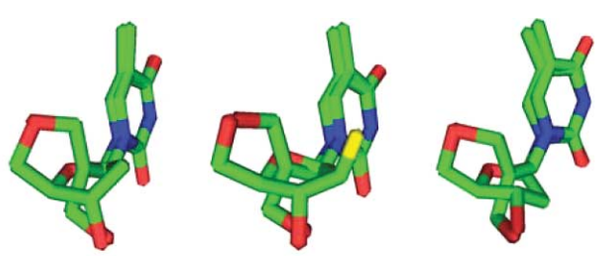
1 **2**

Chart 1 Dihedral angles χ = C2′–C1′–N1–C6; γ = C1′–C2′–C4′–O4′; δ = C1′–C2′–C3′–O3′.

Results and discussion

Model building

At first sight **1** seems far removed from a normal thymine nucleoside structure. In order to illustrate that **1** may be able to mimic a thymine nucleoside in A- and B-type double helices the structure of **1** was drawn with ChemDraw and PM3 minimized with Hyperchem 7.[7] Overlay pictures of the resulting structure **1** with dT or 2′-F-araT structures, taken from published crystal structures of double helices containing dT in a B-helix,[8] dT in an A-helix,[9] and 2′-F-araT in a modified B-helix,[10] are shown in Fig. 1. In these overlay pictures, only rotations around the N1–C1′, C2′–C3′, and C2′–C4′ bonds (dihedral angles χ, δ, and γ, respectively) of **1** were allowed, all bond lengths and other angles were unchanged. The overlay pictures were arrived at by distance minimization, performed with the computer program Macromodel 7,[11] between the same atoms of the bases (N1, O2, N3, and O4), and between O3′ in **1** and O3′ in the sugars, and O4′ in **1** and O5′ in the sugars. No energy minimizations were performed.



A
1 + DNA B-helix thymidine (from a DNA/DNA B-helix[8])

B
1 + 2′F-*ara*-thymidine (from a DNA/DNA B-helix, modified[10])

C
1 + DNA A-helix thymidine (from a RNA/DNA A-helix[9])

Fig. 1

DOI: 10.1039/b307394g

Table 1 Distances between similar atoms in the overlayed structures shown in Fig. 1 and selected dihedral angles as defined for structure **1**

	Distances/Å						Dihedral angles/°		
Structure	O3′–O3′	O4′–O5′	N1–N1	O2–O2	N3–N3	O4–O4	χ	δ	γ
A	0.259	0.098	0.219	0.056	0.079	0.130	−8.4	51.8	136.8
B	0.375	0.288	0.310	0.087	0.157	0.293	−2.8	64.5	176.8
C	0.275	0.089	0.404	0.170	0.082	0.297	−13.0	114.6	114.0

The minimized distances between analogous atoms in the superimposed structures and the dihedral angles χ, δ, and γ are given in Table 1. None of these distances are found to be larger than 0.4 Å, and the dihedral angle χ is between −3 and −13°.

These models suggest that **1** should be able exist in conformations that mimic the conformations of natural nucleosides in A- and B-type helices quite well. Estimates of the energies necessary to reach these conformations from the minimum energy conformation of **1** are given in the following section.

DFT calculations

Density functional theory (DFT) and *ab initio* calculations were used to find the minimum energy conformations of **1** and to estimate the energy barriers to rotation around the N1–C1′ bond (dihedral angle χ). B3LYP/6-31G(d) calculations were used to determine the minimum-energy structure as well as the geometry of other stationary points and to obtain zero-point vibrational energy contributions, and the MP2/6-31G(d) method was used for energy calculations at these stationary points. The calculations were performed with the Gaussian98 suite of programs.[12]

The dihedral angle between the ring and the exocyclic double bond (χ) is 39° in the minimum energy conformation. Rotation around the N1–C1′ bond (*i.e.*, variation of χ) involves an energy barrier of 10 kJ mol^{-1} (MP2-calculations); the highest energy conformation is nearly planar (χ = 4°). In all calculated conformations one of the OH groups forms a hydrogen bond to the other OH group, as expected for a gas phase species; this precludes an estimate of barriers to rotation around the C2′–C3′ and C2′–C4′ bonds. Calculations on a similar structure without the hydroxy groups [1-(2′-methylprop-1-enyl)uracil] gave no indications of any substantial barrier to rotation around these bonds, and showed properties similar to **1** (χ = 52° in the minimum conformation, barrier 21 kJ mol^{-1} (MP2) or 14 kJ mol^{-1} (B3LYP) for the highest energy conformation in which χ is 0°).

These results strongly suggest that there are no significant barriers to rotation around any of the σ bonds in **1**, in particular around the N1–C1′ enamide bond, which was originally expected to have a substantial barrier to rotation due to conjugation between the π system of T and the C1′–C2′ double bond. Any conjugative stabilization is apparently outweighed by other factors which results in a minimum energy conformation with χ far from 0. A similar result was obtained in an X-ray crystal structure of an enamide (*N*-[2-methyl-1-(2-naphthyl)-prop-1-enyl]acetamide) where the enamide dihedral angle was found to 78°.[13] We have so far been unable to obtain crystalline **1** suitable for X-ray crystallography.

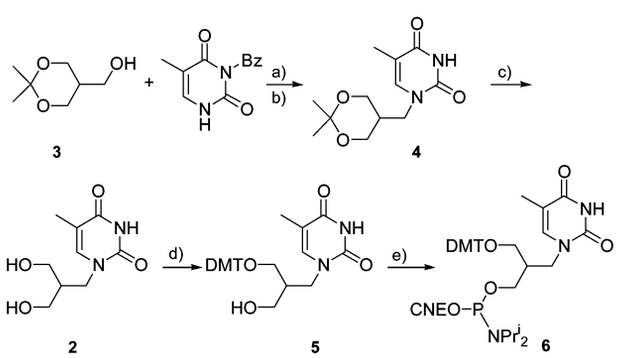
NMR (NOE) and UV experiments

Information on the preferred conformation(s) of **1** in solution can be obtained from ^{1}H NMR NOE (Nuclear Overhauser Enhancements) and UV absorption experiments. For **1** in a nearly planar conformation there should be a large NOE between H6 and H4′(χ = 0°) or H6 and H1′(χ = 180°), and the UV absorption band should move towards longer wavelengths due to conjugation. Irradiation at H6 of **1** (in DMSO-d_6) gave a NOE of 4.5% of the H4′ signals, 3% of the H1′ signal, 2% of the 4′-OH signal, and 7% of the CH_3 thymine signal. The similar NOE's of the H1′ and the H4′ signals corroborate that a population of conformers exists in which χ is substantial, as found by the above calculations; a significant population of nearly planar conformations (χ = 0) should give a much larger NOE of the H4′ signals. However, some conjugation is indicated by UV spectroscopy. Increasing the extent of conjugation in a system results in a bathochromic shift and an increase in intensity of the absorption. The UV spectrum of **1** in MeOH compared with those of some saturated alkyl analogues shows a small bathochromic shift and a small increase in intensity. Thus λ_{max} for the longer-wavelength band of **1** is 273 nm (ε = 1.05·10^4 l mol^{-1} cm^{-1}), compared to 269 nm (ε = 9.55·10^3 l mol^{-1} cm^{-1}) for the saturated analogue 1-(2,3-dihydroxy-2-hydroxymethylpropyl)thymine and 270 nm (ε = 9.50·10^3 l mol^{-1} cm^{-1}) for the saturated analogue **2**.

From these solution experiments we conclude that conformations with a substantial deviation from χ = 0° are significantly populated, although the UV results indicate that some π-orbital overlap is present.

Preparation of the saturated analogue 2 and binding studies of oligonucleotides containing 2

The saturated analogue **2** was prepared from the known alcohol **3**[14](Scheme 1). Thus, 3-benzoylthymine[15] was coupled with **3** under Mitsunobu conditions to give **4**, which was hydrolysed to **2** in a good yield. Alkylation of 3-benzoylthymine at N-1 was confirmed by NOE. The analogue **2** was mono-dimethoxytritylated to give **5** (racemic) in a fair yield using one equivalent of dimethoxytrityl chloride and then **5** was converted to the phosphoramidite **6** in a standard way.



Scheme 1 a) Ph_3P, $Pr^iOOCN{=}NCOOPr^i$, THF, rt overnight; b) 1 M aq. NaOH–dioxane, rt overnight; c) 80% acetic acid, 50 °C, 2 h; d) DMTCl, pyridine, rt overnight; e) $NCCH_2CH_2OP(NPr^i_2)_2$, tetrazole, CH_3CN, rt.

Two oligodeoxyribonucleotides containing the saturated analogue **2** (T^S) were prepared by standard phosphoramidite solid phase oligonucleotide synthesis and their hybridization properties compared with those containing the unsaturated analogue **1** (T^U) in the same positions (Table 2). It is seen that the incorporation of T^S gives a much larger depression of the melting temperature (ΔT_m −10.0 to −12.5 °C) against DNA and RNA complements than T^U (ΔT_m −5.0 to −6.5 °C). Although the T^S unit is racemic, the melting curves had similar shapes (similar hyperchromicity and wideness of the transition)

Table 2 Hybridization data (T_m, °C) for modified and unmodified oligonucleotides with DNA and RNA complements[a]

	dA_{14}	ΔT_m[b]	rA_{14}	ΔT_m[b]
dT_{14}	36.0		33.5	
$dT_7T^UT_6$	31.0	−5.0	28.0	−5.5
$dT_7T^ST_6$	26.0	−10.0	23.5	−10.0

	dGTGAGATGC	ΔT_m[b]	rGTGAGATGC	ΔT_m[b]
dGCATCTCAC	39.0		41.0	
$dGCAT^UCT^UCAC$	28.0	−5.5	27.5	−6.5
$dGCAT^SCT^SCAC$	14.0	−12.5	18.0	−11.5

[a] T_m was determined by measuring absorbance at 260 nm against increasing temperature (0.5 °C steps) on equimolar mixtures (3 μM in each strand) of modified oligomer and its complementary DNA or RNA strand in medium salt buffer (10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0). T^U and T^S are explained in the text. [b] Change in T_m per modification.

for unmodified T, T^U, and T^S, which indicate that both stereoisomers of T^S bind with similar low affinity to their complements.

Conclusion

The analogue **1** was designed to be conformationally restricted by a C=C bond in order to promote preorganization to mimic natural nucleosides. The modeling shown in Fig. 1 indicates that this is indeed successful. DFT and *ab initio* calculations indicate that there is no significant energy barrier to rotation around the σ bonds necessary to attain the modelled conformations. These calculations describe the properties of gas phase species, but NMR NOE and UV experiments in solution (DMSO-d_6 and MeOH, respectively) indicate that conformations which are rotated around the N1–C1′ bond (χ deviating from 0°) are substantially populated. The importance of the C=C bond for the ability of **1** to mimic a natural dT unit in oligonucleotides is shown by comparing the melting temperatures (T_m) of oligonucleotides, modified with **1** or the saturated analogue **2**, when hybridized to their DNA and RNA complements (Table 2). Clearly **1** mimics a dT unit much better than **2**, although the affinity of both analogues is lower than that of natural dT.

The results presented here indicate that nothing in the structure of **1** should prevent good binding of oligonucleotides modified with **1** to both DNA and RNA complements. Nevertheless **1** is a poor substitute for natural dT. Possible reasons for this could be a disruption of solvation at the rather unpolar **1**, or subtle changes in the geometry around the modification. An answer to these questions must await an X-ray structure determination of a modified duplex.

Experimental

1-[(2,2-Dimethyl-1,3-dioxan-5-yl)methyl]thymine (4)

Triphenylphosphine (2.67 g, 10.2 mmol), 3-benzoylthymine[15] (2.32 g, 10.1 mmol), and 2,2-dimethyl-5-(hydroxymethyl)-1,3-dioxane[14] (1.34 g, 9.2 mmol) were coevaporated with acetonitrile and dissolved under nitrogen in dry THF (50 ml). Diisopropyl azodicarboxylate (DIAD) (2.34 g, 11.6 mmol) in dry THF (20 ml) was added, and the mixture stirred overnight at rt. Following evaporation of solvents *in vacuo* the residue was dissolved in 1 M aq. NaOH–dioxane (1 : 1 v/v, 40 ml) and the solution stirred overnight at rt. The basic solution was extracted with diethyl ether (3 × 30 ml), the ether phase extracted with 0.01 M aq. NaOH, the combined aq. phases neutralised to pH *ca*. 8 with acetic acid, and the product extracted with dichloromethane (7 × 30 ml). The dichloromethane solution was dried (Na_2SO_4), the solvent removed *in vacuo*, and the yellow crude oil crystallised from acetonitrile to give **4** (1.39 g, 60%) as colourless crystals, mp 171.5–173 °C. NMR ($CDCl_3$, 300 MHz for ^1H): δ_H 8.29 (1H, br s, NH), 7.07 (1H, q, *J* 1.2, H-6), 3.94 (2H, d, *J* 7.9, CH_2N), 3.86 (4H, ABX system, Δ 127 Hz, J_{AB} 12.6, $J_{ax} = J_{BX}$ 3.1, CH_2O), 2.03 (1H, m, CH), 1.92 (3H, d, *J* 1.2, CH_3T), 1.47 (3H, s, CH_3), 1.43 (3H, s, CH_3). δ_C 164.4, 151.1, 141.4, 110.3, 98.6, 61.0, 48.4, 32.8, 27.8, 20.0, 12.2. FAB^- MS: 253.2 ($M - H^+$), calc. 253.1. Found: C, 56.6; H, 7.1; N, 11.25. Calc. for $C_{12}H_{18}N_2O_4$: C, 56.7, H, 7.1, N, 11.0%.

1-[3-Hydroxy-2-(hydroxymethyl)prop-1-yl]thymine (2)

Compound **4** (0.940 g, 3.70 mmol) was dissolved in 80% aq. acetic acid (50 ml) and stirred at 50 °C for 2 h. The reaction mixture was evaporated *in vacuo* and the solid residue recrystallised from methanol to give **2** (0.596 g, 75%) as colourless crystals, mp 146–147 °C (lit.[6] mp 133–134 °C). NMR (DMSO-d_6): δ_H 11.2 (1H, br s, NH), 7.40 (1H, s, H-6), 4.52 (2H, br s, OH), 3.60 (2H, d, *J* 7.0, CH_2N), 3.37 (4H, d, *J* 5.3, CH_2O), 1.89 (1H, m, CH), 1.73 (3H, s, CH_3). δ_C 164.2, 151.1, 142.0, 108.1, 59.1, 46.8, 42.8, 11.9. FAB^- MS: 212.8 ($M - H^+$), calc. 213.1. Found: C, 50.5; H, 6.6; N, 12.85. Calc. for $C_9H_{14}N_2O_4$: C, 50.5; H, 6.6; N, 13.1%.

1-[3-(Dimethoxytrityloxy)-2-(hydroxymethyl)prop-1-yl]thymine (5)

Compound **2** (0.410 g, 1.92 mmol) was coevaporated with dry pyridine and dissolved under nitrogen in dry pyridine (10 ml). Dimethoxytrityl chloride (0.65 g, 1.92 mmol) was added and the mixture stirred at rt in the dark overnight. Pyridine was removed *in vacuo* and the residue purified by flash chromatography on silica (Merck silica 60, 0.040–0.063 mm, eluted with ethyl acetate–heptane–triethylamine 89 : 10 : 1 v/v/v) to give **5** (0.354 g, 36%) as a colourless foam. NMR ($CDCl_3$, 300 MHz for ^1H): δ_H 8.3 (1H, br s, NH), 7.39 (2H, d, *J* 7.3, Ar), 7.32–7.23 (7H, m, Ar), 6.96 (1H, q, *J* 1.2, H-6), 6.83 (4H, d, *J* 8.8, Ar), 3.95 (2H, ABX system, Δ 21.1 Hz, J_{AB} 14.1, J_{AX} 4.7, J_{BX} 6.0, CH_2N), 3.79 (6H, s, CH_3O), 3.50 (2H, ABX system, Δ 12.8 Hz, J_{AB} 12.1, J_{AX} 6.6, J_{BX} 4.9, CH_2OH), 3.14 (2H, ABX system, Δ 101.9 Hz, J_{AB} 9.7, J_{AX} 4.8, J_{BX} 7.4, CH_2ODMT), 2.19 (1H, m, CH), 1.79 (3H, d, *J* 1.2, CH_3T). δ_C 164.3, 158.3, 151.9, 144.4, 141.2, 135.5, 135.4, 129.7, 127.7, 127.6, 126.7, 112.9, 110.4, 86.3, 61.5, 60.3, 55.0, 46.5, 41.6, 12.1. FAB^+ MS: 517.0 ($M + H^+$), calc. 517.2.

1-[3-(Dimethoxytrityloxy)-2-[2-cyanoethoxy(diisopropylamino)-phosphinoxymethyl]prop-1-yl]thymine (6)

To **5** (0.187 g, 0.362 mmol) in dry acetonitrile (2 ml) under N_2 was added 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.230 g, 0.76 mmol), followed by tetrazole (0.025 g, 0.36 mmol). After stirring for 30 min the solvent was removed *in vacuo*, the residue dissolved in dichloromethane (6 ml), washed with sat. aqueous $NaHCO_3$ (2 ml), dried (Na_2SO_4), and the dichloromethane removed *in vacuo*. The

residue was purified by flash chromatography on silica (Merck silica 60, 0.040–0.063 mm, eluted with ethyl acetate–heptane–triethylamine 60 : 39 : 1 v/v/v) and the product precipitated in pentane to give **6** (0.077 g, 30%) as a colourless foam. 1H NMR ($CDCl_3$): δ_H 8.2 (1H, br s, NH), 7.40–7.20 (9H, m, Ar), 6.93 and 6.91 (1H, s, H-6, 2 diastereomers), 6.82 (4H, d, *J* 8.5, Ar), 3.79 (6H, s, CH_3O), 3.75–3.24 (10H, m, 3 × CH_2O, CH_2N, 2 × CHN), 2.58 and 2.54 (2H, t, *J* 6.3, CH_2CN, 2 diastereomers), 2.43 and 2.36 (1H, m, $CH(CH_3)_2$, 2 diastereomers), 1.78 (3H, s, CH_3T), 1.17 (6H, d, *J* 6.7, $CH(CH_3)_2$), 1.11 (6H, d, *J* 6.7, $CH(CH_3)_2$). δ_C 164.3, 158.7, 151.0, 144.9, 141.8, 141.6, 136.1, 130.1, 128.2, 128.0, 127.0, 117.8, 113.3, 110.1, 86.4, 62.7, 62.5, 62.3, 62.1, 61.8, 61.7, 58.7, 58.5, 55.4, 48.4, 48.2, 43.5, 43.2, 40.6, 40.5, 24.9, 24.8, 20.7, 12.4. δ_P 149.1, 149.0. FAB^- MS: 716.1 ($M - H^+$), calc. 715.3.

Oligonucleotide syntheses. Oligonucleotide syntheses were performed in 0.2 μmol scale on a Biosearch 8750 DNA Synthesizer using standard conditions with unmodified phosphoramidites (Cruachem), or **6** and tetrazole activation. The modified phosphoramidite **6** (0.05 M in acetonitrile) was manually coupled for 6 min with a DMT efficiency of 98–99%. The oligonucleotides (precipitated by ethanol), $dT_7T^ST_6$, >95% pure (CE), M 4167.7 (ESI-MS, calc. 4167.7), and $dGCAT^SCT^SCAC$, *ca.* 95% pure (CE), M 2602.0 (ESI-MS, calc. 2602.5), were used for T_m measurements under conditions specified in Table 2.

Acknowledgements

Per-Ola Norrby, The Technical University of Denmark, is thanked for help with modeling, Britta M. Dahl for oligonucleotide synthesis, Jette Poulsen and Anette W. Jørgensen for technical assistance, and Cureon A/S for ESI-MS measurements.

References

1 C. Simons, *Nucleoside Mimetics: Their Chemistry and Biological Properties,* Gordon and Breach Science Publishers, Amsterdam, 2001.
2 *Oligonucleotides as Therapeutic Agents*, ed. D. J. Chadwick and G. Cardew, Ciba Foundation, John Wiley & Sons Ltd, Chichester, England, 1997; *Antisense Research and Application*, ed. S. T. Crooke, Springer Verlag, Berlin, 1998; E. Uhlmann, *Curr. Opin. Drug Discovery Dev.*, 2000, **3**, 203.
3 P. Nielsen, L. H. Dreiøe and J. Wengel, *Bioorg. Med. Chem.*, 1995, **3**, 19.
4 D. S. Pedersen, T. Boesen, A. B. Eldrup, B. Kiær, C. Madsen, U. Henriksen and O. Dahl, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1656.
5 T. Boesen, D. S. Pedersen, B. M. Nielsen, A. G. Petersen, U. Henriksen, B. M. Dahl and O. Dahl, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 847.
6 After the completion of our work **2** has been prepared by another route: S. Guillarme, S. Legoupy, A.-M. Aubertin, C. Olicard, N. Bourgougnon and F. Huet, *Tetrahedron*, 2003, **59**, 2177.
7 ChemDraw Ultra version 6.01, www.cambridgesoft.com; HyperChem7, www.hyper.com.
8 C. L. Kielkopf, S. Ding, P. Kuhn and D. C. Rees, *J. Mol. Biol.*, 2000, **296**, 787 (Nucleic Acid Database BD0023).
9 Y. Xiong and M. Sundaralingam, *Nucleic Acids Res.*, 2000, **28**, 2171 (Nucleic Acid Database AH0005).
10 V. Tereshko, G. Minasov and M. Egli, *J. Am. Chem. Soc.*, 1999, **121**, 470 (Nucleic Acid Database BD0007).
11 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caulfield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **19**, 440 www.schroedinger.com.
12 Gaussian 98 (Revision A.7), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 1998; www.gaussian.com.
13 X. Chen, R. Guo and Z. Zhou, *Acta Crystallogr. Sect. E*, 2002, **58**, 568.
14 M. R. Harnden, P. G. Wyatt, M. R. Boyd and D. Sutton, *J. Med. Chem.*, 1990, **33**, 187.
15 J. Zhou and P. B. Shevlin, *Synth. Commun.*, 1997, 3591.