Synthesis and properties of DNA oligomers containing an O4-ethylated thymine. d(e^{4}TpA), d(Ape^{4}T), d(Ape^{4}TpA), and d(Tpe^{4}TpT)[1]

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Received February 21, 1990

GARRY W. BUCHKO, FRANK E. HRUSKA, and KRISHAN L. SADANA. Can. J. Chem. **68**, 2011 (1990).

Syntheses via the phosphotriester method are described for DNA oligonucleotides containing O4-ethylated thymine (e^{4}Thy) and adenine (Ade) bases, d(e^{4}TpA), d(Ape^{4}T), and d(Ape^{4}TpA), for their unmodified analogs, d(TpA), d(ApT), and d(ApTpA), and for d(Tpe^{4}TpT). ^{1}H NMR chemical shift data obtained from 10 to 70°C at 300 and 500 MHz show that these molecules form right-handed minihelices at low temperature in aqueous solution; the presence of the O4-ethyl group does not seem to have a drastic effect on the stacking geometry of the thymine base. The presence of right-handed stacking is confirmed by circular dichroic data obtained over a similar temperature range. Coupling constants for the sugar ring indicate that the e^{4}Tp unit of d(e^{4}TpA) does not show the shift towards the 3′-*endo* (*N*) pucker noted for the corresponding unit of d(e^{4}TpT). A prominent quasimolecular ion peak $[M - H]^-$ is observed for the modified and unmodified molecules in the spectra obtained by Fast Atom Bombardment mass spectrometry (FAB-MS) operating in the negative ion mode, indicating that the labile O4-ethyl group is sufficiently stable to withstand the ionization process used in this method. In addition, a number of fragment ion peaks such as [O4-ethylthymine − H]$^-$ are observed that reveal the potential of FAB-MS for characterizing DNA oligomers modified by alkylating agents.

Key words: oligonucleotides, O-alkylation, stereochemistry, NMR, mass spectrometry.

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On décrit la synthèse, par la méthode du phosphotriester, des oligonucléotides de l'ADN contenant les bases O4-éthylées de la thymine (e^{4}Thy) et de l'adénine (Ade), d(e^{4}TpA), d(Ape^{4}T) et d(Ape^{4}TpA), et leurs analogues qui ne sont pas modifiés, d(TpA), d(ApT), d(ApTpA) et d(Tpe^{4}TpT). Les données relatives aux déplacements chimiques en RMN du ^{1}H, obtenues de 10 à 70°C à 300 et à 500 MHz, montrent que, en solution, à basse température, ces molécules forment des minihélices tournant vers la droite; la présence de groupements O-éthyles ne semble pas affecter beaucoup la géométrie de l'empilement de la base thymine. La présence d'un empilement tournant à droite est confirmé par les données de dichroïsme circulaire obtenues sur la même plage de températures. Les constantes de couplage dans les cycles des sucres indiquent que l'unité e^{4}Tp de d(e^{4}TpA) ne présente pas le déplacement vers la forme 3′-*endo* (*N*) replissée qui a été observée pour l'unité correspondante de d(e^{4}TpT). On a déterminé les spectres de masse par bombardement avec des atomes rapides (SM-BAR) en mode d'ion négatif des molécules tant modifiées que non-modifiées et on observe toujours un pic prédominant qui est pratiquement moléculaire $[M - H]^-$; ce résultat indique que le groupement labile O-éthyle est suffisamment stable pour supporter le processus d'ionisation utilisé dans cette méthode. De plus, on a observé un certain nombre de pics correspondants à des ions de fragmentation, comme [O4-éthylthymine − H]$^-$, qui révèlent le potentiel de la SM-BAR pour caractériser des oligomères d'ADN qui ont été modifiés par des agents alkylants.

Mots clés : oligonucléotides, O-alkylation, stéréochimie, RMN, spectrométrie de masse.

[Traduit par la revue]

Introduction

Alkylating agents such as alkyl nitrosoureas react with the oxygen atoms of DNA bases to form the O2- and O4-alkylthymines (r^{2}Thy, r^{4}Thy) and O6-alkylguanine (r^{6}Gua), or with the polynucleotide backbone to form alkyltriesters (1). Experimental evidence indicates that the altered thymine (Thy) and guanine (Gua) bases can mispair during replication and lead to mutations (1). On the other hand, the formation of the alkyltriesters does not lead to misincorporation of bases, though their presence has been shown to inhibit in vitro replication by *E. coli* DNA polymerase I (2). Some insight into the action of these agents is provided by the structural information obtained at the monomer level by X-ray crystallographic (3–8) and NMR (9, 10) investigations of O-alkylated nucleosides. More useful are the structural data for oligomer duplexes containing an O-alkylated base, but studies on such duplexes are limited (11, 12).

As part of our study of the structural consequences of base modification, we have been synthesizing DNA oligomers[3] containing r^{4}Thy. Since this base is labile in acid and basic solution (9, 13), these syntheses require considerable care, and for this reason our investigation was begun with molecules containing only r^{4}Thy and the parent Thy base. Initially we described (14) the synthesis of e^{4}TpT and Tpe^{4}T, the O4-ethylated analogues of TpT. This was followed (15) by the synthesis of the corresponding O4-isopropylated dimers, i^{4}TpT and Tpi^{4}T, and the trimer Tpi^{4}TpT. Also provided was their characterization by NMR, Fast Atom Bombardment mass spectrometry (FAB-MS), and circular dichroism (CD). Here we extend our work to oligomers containing an adenine (Ade) as well as an e^{4}Thy base: e^{4}TpA (**1**), Ape^{4}T (**2**), and Ape^{4}TpA (**3**) (Fig. 1). In addition, we report our methods for the synthesis of Tpe^{4}TpT (**7**). ^{1}H NMR as well as FAB-MS and CD data are provided for comparison with the unmodified analogues, TpA (**4**), ApT (**5**), and ApTpA (**6**), and with the oligomers discussed in our earlier manuscripts (14, 15). The data are discussed in terms of the conformational properties of the molecules in aqueous solution.

[1]Presented in part at the XIII International Conference on Magnetic Resonance in Biological Systems, Madison, WI, August 14–19, 1988.

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[3]Since we discuss only DNA oligomers, for convenience TpT will be used to represent d(TpT), and so on. The "d" will be retained for all monomers (dT, e^{4}dT, 5′-dTMP, etc.).

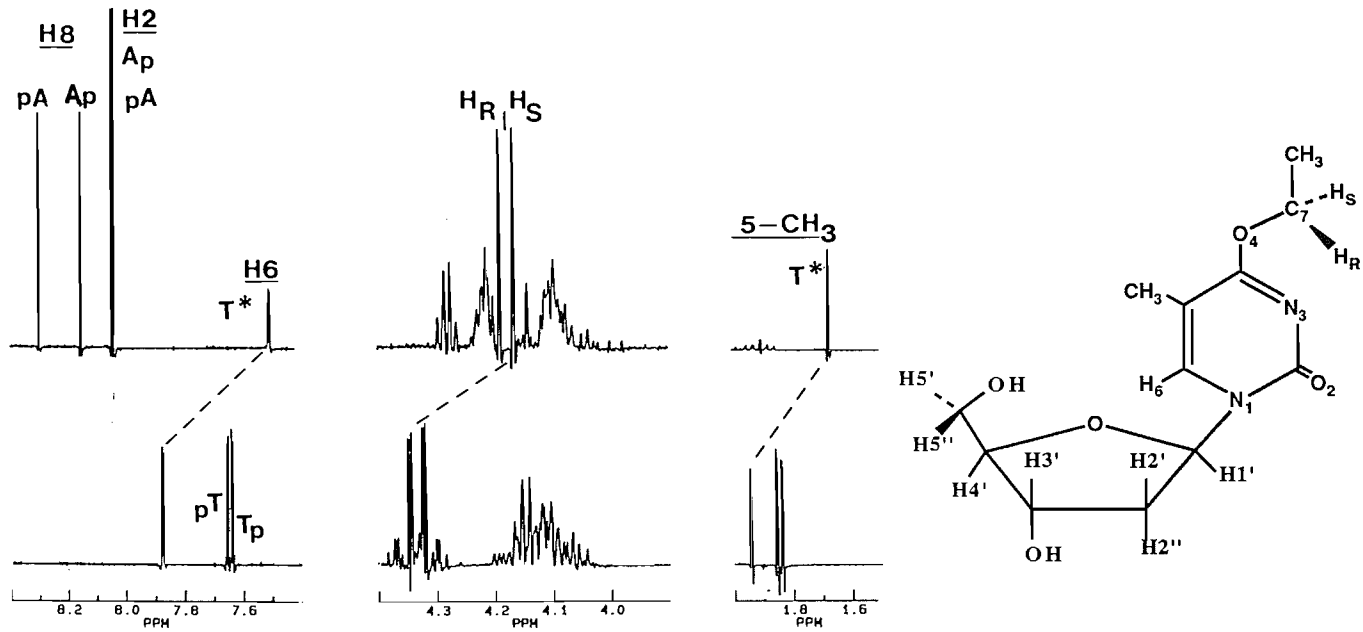


FIG. 1. Right, structure and numbering of e^4dT (O4-ethylthymidine). The methylene and methyl carbons of the O4-ethyl group are denoted C7 and C8, respectively; H_R and H_S refer to the *pro-R* and *pro-S* methylene protons of C7. Left, partial 300 MHz ^{1}H NMR spectra (27°C) of Ape^{4}TpA (**3**) (upper) and of Tpe^{4}TpT (**7**) (lower). The chemical shifts (δ) are in ppm relative to DSS. Note the intense H_R, H_S bands (joined by dashed lines) at 4.18 ppm and 4.33 ppm in the spectra of **3** and **7**, respectively. T* denotes the e^4Thy base.

Experimental

Materials and methods

2′-Deoxythymidine (dT), 2′-deoxyadenosine (dA), and the reagents used for protection, coupling, and deblocking were purchased (Aldrich or Sigma Chemical Co.). 5′-*O*-4,4′-Dimethoxytrityl-6-*N*-benzoyl-adenosine was obtained from Cruachem Inc. whereas the protected thymidines were synthesized and purified as described by Buchko *et al.* (14). Chromatographic solvents were purchased from Fisher Scientific. Thin-layer chromatography (TLC) was carried out on Kieselgel 60 F_{254} plates (Merck). Kieselgel 60 (Merck) was used for dry-column flash chromatographic (DCFC) purification of the precursors. Deprotected oligomers were purified initially by descending paper chromatography (Whatman paper No. 1) using solvent B′ (*n*-butanol:ethanol:water = 4:1:5 v/v, organic phase), followed by purification by HPLC (Perkin Elmer Series 4 liquid chromatograph) using a semipreparative ODS-Hypersil column and a gradient of methanol and 0.1 M aqueous ammonium acetate (pH 6.0). The strategy used for the synthesis of **1**, **2**, and **3** is shown in Scheme 1.

Synthetic methods

3′-Acetyl-4-(1,2,4-triazol-1-yl)-thymidine (**8**) and *5′*-O-*4,4′-dimethoxytrityl*-O-*4-ethylthymidine* (**9**) were synthesized according to Buchko *et al.* (14), except that the 4,4′-dimethoxytrityl group (DMT) was used in place of the *tert*-butyldimethylsilyl (TBDMS) group for 5′-protection in **9**. This change in the 5′-protecting group was necessary for our synthesis of **3**, which involves 5′-extension of the appropriately blocked **1** (see **15** below). The extension initially requires the removal of the 5′-protecting group from **15**. If TBDMS had been used for 5′-protection, then 5′-deprotection of **15** would have required tetra-*n*-butylammonium fluoride (TBAF), a reagent that also effects an undesirable removal of the 2-chlorophenyl group used for phosphate protection (14, 16). On the other hand, the 5′-DMT group is conveniently removed by a reagent, $ZnBr_2$, that leaves the protected phosphate intact.

3′-O-*Acetyl-6*-N-*acetyl-2′-deoxyadenosine* (**10**)

5′-*O-tert*-Butyldimethylsilyl-2′-deoxyadenosine (**11**) was prepared following Ogilvie (17), and converted to 5′-*O-tert*-butyldimethylsilyl-3′-*O*-acetyl-6-*N*-acetyl-2′-deoxyadenosine (**12**) using acetic anhydride. Molecule **10** was obtained by desilylation of **12** using TBAF (17).

6-N-*Benzoyl-2′-deoxyadenosine* (**13**) was prepared via Schaller *et al.* (18) and converted to 5′-*O-tert*-butyldimethylsilyl-6-*N*-benzoyl-2′-deoxyadenosine (**14**) following Ogilvie (17).

e^4TpA (**1**)

Molecule **9** was phosphorylated with 2-chlorophenyl phosphodichloridate and condensed with **10** using 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and tetrazole (14) to give **15**, the fully protected dimer. Molecule **15** was purified and then deprotected by sequential treatment with (i) $ZnBr_2$ for detritylation (19), (ii) oximate for phosphate deprotection, and (iii) ethanolic sodium ethoxide for removal of the 6-*N*- and 3′-*O*-acetyl groups.

Ape^{4}TpA (**3**)

Molecule **15** was detritylated with $ZnBr_2$ and purified by DCFC. The product was phosphorylated with 2-chlorophenyl phosphodichloridate and coupled with 5′-*O*-4,4′-dimethoxytrityl-6-*N*-benzoyl-2′-deoxyadenosine. The fully protected trimer **16** was then deblocked sequentially as described above.

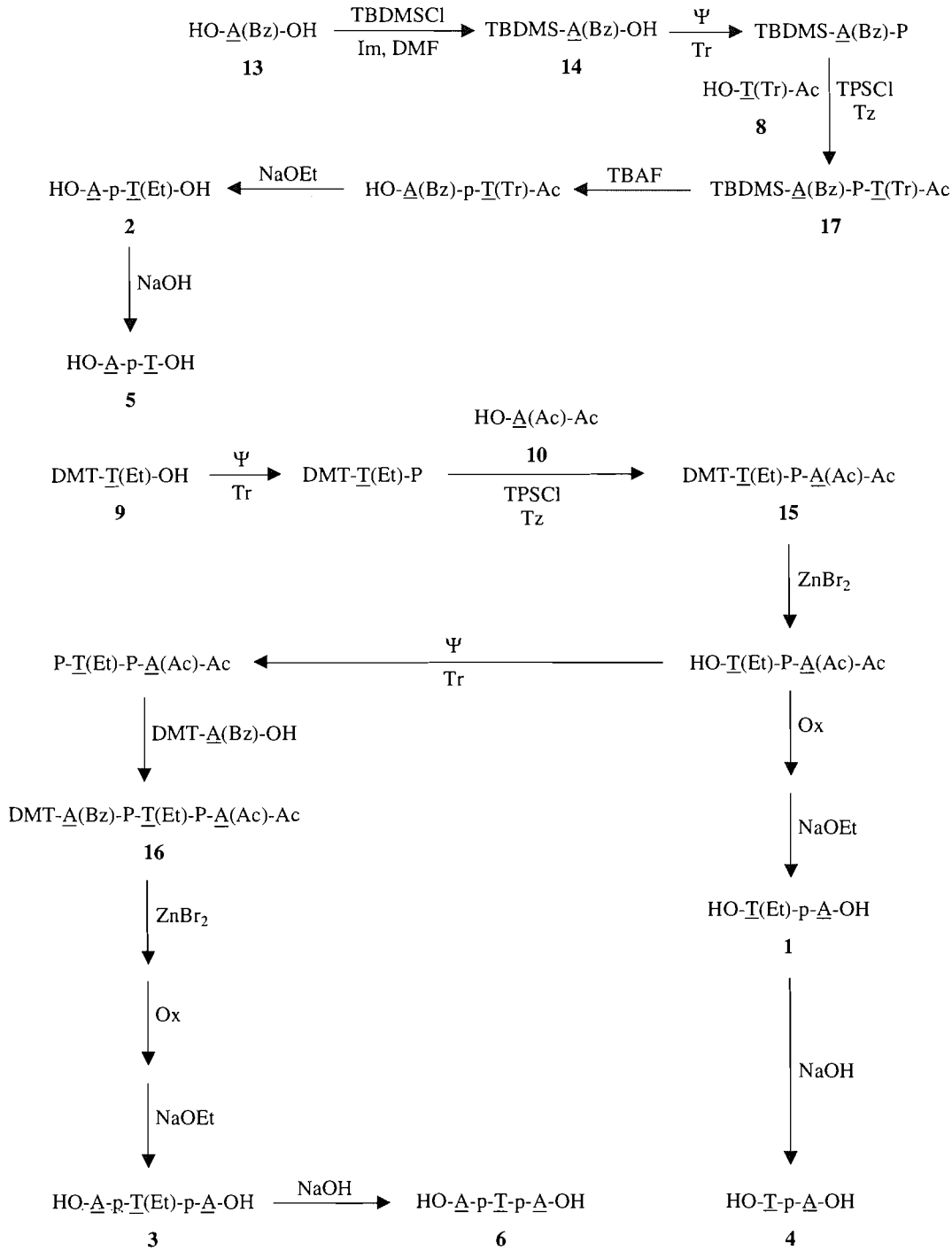
Ape^{4}T (**2**)

Molecule **14** was phosphorylated and condensed with **8** to give **17**. Molecule **17** was deprotected, as described in the synthesis of Tpe^{4}T (14), by (a) TBAF for 1 min and (b) sodium ethoxide. Note that, because of the presence of the 6-*N*-benzoyl group, the sodium ethoxide treatment had to be extended to 12 h from the 1 h required for deprotection of Tpe^{4}T (14).

Molecules **4**, **5**, and **6**, the unmodified analogues of **1**, **2**, and **3**, were prepared by replacing sodium ethoxide with sodium hydroxide (pH > 13) during deblocking of the fully blocked forms of **1**, **2**, and **3**.

Tpe^{4}TpT (**7**)

This molecule was prepared from the fully blocked form of the O4-isopropylated trimer Tpi^{4}TpT (**18**), the synthesis of which was described by Buchko *et al.* (14). Treatment of **18** with $ZnBr_2$, oximate reagent, and sodium ethoxide yielded **7**. Note that the sodium ethoxide both removes the 3′-*O*-acetyl protecting group and effects an alkoxy exchange, replacing the O4-isopropyl with the O4-ethyl function on the central base. Exchange of O4-isopropyl groups by O4-methyl



Abbreviations: T, thymidine; A, 2'-deoxyadenosine; TBAF, *n*-tetrabutylammonium fluoride; (Bz), 6-*N*-benzoyl; DMF, dimethylformamide; Ac, 3'-acetyl; (Ac), 6-*N*-acetyl; DMT-A(Bz)-OH, 5'-*O*-4,4'-dimethoxy-6-*N*-benzoyl-2'-deoxyadenosine; (Et), O4-ethyl; HO-A(Ac)-Ac, 3'-*O*-acetyl-6-*N*-acetyl-2'-deoxyadenosine (**10**); HO-T(Tr)-Ac, 3'-acetyl-4-(1,2,4-triazol-1-yl)-thymidine (**8**); Im, imidazole; NaOEt, ethanolic sodium ethoxide; Ox, oximate reagent; p, unprotected, diesterified phosphate; P, phosphate protected by *o*-chlorophenyl; TBDMSCl, *tert*-butyldimethylsilyl chloride; TPSCl, 2,4,6-triisopropylbenzenesulfonyl chloride; Tr, 1,2,4-triazole; (Tr), 4-(1,2,4-triazol-1-yl); Tz, tetrazole; Ψ, 2-chlorophenyl phosphodichloridate.

SCHEME 1

was reported initially by Singer *et al.* (20); Borowy-Borowski and Chambers (21) are using a DBU-catalyzed exchange for synthesis of oligomers containing *O*-alkylthymine residues.

The following UV absorption data for H_2O solutions were obtained on a Cary 219 spectrophotometer: **1**: λ_{max} = 262 nm, λ_{min} = 235 nm; **2**: λ_{max} = 262 nm, λ_{min} = 235 nm; **3**: λ_{max} = 261 nm, λ_{min} = 233 nm; **4**: λ_{max} = 260 nm, λ_{min} = 231 nm; **5**: λ_{max} = 260 nm, λ_{min} = 231 nm; **6**: λ_{max} = 259 nm, λ_{min} = 230 nm; **7**: λ_{max} = 269 nm, λ_{min} = 237 nm.

Mass spectrometric experiments

Negative-ion FAB mass spectra (glycerol matrix, 8-keV xenon atoms) were obtained on a VG 7070E-HF spectrometer. Some of the

TABLE 1. ^{1}H chemical shifts (δ ppm from DSS) in D_2O[a,b]

Nucleotide		Temperature (°C)	1′	2′	2″	3′	4′	5′	5″	6(2)	5M(8)	7A	7B	8M
1. e^{4}TpA	e^{4}Tp	10	5.937	1.545	2.261	4.542	4.052	3.689	3.642	7.541	1.892	4.304	4.281	1.384
		70	6.053	1.866	2.404	4.608	4.073	3.721	3.654	7.600	1.916	4.380	4.365	1.372
	pA	10	6.351	2.820	2.572	4.768	4.215	4.06	4.06	8.037	8.364	—	—	—
		70	6.425	2.809	2.599	4.724	4.223	4.087	4.066	8.175	8.349	—	—	—
2. Ape^{4}T	Ap	10	6.301	2.83	2.83	4.837	4.292	3.889	3.831	8.053	8.267	—	—	—
		60	6.342	2.789	2.746	4.891	4.291	3.841	3.790	8.159	8.243	—	—	—
	pe^{4}T	10	6.153	2.242	2.358	4.527	4.106	4.253	4.099	7.656	1.614	4.214	4.214	1.321
		60	6.228	2.268	2.418	4.536	4.150	4.207	4.105	7.718	1.745	4.272	4.247	1.299
3. Ape^{4}TpA	Ap	10	6.215	2.71	2.71	4.875	4.286	3.844	3.808	7.997	8.141	—	—	—
		60	6.273	2.719	2.682	4.893	4.272	3.814	3.773	8.110	8.180	—	—	—
	pe^{4}Tp	10	6.026	1.847	2.304	4.774	4.28	~4.1	~4.1	7.483	1.629	4.159[c]	4.138	1.338
		60	6.120	1.998	2.418	4.783	~4.3	4.11	4.06	7.576	1.741	4.227	4.242	1.314
	pA	10	6.312	2.699	2.529	4.715	~4.1	~4.1	~4.1	8.008	8.296	—	—	—
		60	6.372	2.720	2.559	4.697	~4.1	~4.1	~4.1	8.116	8.312	—	—	—
4. TpA	Tp	10	5.935	1.511	2.126	4.568	4.002	3.640	3.618	7.313	1.834	—	—	—
		70	6.030	1.858	2.276	4.619	4.022	3.684	3.631	7.372	1.853	—	—	—
	pA	10	6.382	2.883	2.582	4.798	4.217	4.05	4.05	8.100	8.392	—	—	—
		70	6.439	2.849	2.605	4.738	4.222	4.06	4.06	8.207	8.358	—	—	—
5. ApT	Ap	10	6.347	2.810	2.832	4.840	4.310	3.889	3.831	8.106	8.284	—	—	—
		60	6.391	2.817	2.782	4.896	4.309	3.856	3.802	8.194	8.266	—	—	—
	pT	10	6.144	2.26	2.26	4.552	4.062	4.219	4.087	7.445	1.594	—	—	—
		60	6.202	2.31	2.31	4.544	4.103	4.178	4.092	7.505	1.713	—	—	—
6. ApTpA	Ap	10	6.273	2.72	2.72	4.888	4.303	3.846	3.809	8.037	8.166	—	—	—
		70	6.326	2.753	2.704	4.898	4.284	3.824	3.779	8.135	8.205	—	—	—
	pTp	10	5.999	1.819	2.182	4.780	4.168	4.089	4.045	7.297	1.619	—	—	—
		70	6.076	1.995	2.294	4.779	4.193	4.076	4.035	7.386	1.723	—	—	—
	pA	10	6.335	2.748	2.541	4.730	4.211	~4.1	~4.1	8.046	8.308	—	—	—
		70	6.377	2.746	2.560	4.700	4.213	4.09	4.08	8.140	8.313	—	—	—
7. Tpe^{4}TpT	Tp	10	6.109	2.339	2.517	4.738	4.151	3.810	3.769	7.675	1.849	—	—	—
		70	6.150	2.307	2.502	4.760	4.141	3.801	3.749	7.582	1.863	—	—	—
	pe^{4}Tp	10	6.222	2.351	2.609	4.834	4.35	4.176	4.094	7.897	1.932	4.327	4.304	1.371
		70	6.271	2.313	2.629	4.847	4.26	4.151	4.084	7.852	1.958	4.380	4.366	1.355
	pT	10	6.270	2.33	2.33	4.561	4.106	4.149	4.064	7.663	1.820	—	—	—
		70	6.257	2.331	2.357	4.541	4.125	4.125	4.072	7.636	1.861	—	—	—

[a]Temperature in 0°C. Spectra acquired at 300.1 MHz (**1**, **2**, **4**) and 500.1 MHz (**3**, **5**, **6**, **7**). Proton labelling (Fig. 1): 5M, 5-methyl; 7A and 7B: diastereotopic C7 methylene protons at low and high field, respectively (no absolute assignment to H_R and H_S can be made here); 8M: 8-methyl protons of the O4-ethyl group.

[b]δ is given to only three significant figures when the analysis was complicated by strong coupling or peak overlap. If the overlap is particularly severe, only the general location of the proton is indicated to two significant figures.

[c]A crossover in the H_R and H_S protons of **3** is suggested by the temperature dependence of their spectral band.

more prominent peaks are reported here as follows: m/z (relative intensity, [ionic species]), with the relative intensity normalized to the molecular ion $[M - H]^-$. *e^4TpA*: 582 (100, $[M - H]^-$); 674 (5.6, [M + glycerol − H]$^-$); 554 (9.7, $[M - CH_2CH_3]^-$); 153 (58, [O4-ethylthymine − H]$^-$); 349 (39, [3′-e^{4}dTMP − H]$^-$); 330 (20, [5′-dAMP − H]$^-$); 134 (39, [adenine − H]$^-$). *Ape^{4}T*: 582 (100, [M − H]$^-$); 674 (5.5, [M + glycerol − H]$^-$); 554 (12, $[M - CH_2CH_3]^-$); 153 (24, [O4-ethylthymine − H]$^-$); 349 (60, [5′-e^{4}dTMP − H]$^-$); 330 (14, [3′-dAMP − H]$^-$); 134 (94, [adenine − H]$^-$). *Ape^{4}TpA*: 895 (100, [M − H]$^-$); 867 (13, $[M - CH_2CH_3]^-$); 662 (78, [Ape^{4}Tp − H]$^-$ + [pe^{4}TpA − H]$^-$); 330 (217, [3′-dAMP − H]$^-$ + [5′-dAMP − H]$^-$); 153 (126, [O4-ethylthymine − H]$^-$); 134 (435, [adenine − H]$^-$). *TpA*: 554 (100, [M − H]$^-$); 646 (6.8, [M + glycerol − H]$^-$); 125 (49, [thymine − H]$^-$); 321 (22, [3′-dTMP − H]$^-$); 330 (22, [5′-dAMP − H]$^-$); 134 (19, [adenine − H]$^-$). *ApT*: 554 (100, [M − H]$^-$); 646 (12, [M + glycerol − H]$^-$); 330 (19, [3′-dAMP − H]$^-$); 321 (69, [5′-dTMP − H]$^-$); 134 (57, [adenine − H]$^-$); 125 (52, [thymine − H]$^-$). *ApTpA*: 867 (100, [M − H]$^-$); 634 (80, [ApTp − H]$^-$ + [pTpA − H]$^-$); 330 (146, [3′-dAMP − H]$^-$ + [5′-dAMP − H]$^-$); 134 (185, [adenine − H]$^-$); 125 (131, [thymine − H]$^-$). *Tpe^{4}TpT*: 877 (100, [M − H]$^-$); 653 (36, [Tpe^{4}Tp − H]$^-$ + [pe^{4}TpT − H]$^-$); 321 (80, [3′-dTMP − H]$^-$ + [5′-dTMP − H]$^-$); 573 (5, [Tpe^{4}T − H]$^-$ + [e^{4}TpT − H]$^-$); 153 (27, [O4-ethylthymine − H]$^-$); 125 (182, [thymine − H]$^-$).

^{1}H NMR experiments

^{1}H NMR spectra were obtained at 300.1 MHz (Bruker AM300) and 500.1 MHz (Bruker AM 500, McMaster University). The samples were contained in 5 mm (o.d.) tubes at concentrations in D_2O (3.0–12.0 mM) that preclude significant intermolecular interaction. The concentrations were obtained from UV absorbance measurements at 260 nm. The molar extinction coefficients per residue (ε_{260}) were taken from ref. 22 (**4**: 11 700; **5**: 11 400) or calculated by the method described therein (**6**: 12 500). The ε_{260} of **1** (9030), **2** (8800), **3** (10 800), and **7** (6570) were obtained by correcting the ε_{260} of the corresponding *unmodified* molecule for the Thy to e^{4}Thy substitution. For this correction, we used ε_{260} = 3200 for an e^{4}dT unit, which we estimated from the UV absorption profile of this monomer and the ε_{max} provided by Singer *et al.* (20). The solutions contained 0.01 M sodium phosphate buffer (pH = 6.9), EDTA (0.001 M) to chelate paramagnetic ions, 0.1 M NaCl to maintain a constant ionic strength, and, for an NMR reference, a trace of *tert*-butanol (assigned a chemical shift of 1.231 ppm on the DSS scale). The samples were lyophilized twice from 99.8% D_2O and taken up in 99.9% D_2O. One-dimensional (1D) FIDs were collected at 10°C intervals from 10 to 70°C in 16K of computer memory and processed with a Lorentz-to-Gaussian lineshape transform prior to zero-filling to 32K. The HDO resonance was suppressed by presaturation (Bruker PRESAT. AU microprogram). Conventional homonuclear decoupling, nOe, and two-dimensional (COSY-45°) (23) and relayed coherence transfer (24) experiments were carried out for spectral assignments. Spectra for the trimers at 10 and 70°C were also obtained at 500 MHz (Bruker AM500). Spectra were analyzed (NUMARIT) (25) at two temperatures (10 or 20°C, and 70°C), and computer-generated spectra confirmed the data.

Circular dichroism experiments

Circular dichroic spectra were obtained on a Jasco J-500A spectropolarimeter using a cell equipped for temperature control with a water bath. The molecules were studied at concentrations (0.46–3.64) × 10^{-4} M (calculated from UV absorbance measurements) in the phosphate buffer solution used for the NMR experiments.

Results and discussion

Nuclear magnetic resonance spectra, data, and assignments

Tables 1 and 2 provide ^{1}H chemical shift (δ) and coupling constant (J) data; partial 1D NMR spectra of the trimers **3** and **7** are shown in Fig. 1. The methods used to assign the spectral bands have been listed by Buchko *et al.* (14, 15); our assignments for **4** and **5** are in agreement with those of Kan *et al.* (26), and Cheng and Sarma (27). Data provided by Mellema *et al.* (28) were useful for assigning our trimer spectra. As noted earlier (14), the C7 methylene protons of the O4-ethyl group of an e^{4}dT fragment are diastereotopic because of the chiral centres in the sugar; the *pro-R* and *pro-S* atoms are designated H_R and H_S (Fig. 1). At the nucleoside level (e^{4}dT), H_R and H_S are isochronous (A_2X_3 pattern for the O4-ethyl group); this isochronicity indicates that the chiral sugar is too distant from the ethyl group to induce detectable differential shielding (Δ) in these protons (5, 14). A temperature-dependent Δ was evident (14) in the ABX_3 patterns observed for e^{4}TpT (**19**) and Tpe^{4}T (**20**) and attributed to intramolecular base stacking. A temperature-dependent Δ is also apparent in the H_R, H_S band of the all-pyrimidine trimer (**7**) (4.32 ppm, Fig. 1; Fig. 7 below) and in the corresponding bands of the adenine-containing dimers **1** and **2**. Note that no Δ is apparent in the spectrum of **3** at 27°C (intense quartet at 4.18 ppm; Fig. 1) though differential shielding is observed in **3** at other temperatures. We are not able to provide an absolute assignment of H_R and H_S, and so they are denoted in Tables 1 and 2 as 7A (less shielded) and 7B (more shielded).

Chemical shift temperature profiles: pyrimidine units

Temperature profiles of the chemical shifts are useful for assessing which conformations prevail in solution (29, 30). At our concentrations, intermolecular association should not be serious for the dimers and trimers, although Altona (29) has suggested that some association can occur at concentrations of ~10 mM, particularly when the oligomer contains bases such as methylated adenines, which have a strong tendency to stack. Even in the absence of complications from association effects, deriving conformations for single-stranded oligomers from δ data is complicated because the molecules exhibit considerable flexibility by interconverting between several stacked and unstacked forms that differ in their sugar pucker, in the orientation of the bases about the glycosyl link (χ), and so on. Thus, we shall restrain ourselves to more or less qualitative statements regarding the influence of the O4-alkylation on the positioning of the Thy base in any ordered structure that may form. The chemical shifts of the pyrimidine base should be determined mainly by intramolecular factors, particularly for our trimers in which the pyrimidine is sandwiched between the terminal bases. The profiles discussed below suggest that the e^{4}Thy and Thy derivatives form right-handed (RH) base-stacked structures at low temperature and that the O4-ethyl group has no large effect on the orientation of the pyrimidine base within the stack.

(a) *5-Methyl (5M) protons*

These profiles (Fig. 2) reveal association of the Pyr bases with an Ade on their 5′ side. At the monomer level, e^{4}dT and dT, O4-ethylation leads to a deshielding of 0.09 ppm for the 5M protons; neither monomer shows any temperature dependence. If Thy or e^{4}Thy is located at the 5′ end of an adenine-containing dimer (**1** and **4**), or in the central position of an all-pyrimidine trimer, **7** and **21** (TpTpT), the 5M protons experience only small upfield shifts ($\Delta\delta < 0.07$ ppm, where Δδ is the chemical shift of a proton in an oligomer relative to the corresponding monomer). In contrast, when the pyrimidine is located at the 3′ end of an adenine-containing dimer (**2** and **5**), or is flanked by two Ade bases as in the trimers **3** and **6**, the 5M protons are shifted upfield at 10°C relative to the monomers: ~0.35 ppm for e^{4}Thy of **2** and

TABLE 2. Coupling constants (J, Hz)[a,b]

Nucleotide		Temperature (°C)	1′2′	1′2″	2′3′	2″3′	3′4′	4′5′	4′5″	3′P	4′P	5′P	5″P
1. e^4TpA	e^4Tp	10	7.8	5.9	6.2	3.0	3.1	3.2	4.5	6.3	—	—	—
		70	7.4	6.1	6.7	3.4	3.4	3.6	4.9	6.7	—	—	—
	pA	10	6.6	6.6	6.5	4.4	4.1	2.6	2.6	—	2.6	—	—
		70	6.6	6.6	6.6	4.4	4.2	2.8	4.0	—	2.0	4.8	4.7
2. Ape^4T	Ap	10	6.4	6.4	5.4	5.4	3.8	2.8	3.7	5.8	—	—	—
		60	7.4	6.0	6.2	3.4	3.6	3.4	4.1	5.8	—	—	—
	pe^4T	10	5.7	6.7	7.3	5.6	5.4	2.4	3.1	—	2.8	3.6	3.1
		60	6.6	6.6	6.9	4.7	4.6	2.6	3.9	—	2.2	4.3	4.4
3. Ape^4TpA	Ap	10	6.7	6.7	4.6	4.6	3.0	2.8	3.5	5.5	—	—	—
		60	7.8	6.0	5.9	3.0	3.1	3.1	4.2	6.1	—	—	—
	pe^4Tp	10	7.9	6.0	6.5	3.1	3.3	[b]	[b]	6.5	[b]	[b]	[b]
		60	7.8	6.1	6.5	3.2	3.3	**2.5**	**3.9**	6.6	[b]	**4.6**	**3.9**
	pA	10	6.6	6.6	6.4	4.2	4.1	[b]	[b]	—	[b]	[b]	[b]
		60	6.6	6.6	6.5	4.2	4.1	[b]	[b]	—	[b]	[b]	[b]
4. TpA	Tp	10	8.5	5.8	6.0	2.2	2.4	3.7	4.1	6.0	—	—	—
		70	7.8	6.2	6.6	3.0	3.1	3.6	5.0	6.6	—	—	—
	pA	10	6.8	6.6	6.6	4.2	4.0	2.6	2.6	—	2.6	3.5	3.5
		70	6.6	6.7	6.6	4.3	4.0	3.5	3.5	—	2.1	4.8	4.8
5. ApT	Ap	10	6.7	6.1	5.9	4.5	3.5	2.8	3.7	5.2	—	—	—
		60	7.4	6.0	6.1	3.4	3.4	3.2	4.3	6.1	—	—	—
	pT	10	6.7	6.7	5.9	5.9	4.9	2.0	2.9	—	2.6	3.9	2.8
		60	6.9	6.9	5.5	5.5	4.9	2.9	3.8	—	2.2	4.4	4.3
6. ApTpA	Ap	10	6.7	6.7	4.3	4.3	2.7	3.0	3.4	6.1	—	—	—
		60	7.7	6.0	6.1	3.0	3.1	3.1	4.2	6.0	—	—	—
	pTp	10	8.6	6.0	6.2	3.0	3.2	2.0	3.0	6.4	3.3	4.1	2.8
		60	8.3	6.1	6.5	2.6	2.7	2.6	3.9	6.6	2.6	4.2	3.9
	pA	10	6.6	6.5	6.3	3.9	3.7	3.1	3.1	—	2.5	[b]	[b]
		60	6.6	6.6	6.5	4.1	3.9	3.4	3.4	—	1.9	4.8	4.8
7. Tpe^4TpT	Tp	10	6.6	6.3	6.4	3.9	3.8	3.3	4.3	6.4	—	—	—
		70	7.4	6.3	6.6	3.5	3.5	3.5	4.7	6.7	—	—	—
	pe^4Tp	10	6.6	6.4	6.5	4.3	4.2	2.2	3.1	6.3	2.7	4.4	3.2
		70	7.2	6.4	6.8	3.4	3.4	2.8	3.6	6.6	2.3	4.9	4.6
	pT	10	6.8	6.8	5.5	5.5	4.0	2.5	3.1	—	2.5	3.9	4.0
		70	7.0	6.7	6.6	3.7	4.1	2.9	4.4	—	2.9	4.8	4.8

[a]Temperature in 0°C. Spectra acquired at 300.1 MHz (**1**, **2**, **4**) and 500.1 MHz (**3**, **5**, **6**, **7**). Proton labelling (Fig. 1). Other couplings showing little variation: $^2J(2'2'')$: −14.1 ± 0.2 Hz; $^2J(5'5'')$: −12.6 ± 0.2 Hz (Np) or −11.6 ± 0.2 Hz (pNp, pN); for the O4-ethyl, $^2J(RS)$ = −10.6 Hz and $^3J(78)$ = 7.1 Hz; $^4J(56)$: 1.1–1.2 Hz (e^4Thy) and 1.2–1.3 Hz (Thy). Only the sums of connected coupling constants are significant if δ2′ ~ δ2″ or if δ5′ ~ δ5″. Bold-faced Js are from first-order analyses.

[b]J cannot be obtained due to spectral overlap.

3, and ~0.28 ppm for Thy of **5** and **6**. Note that the Δδ for e^4Thy on the one hand, and for Thy on the other, are identical to within 0.04 ppm at the dimer and trimer levels; this indicates that the Ade base on the 5′ side of the pyrimidine is the main source of the 5M shielding in the trimers, with little, if any, shielding derived from the Ade on the 3′ side. Increasing the temperature to 70°C leads to a downfield shift of 0.12–0.15 ppm for the 5M protons of **2**, **3**, **5**, and **6**, and their shifts approach those of the monomers. The trends in Fig. 2 suggest that e^4Thy and Thy of **2**, **3**, **5**, and **6** participate in a RH base-stacked structure. In such a conformation, the 5M group lies over the Ade in the 5′ direction and would experience the substantial ring current shielding of this purine. On the other hand, an Ade located on the 3′ side of the pyrimidine is not expected to cause significant shielding of the 5M group. Earlier NMR data were interpreted in terms of RH folding for TpA (26, 27) and for the pTpA segment of TpTpA (31). Also, the small Δδ for the NpA and large Δδ for the ApN are strong indications that the Thy and e^4Thy bases are *anti* about the *N*-glycosyl bond (26–31); both e^4dT and dT assume this conformation in the crystal state (5, 32). In the trimers **7** or **21**, the central base is flanked by pyrimidines that sustain a smaller ring current and are less inclined than purines to base stack; in these cases, Δδ for the central base is slight (<0.03 ppm) and shows little temperature dependence (Fig. 2). The somewhat larger Δδ(e^4Thy) for **2** and **3** relative to Δδ(Thy) for **5** and **6** could be due to an effect of O4-ethylation on the extent of base stacking, or on the orientation of the pyrimidine relative to the stacked Ade base in the 5′ direction; our data do not warrant further comment on this matter.

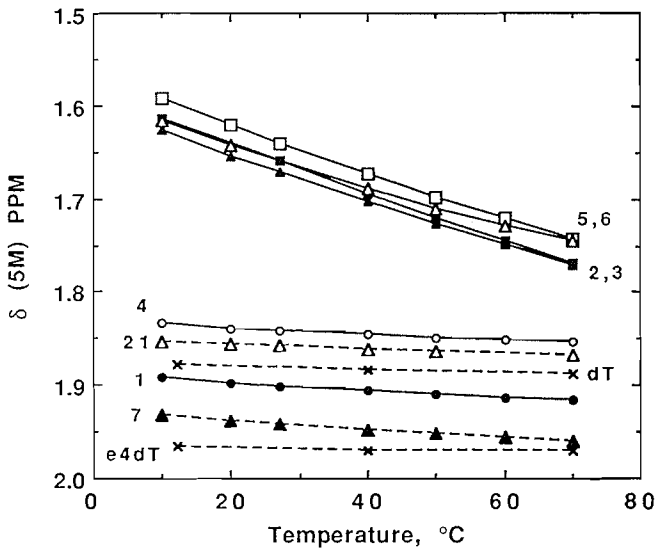


FIG. 2. Temperature profile of the chemical shifts (δ in ppm from DSS) of the 5-methyl (5M) protons of e⁴Thy (solid symbols) and Thy (open symbols). Connected by solid lines: e⁴TpA (**1**, ●), TpA (**4**, ○), Ape⁴T (**2**, ■), ApT (**5**, □), Ape⁴TpA (**3**, ▲), and ApTpA (**6**, △). Connected by dashed lines: pe⁴Tp unit of Tpe⁴TpT (**7**, ▲) and pTp unit of TpTpT (**21**, △); ×: monomers e⁴dT and dT.

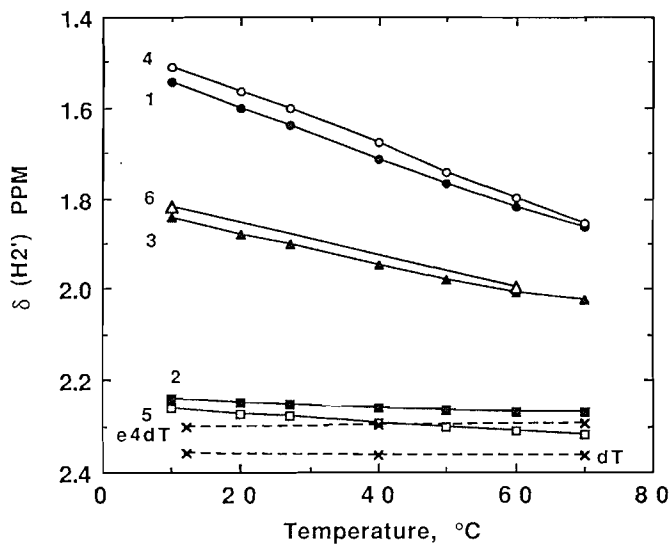


FIG. 3. Temperature profiles for the H2′ chemical shifts (δ in ppm from DSS) for the e⁴Thy (solid symbols) and Thy (open symbols) nucleotidyl units. Connected by solid lines: e⁴TpA (**1**, ●), TpA (**4**, ○), Ape⁴T (**2**, ■), ApT (**5**, □), Ape⁴TpA (**3**, ▲), and ApTpA (**6**, △). Connected by dashed lines: monomers e⁴dT and dT.

(b) *Sugar H2′ protons*

These profiles (Fig. 3) reveal association of Thy and e⁴Thy with an Ade base on the 3′ side. The δ(H2′) of dT and e⁴dT are identical to within 0.07 ppm and independent of temperature. In dimers **1** and **4**, an Ade base is on the 3′ side of the e⁴Tp and Tp units, and molecular models of RH structures show that H2′(Np) can enter the shielding regions above the plane of this purine. Consistent with RH helix formation are the large Δδ(H2′) at 10°C for **1** (0.76 ppm) and **4** (0.85 ppm), as are the large downfield shifts (~0.34 ppm) observed when the temperature is increased to 70°C. On the other hand, in the RH stack of dimers **2** and **5**, H2′ of the pe⁴T and pT moieties is distant from the Ade of the Ap unit; these protons are not expected to sense its ring current and indeed their δ behave like those of the monomers. The behaviour of H2′(Np) of **1** and **4** also contrasts with that of H2′(Np) of their all-pyrimidine analogs, e⁴TpT and TpT, which show a greatly reduced temperature dependence in the 10–70°C range (14), in line with smaller ring currents of the pT base.

Consistent with a RH structure for the pNpA segments of the trimers **3** and **6** is the upfield shift, Δδ, of H2′ for their pNp moieties at 10°C (pe⁴Tp: 0.46 ppm; pTp: 0.54 ppm) and the downfield shift (~0.20 ppm) when the temperature is elevated to 70°C. No such effects are observed for the pNp fragment of the all-pyrimidine trimers **7** (Table 1) and **21** (33). However, it is surprising that Δδ(H2′) for the trimers at 10°C is only 60% of that for the dimers **1** and **4**. Initially we thought that this reduction might be indicative of a competition between the terminal Ade bases for stacking with the central base. This view, however, is not consistent with the data in Fig. 2, which show that Δδ(5M) are similar at the dimer and trimer levels. Oltshoorn *et al.* (34) also observed an anomalous H2′ shift of similar magnitude for H2′ of the central pAp unit of ApApA relative to the Ap unit of ApA. They presented a rationale for this anomaly based on (a) the populations of the g^+ conformers of the C4′—C5′ (γ) bond and (b) the deshielding of H2′ by O5′ when γ is g^+. Unfortunately, the complexity of the NMR spectrum of **3** did not permit determination of accurate values of $J(4'5')$ and $J(4'5'')$ of the pe⁴Tp unit (Table 2) and so a comparison of the g^+ populations of the e⁴Thy units of **1** and **3** cannot be made. On the other hand, accurate values for these couplings could be determined for **4** and **6** (Table 2); these indicate that the g^+ population is larger for pTp (**6**) relative to Tp (**4**), and so the Oltshoorn interpretation may account for our δ(H2′) anomalies. But, whatever the cause of the reduced H2′(pNp) shielding at the trimer level, the important message is that it operates whether the pNp base is Thy or e⁴Thy, and so we have further support for the contention that O4-ethylation has no drastic effects on the conformation of our molecules.

(c) *Sugar H2″ protons*

In Fig. 4 we compare H2″ profiles for the Np fragments of e⁴TpA (**1**) and TpA (**4**) with those for H2″(Np) of the all-pyrimidine dimers e⁴TpT (**19**) and TpT (**22**) (data from refs. 14 and 35). Also compared are profiles of H2″(pNp) of Ape⁴TpA (**3**) and ApTpA (**6**) with the H2″(pNp) protons of the all-pyrimidine trimers Tpe⁴TpT (**7**) and TpTpT (**21**). For the all-pyrimidine molecules, we note that δH2″ is independent of temperature in the 10–70°C range. (Note also that H2″ for an e⁴Thy unit is shifted downfield (~0.1 ppm) relative to the corresponding Thy data; a comparable effect was observed at the monomer level (9), and so must be an intranucleoside effect.) In the Ade-containing molecules at 10°C, H2″ is shifted upfield, the shift being slightly larger (10–15%) for the Thy units (**4**: 0.43 ppm; **6**: 0.35 ppm) than for e⁴Thy units (**1**: 0.38 ppm; **3**: 0.31 ppm). At 70°C, the shielding effect is reduced by about 40%, and the δ(H2″) approach those of the all-pyrimidine molecules. Profiles are not given for the H2″(pN) of the isomers Ape⁴T (**2**) and TpT (**22**), but our data for these dimers indicate that an Ade base on the 5′ side has much smaller shielding effect (<0.08 ppm) on H2″(pN). Thus, the data in Fig. 4 confirm stacking of a e⁴Tp and Tp base with an Ade on its 3′ side.

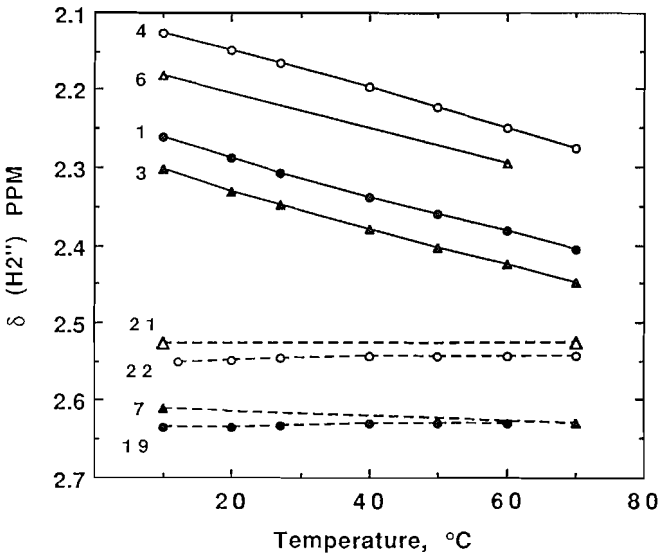


FIG. 4. Temperature profiles for the H2″ chemical shifts (δ in ppm from DSS) for the e⁴Thy (solid symbols) and Thy (open symbols) nucleotidyl units. Connected by solid lines: e⁴TpA (**1**, ●), TpA (**4**, ○), Ape⁴TpA (**3**, ▲), and ApTpA (**6**, △). Connected by dashed lines: e⁴Tp of e⁴TpT (**19**, ●), Tp of TpT (**22**, ○), pe⁴Tp of Tpe⁴TpT (**7**, ▲), and pTp of TpTpT (**21**, △). Data for **19** and **22** are from ref. 14.

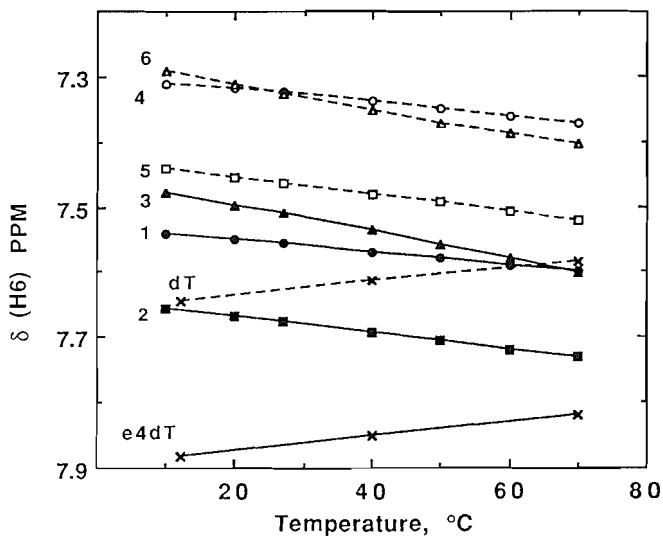


FIG. 5. Temperature profile for the H6 chemical shifts (δ in ppm from DSS) for e⁴Thy (solid lines and symbols) and Thy (dashed lines, open symbols). e⁴TpA (**1**, ●), TpA (**4**, ○), Ape⁴T (**2**, ■), ApT (**5**, □), Ape⁴TpA (**3**, ▲), and ApTpA (**6**, △). ×: monomers e⁴dT and dT.

(d) *Base H6 protons and sugar H1′*

The e⁴dT and dT data in Fig. 5 show that O4-ethylation effects a deshielding of 0.23 ppm in the H6 chemical shift, and in each monomer, H6 shifts upfield by ~0.06 ppm as the temperature is increased to 70°C. Relative to the corresponding monomer, the δ(H6) at 10°C are shifted upfield in the NpA (**1**: 0.34 ppm; **4**: 0.33 ppm), the ApN (**2**: 0.23 ppm; **5**: 0.20 ppm), and the ApNpA (**3**: 0.41 ppm; **6**: 0.36 ppm), and in each instance H6 shifts downfield as the temperature increases. It is remarkable that, if a correction of 0.23 ppm is made for the effect of O4-alkylation, the corresponding profiles for the e⁴Thy and Thy bases (NpA, etc.) are identical to within ~0.05 ppm over the entire temperature range. The H6 chemical shifts relative to the monomer reflect a variety of factors including sugar phosphorylation, ring current effects from the adjacent Ade bases, and solvent effects (14), and so are not easily interpreted in conformational terms. However, the correspondence of the two sets of profiles provides support for the contention that the stacking geometries are not drastically affected by O4-ethylation. This contention is further supported by the correspondence in the H1′ profiles (Fig. 6), which show similar upfield shifts for the e⁴Thy and Thy nucleotidyl fragments of the NpA, ApN, and ApNpA relative to the monomers. In each case, this shift is slightly larger for the Thy, but never by more than 0.05 ppm in the 10–70°C range.

(e) *O-Alkyl chemical shifts*

It would be useful to discuss the chemical shifts of the diastereotopic C7 methylene (H_R, H_S) and C8 methyl protons of the *O*-ethyl group in light of the conclusions based on the temperature profiles discussed above. In general, this group in the oligomers shows an ABX_3 pattern (Fig. 1 and Table 1), that is, the C7 protons are not identically shielded, but have a differential shielding (Δ) that depends on the molecule and the solution temperature. Figure 7 shows that at 10°C both C7 protons are shielded relative to e⁴dT and shift downfield as the temperature increases, presumably due to the stacking–destacking process (14). In the case of the all-pyrimidine trimer **7**, the upfield shifts at 10°C are small (0.07 ppm, 0.04 ppm), no doubt a reflection of the reduced ring currents and propensity for stacking of the flanking Thy bases; small upfield shifts (<0.07 ppm) were observed for the dimers **19** and **20** as well (14). The upfield shifts are also small in dimer **1** (0.07 ppm, 0.09 ppm), and so, bearing in mind the striking upfield shift experienced at 10°C by the H2′ of e⁴Tp (**1**) (0.76 ppm; Fig. 3), we conclude that the C7 protons do not enter the strongly shielding regions above an Ade base stacked on the 3′ side of the e⁴Thy base. However, larger shielding is experienced by the C7 protons in dimer **2** (0.16 ppm, 0.18 ppm) and in trimer **3** (0.22 ppm, 0.23 ppm). These trends, along with the 5M profiles (Fig. 2), point to a better overlap of the 5M-C5-C4-O4-C7 segment of the e⁴Thy base with an Ade on the 5′ side than with an Ade on the 3′ side, a result easily reconciled with a RH helical stack.

On the other hand, the C8 methyl protons show much less variation (Table 2; refs. 14, 35). In e⁴dT, δ(H8) = 1.36 ppm and is constant to within 0.001 ppm from 10 to 70°C; for all dimers and trimers in this temperature range, δ(H8) differs by ≤0.05 ppm from the monomer value. This constancy is consistent with a predominantly extended conformation for the *O*-ethyl group (anti-periplanar C4-O4-C7-C8), which projects the methyl group away from the stacked bases. Such an extended conformer was observed in the crystal structure of e⁴dT (5).

A result which surprised us is that, for all of the molecules whose profiles are given in Fig. 7, the two C7 protons are shifted upfield to quite similar extents over the temperature range examined. In the anti-periplanar orientation of the C4-O4-C7-C8 bond, the H_S proton flanks the e⁴Thy base on its 5′ face whereas H_R does so on the 3′ face (5),[4] and so we expected in molecule

[4]We denote as 5′ or 3′ the face that points to the 5′ or 3′ end of a strand in a normal B-DNA.

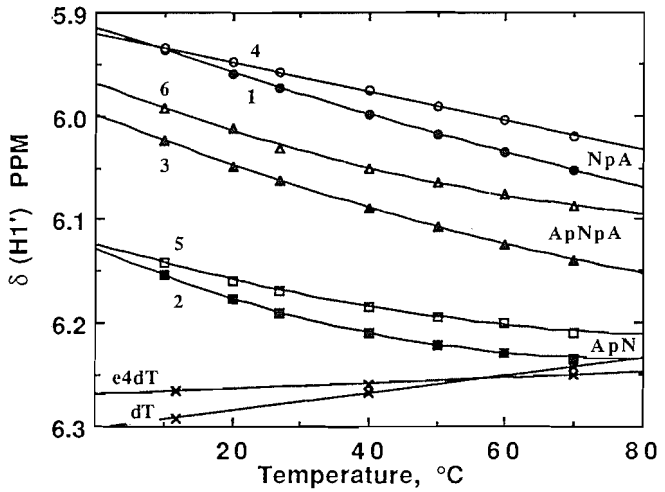


FIG. 6. Temperature profiles for the H1′ chemical shifts (δ in ppm from DSS) for e⁴Thy (solid symbols) and Thy (open symbols) nucleotidyl units. e⁴TpA (**1**, ●), TpA (**4**, ○), Ape⁴T (**2**, ■), ApT (**5**, □), Ape⁴TpA (**3**, ▲), and ApTpA (**6**, △). ×: monomers e⁴dT and dT.

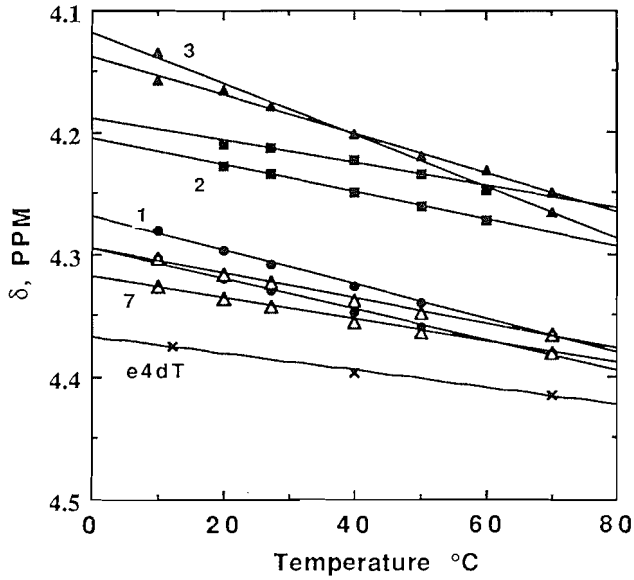


FIG. 7. Temperature profiles for the ^{1}H chemical shifts (δ ppm from DSS) of the H_R and H_S methylene protons of the O4-ethyl group. Solid symbols: e⁴TpA (**1**, ●), Ape⁴T (**2**, ■), and Ape⁴TpA (**3**, ▲). Open triangles: Tpe⁴TpT (**7**, △). An absolute assignment of the H_R and H_S protons could not be made; they are referred to as 7A (less shielded) and 7B (more shielded) in th bles. The protons are equivalent in the monomer e⁴dT (×).

2, for example, that one C7 proton, namely H_S, would be more influenced by the stacking of the Ap base, which occurs on the 5′ face. However, the similarity in upfield shifts indicates that the association of an Ade base on one particular face exerts comparable shielding on the two C7 protons. Theory (36) indicates that a number of factors (ring current, etc.) contribute to the magnetic fields near a purine base. This leads to a complex dependence of the magnetic shielding on position, and so we will not attempt to derive more precise information about stacking geometry of our flexible systems. Thus, at this point, we are uncertain whether Δ, in addition to the individual H_R and H_S chemical shifts, will prove, as we originally hoped (14), to be a generally useful quantity for monitoring local melting in e⁴Thy-containing oligomers.

(f) *Purine nucleotidyl shifts*

No temperature profiles are given for the Ap and pA units. However, the data in Table 1 show that the effect of replacing a Thy base by e⁴Thy on proton shifts of these fragments is never larger than 0.06 ppm. Overall, the purine δ data provide no evidence for any large conformational changes attributable to this replacement. Nor are they consistent with any significant purine–purine stacking, which could result from looping out of the central pyrimidine base.

Sugar conformation

Using plots of $\Sigma 1' (= J(1'2') + J(1'2''))$ to monitor changes in the sugar pucker, we noted that the modified Np fragment of e⁴TpT and of i⁴TpT (14, 15, 37) shows an unusual temperature dependence in the range 10–70°C, shifting from 2′-*endo* (*S*) towards 3′-*endo* (*N*) at low temperature. Figure 8 provides $\Sigma 1'$(Np) and $\Sigma 1'$(pNp) profiles that allow us to assess the influence of O4-ethylation in our Ade-containing dimers and trimers. The vertical axis of Fig. 8 also provides the %*S* (2′-*endo*) conformation calculated according to Rinkel and Altona (38); these populations are only approximate (±(10–15)%), and are included to provide the reader with an alternate view of the major trends defined by the primary data, $\Sigma 1'$. Considering first e⁴TpA and TpA at 70°C, we note that O4-ethylation reduces $\Sigma 1'$(Np) by about 0.6 Hz, a reduction comparable to that observed at the nucleoside level (14). Lowering the temperature to 10°C has little effect (<0.3 Hz) on these $\Sigma 1'$. Similarly, for the e⁴TpT and TpT at 70°C, we note a small decrease (~0.7 Hz) in $\Sigma 1'$(Np) due to the ethylation. However, $\Sigma 1'$(e⁴Tp) of e⁴TpT shows a large decrease (1.5 Hz) as the temperature is reduced to 10°C; this decrease is not seen for the Np unit of TpT. Since the $\Sigma 1'$ for the monomers (e⁴dT, dT) is constant in the 10–70°C range (14), the differences in behaviour of the $\Sigma 1'$ are reasonably related to intramolecular base stacking. In other words, the O4-ethylation of a Tp unit of a stacked TpN dimer causes a shift towards 3′-*endo* (*N*) if pN = pT, but not if pN = pA.

Another striking feature of the 10°C dimer data is the large difference (~3.0 Hz) between $\Sigma 1'$(Np) for e⁴TpT and TpA. This shows that the sugar of the 3′-thymidylyl unit can show a wide range of *N*,*S* populations depending on whether the base is alkylated or not, and on whether a Thy or Ade base occupies the pN unit.

The effects of O4-ethylation on the central pNp of the trimers parallel those for the dimer Np units discussed above. Thus, at 10°C, O4-ethylation effects a decrease of 1.5 Hz in $\Sigma 1'$(pNp) for the TpNpT pair whereas at 70°C these $\Sigma 1'$(pNp) differ by only 0.2 Hz. In contrast, the $\Sigma 1'$(pNp) for the ApNpA pair remain constant over the entire temperature range. We have not included in Fig. 8 any $\Sigma 1'$ data for dimer pN units. However, available data for these fragments of TpT, Tpe⁴T, ApT, and Ape⁴T (Table 2; refs. 14, 35) reveal that O4-ethylation in the 10–70°C interval leads to only a small (≤0.6 Hz) decrease in $\Sigma 1'$(pN), an effect comparable to that observed at the monomer level. In addition, our data (Table 2; refs. 14, 15, 35) show that O4-alkylation has little effect on the sugar pucker of either a thymidylyl or adenosyl residue adjacent to the modified segment.

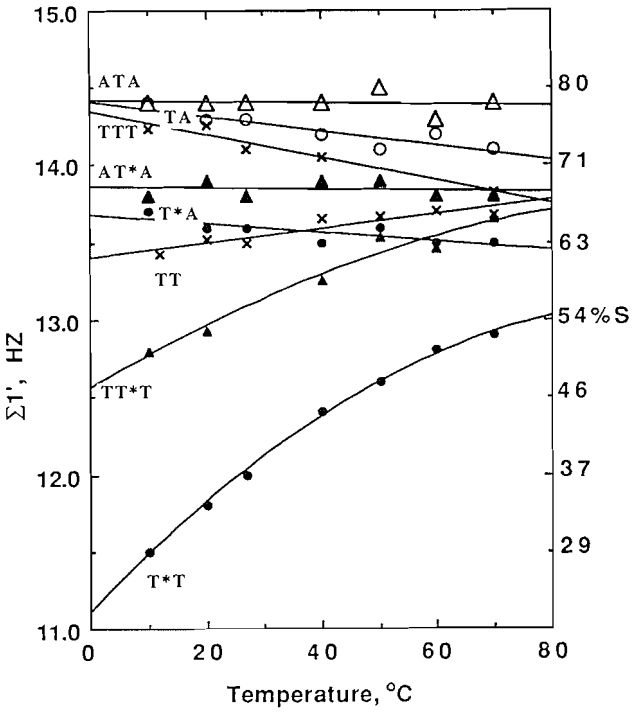


FIG. 8. Temperature profiles of $\Sigma 1' = J(1'2') + J(1'2'')$ in Hz for (a) the Np sugars of e^4TpA (**1**, ●), TpA (**4**, ○), e^4TpT (**19**, ●), and TpT (**22**, x), and (b) the pNp sugars of Ape^{4}TpA (**3**, ▲), ApTpA (**6**, △), Tpe^{4}TpT (**7**, ▲), and TpTpT (**21**, ×). The data are from Table 2, or from the width of the H1' band; data for **19**, **21**, and **22** are from refs. 14, 15, and 35. The right vertical axis provides the *%S* (2'-*endo*) calculated from $\Sigma 1'$ according to ref. 38. T* represents an e^4Thy unit.

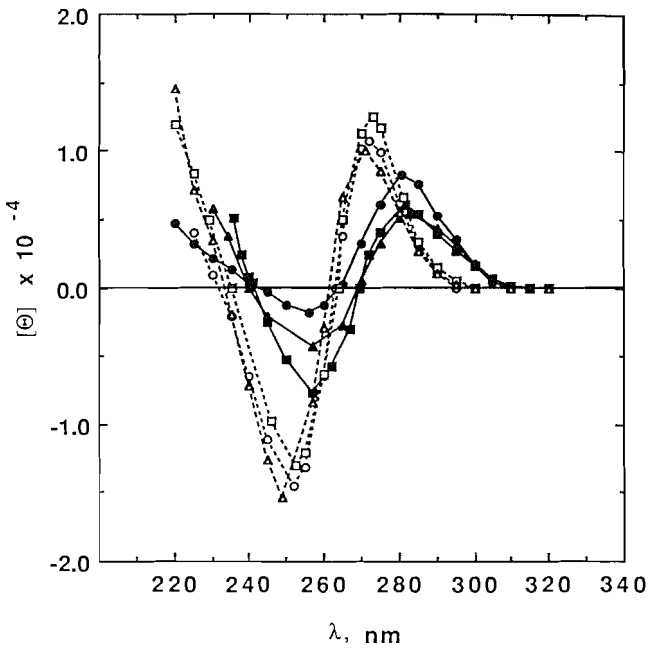


FIG. 9. Circular dichroism (CD) spectra (10–11°C). Solid symbols and lines: e^4TpA (**1**, ●), Ape^{4}T (**2**, ■), and Ape^{4}TpA (**3**, ▲). Open symbols and dashed lines: TpA (**4**, ○), ApT (**5**, □), and ApTpA (**6**, △). The vertical axis gives [θ] in deg M^{-1} cm^{-1}, the molar ellipticity per residue.

In summary, our $\Sigma 1'$ data reveal an interesting sequence-dependent effect of O4-alkylation on sugar pucker that is manifest at low temperature, presumably as a consequence of the formation of minihelical conformations. Whether this phenomenon will appear in molecules larger than our dimers and trimers remains to be seen. Patel and co-workers (12, 39) have, in their NMR work, shown that e^4Thy can be accommodated into RH double helical DNA, but their data were not interpreted in terms of a precise sugar conformation for the modified nucleotide.

Populations for the *gauche*$^+$ (g^+), *trans* (t), and *gauche*$^-$ (g^-) conformers of the C4'—C5' (γ) bond can be calculated from the $J(4'5')$ and $J(4'5'')$ data (Table 2) following Haasnoot *et al.* (40). At the low temperature, the Np fragments of the dimers and trimers show preferences for g^+ in the narrow ranges 59 ± 1% (Tp, e^4Tp) and 72 ± 1% (Ap), whereas the pN and pNp lie in the range 80 ± 5%; increasing the temperature to 60–70°C lowers the g^+ population by ~10%. The g^- conformer is always least populated. Calculations (according to Buchko *et al.* (15)) with the available $\Sigma' = J(5'P) + J(5''P)$ data in Table 2 show that the commonly observed *trans* conformer of the C5'—O5' bond predominates (80–90%) at the lower temperature, and shows a small destabilization (~10%) at the high temperature. Similar trends in β and γ are generally observed for dimers and trimers containing only the normal bases, and so it seems that, for our modified oligomers, O4-alkylation of thymine is not reflected in the conformation of these bonds.

Circular dichroism

The CD spectra of **1**–**6** at 10–11°C are shown in Fig. 9. For the unmodified molecules, positive and negative maxima are observed at 271–273 nm and 250–253 nm, respectively. The general features of the dimer spectra **4** and **5** are in good agreement with literature data (41, 42); we are not aware of published data for **6**. Upon O4-ethylation, the long-wavelength maxima are red-shifted by about 10 nm, in contrast with the small (~1 nm) red shift in λ_{max} observed in the UV absorption spectra (see above); a smaller (~5 nm) red shift occurs in the negative maxima. On the other hand, CD spectra reported by Weinfeld *et al.* (42) reveal little effect of *phosphate* ethylation on the positions of the positive and negative bands of a number of dimers. As the temperature is increased to 70°C, the magnitudes of the CD maxima of **1**–**6** decrease by 30–50%. Overall, these results are consistent with the formation of right-handed minihelices at low temperature, as indicated by the δ data discussed above.

Mass spectrometric experiments

FAB-MS data for molecules **1**–**7** provided in the experimental section, as well as those for the homopyrimidine dimers reported by Buchko *et al.* (14, 15, 35), demonstrate the utility of this technique for characterizing DNA oligomers containing an r^4T base as well as their unmodified analogs. In the negative ion mode, the molecular mass was easily confirmed for molecules **1**–**7** by the presence of a prominent quasimolecular ion peak $[M - H]^-$; in some instances a glycerol adduct peak, $[M + \text{glycerol} - H]^-$, was also observed and served as further confirmation. Also of diagnostic value was the presence of a variety of intense ion peaks assignable to fragments bearing an O4-ethylthyminyl unit.

Recently, Buchko (35) reported FAB-MS data (negative ion

mode) for the largest of our molecules, e^4TpApe^4TpA, a tetramer with two e^4Thy units. Its mass was confirmed by the intense $[M - H]^-$ ion at m/z 1228. The presence of three prominent fragment ion peaks provided some sequence information: the first (m/z 994) is assignable to the fragment $[M - A - H]^-$, resulting from the loss of the terminal 3′-adenosyl unit by cleavage of its O—C5′ bond; the second (m/z 662) corresponds to $[M - e^4TpA - H]^-$, which would result from the cleavage of either the O—C5′ bond of the pe^4Tp unit or the C3′—O bond of the pAp unit; the third (m/z 349) corresponds to [3′-e^4dTMP − H]$^-$ and confirms the presence of a terminal e^4Thy. Thus, our data are demonstrating that, though O4-alkyl groups are labile (1, 9), they are sufficiently stable to withstand the mild ionizing conditions employed in FAB-MS. Thus we are optimistic about the potential of this method for characterizing longer DNA oligomers containing r^4Thy bases.

Acknowledgements

We acknowledge the support of the following: NSERC (for operating grants to F.E.H. (A1549); The University of Manitoba (research funds for F.E.H. and K.L.S., and a Manitoba Fellowship for G.W.B.); Mr. W. Buchanon, K. Marat, and R. Sebastian for technical support; F. LaFortune, B. Singer, and J. B. Westmore (for discussions).

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