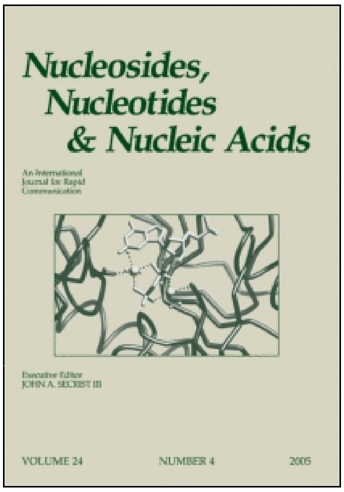

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Base-Modified 2'-Deoxyribonucleosides in Bended DNA Fragments: Differential Bending at 3'- and 5'- Junctions of H- and B-DNA

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BASE-MODIFIED 2'-DEOXYRIBONUCLEOSIDES IN BENDED DNA FRAGMENTS: DIFFERENTIAL BENDING AT 3'- AND 5'- JUNCTIONS OF H- AND B-DNA

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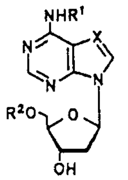
DNA-Sequences containing repeated $(dA)_n \cdot (dT)_n$ tracts in phase with B-DNA helical repeat show strongly decreased mobility in polyacrylamide gel electrophoresis. This phenomenon is the result of intrinsic, sequence-directed bending of DNA and was first observed by Marini et al. [1]. As the $d(GGCA_6C) \cdot d(GT_6GCC)$ duplex contains a tract of 6 dA residues it is bended. We have oligomerized duplexes containing 2'-deoxytubercidin (c^7A_d, 1a) instead of dA at various positions of the $d(A)_6$ tract with DNA-ligase. This replacement should allow the correlation of DNA-bending with stacking interactions of the nucleobase.

As automated solid-phase technique was chosen for the synthesis of oligonucleotides appropriately protected monomeric building blocks had to be prepared. Earlier, we have reported on phosphoramidites of 1a protected by a benzoyl group at the 6-amino function [2]. As this group is rather difficult to remove from oligonucleotides we have now used the phenoxyacetyl (Pac) residue as protecting group [3]. For this purpose 2'-deoxytubercidin as well as 2'-deoxyadenosine were peracylated with phenoxyacetic anhydride. Partial deprotection of the sugar moiety with Et_3N-pyridine-H_2O (1:1:3) yielded crystalline 1b and 2b, respectively [9].

In order to test the utility of the Pac group on compound 1a hydrolysis experiments were carried out in conc. ammonia at 40°C. The kinetics was followed UV-spectrophotometrically. Hydrolysis of the Pac-group of 1b is faster ($\tau/2$ = 6.6 min) compared to the benzoyl-protected compound ($Bz^6c^7A_d$, $\tau/2$ = 320 min) by a factor of 50 but still slower as that of 2b by a factor of 2 ($\tau/2$ = 2.9 min). As the Pac group was a promising alternative to the benzoyl group it was used for further experiments.



1a X = CH, $R^1 = R^2 = H$

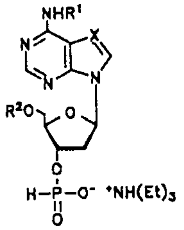
1b X = CH, $R^1 = COCH_2OPhe$, $R^2 = H$

1c X = CH, $R^1 = COCH_2OPhe$, $R^2 = (MeO)_2Tr$

2a X = N; $R^1 = R^2 = H$

2b X = N, $R^1 = COCH_2OPhe$, $R^2 = H$

2c X = N, $R^1 = COCH_2OPhe$, $R^2 = (MeO)_2Tr$



1d X = CH, $R^1 = COCH_2OPhe$, $R^2 = (MeO)_2Tr$

2d X = N, $R^1 = COCH_2OPhe$ $R^2 = (MeO)_2Tr$

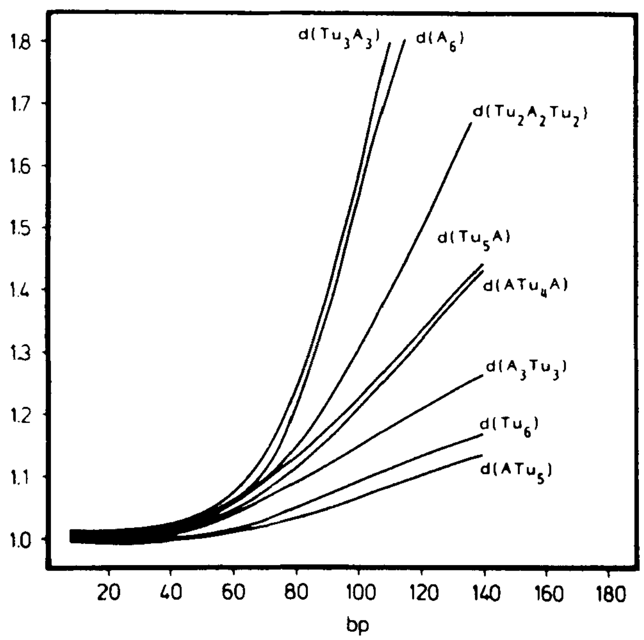


Figure 1

Table Relative DNA Bending at 25 and 5°C of Oligonucleotide Duplexes.

Compd.	Bending Element	R$_L$(5°C)	R$_L$(25°C)
3 · 5	d(ATu$_5$)	1.06	1.04
3 · 6	d(Tu$_6$)	1.09	1.06
3 · 7	d(A$_3$Tu$_3$)	1.15	1.15
3 · 8	d(ATu$_4$A)	1.21	1.11
3 · 9	d(Tu$_5$A)	1.22	1.12
3 · 10	d(Tu$_2$A$_2$Tu$_2$)	1.31	1.17
3 · 4	d(A$_6$)	1.55	1.28
3 · 11	d(Tu$_3$A$_3$)	1.57	1.31
12 · 12	-	1.00	1.00

The ratio R$_L$ of apparent length to real length indicates the extent of gel electrophoretic mobility and is an increasing function of bending extent.

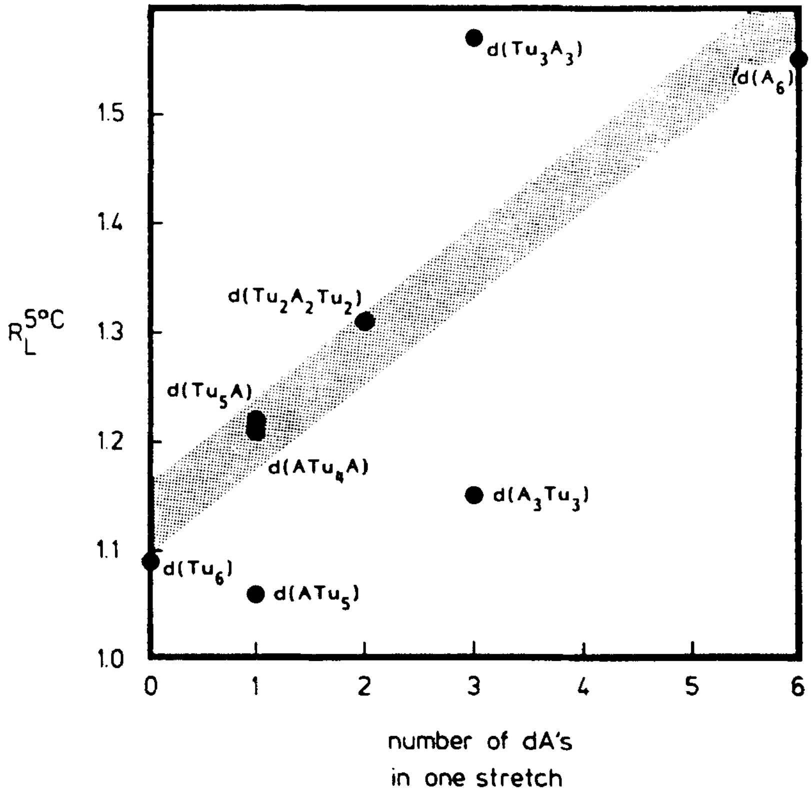


Figure 2

Next, the 5'-OH of 1b and 2b was protected with the 4,4'-dimethoxytrityl ($(MeO)_2Tr$) residue affording compounds 1c and 2c. Subsequent treatment with PCl_3/N-methylmorpholine/1,2,4-triazole afforded the H - phosphonates 1d and 2d [4]. They were isolated as the triethylammonium salts and characterized by elemental analysis as well as ^1H- and ^{31}P NMR spectroscopy. The phosphonate 1d together with those of regular 2'-deoxyribonucleosides were then employed in automated DNA synthesis on solid support. The protocol of detritylation, activation (adamantoylchloride), coupling, and capping followed a procedure recently described [5]. Oxidation with I_2/pyridine/H_2O/THF was carried out on the oligomeric level. The oligonucleotides were removed from the support according to [6]. The 5'-protected oligonucleotides were purified by reverse-phase HPLC, the detritylated compounds (acetic acid, 80%) again submitted to reverse-phase chromatography, and lyophilized. Enzymatic tandem hydrolysis by snake venom phosphodiesterase and alkaline phosphatase followed by HPLC analysis resulted in nucleoside pattern from which the content of the monomeric units was determined [7]. The following oligomers have been synthesized:

d(GGCAAAAAAC) 4

d(CCGTTTTTTG) 3

d(GGCATuTuTuTuTuC) 5

d(GGCTuTuTuTuTuTuC) 6

d(GGCAAATuTuTuC) 7

d(GGCATuTuTuTuAC) 8

d(GGCTuTuTuTuTuAC) 9

d(GGCTuTuAATuTuC) 10

d(GGCTuTuTuAAAC) 11

d(CGGGATCCCG) 12

The single-stranded oligonucleotides were 5'-phosphorylated with polynucleotide kinase, hybridized to its complementary strand (3) yielding duplexes with a two base pair overhang at the 5'-end of each strand, and then self-ligated to multimers with T4 DNA ligase. The DNA fragments were analyzed on nondenaturating 8 % polyacrylamide gels either at ambient temperature or at 5 °C [6]. Figure 1 shows a plot of the $R_L^{5°C}$ factor [8] vs the number of repeats of the oligonucleotides; for comparison of DNA curvature the R_L values of corresponding 100 bp multimers are listed in the Table using the BAM H1 linker (12·12) as non-bended standard.

In a preceeding paper [6] describing the replacement of 1-3 dA residues by dTu within a $d(A)_6$ tract we were able to show that bending depends strongly on the position of nucleobase replacement. Strong bending was observed if at least a $d(A)_4$ tract was present while a break of the $d(A)_6$ tract in the middle reduced curvature strongly. Now, from figure 2 it can be seen that there is a rough linear correlation between the R_L factor of 3 · 4, 3 · 8, 3 · 9, 3 · 10, and 3 · 6 and the number of dA's either in one stretch or totally. On the other hand, however, multimers of oligonucleotides with the bending elements $d(ATu_5)$, $d(A_3Tu_3)$, and $d(Tu_3A_3)$ exhibit extraordinary gel electrophoretic mobilities implying a special duplex structure. In particular, $d(Tu_3)$ sub-tract at the 3'-end of a $d(A^*)_6$ run (3 · 7) reduces DNA curvature excessively while a substitution of three dA residues by dTu's at the 5'-end ($d(Tu_3A_3)$) does not affect the curvature of a $d(A)_6$ - tract. The same tendency can be seen for the duplexes 3 · 5 ($d(ATu_5)$) and 3 · 9 ($d(Tu_5A)$).

This strongly confirms the experimental evidence of Koo et al. [8] that the 3'- and 5'- junctions between the polymorphic H-DNA $d(A)_6$ tract (heteronomous DNA) and adjacent B-DNA fragments are not equivalent. The helix axis bending angle at the 3'-end seems to be larger than at the 5'-end and is therefore more sensitive towards chemical modification.

Crothers and coworkers [8] have shown that significant DNA bending occurs only if the $d(A)_n$ run is at least 4 bp long. From our results it becomes apparent that 3'-extension of a $d(A)_3$ tract by a molecule have the same spatial requirements but altered stacking behaviour (dTu) does not enhance its curvature; on the other hand, additional dTu's at the 5'-end behave structurally similar as dA residues and form an apparently longer oligo(dA) tract resulting in almost identical bending of $d(Tu_3A_3)$ and $d(A)_6$ fragments.

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9. NMR spectra were measured in D_6DMSO on a AC-250 spectrometer (Bruker, West-Germany), δ-values are rel. to internal tetramethylsilane for ^{1}H and to external 85% phosphoric acid for ^{31}P. UV spectra and reaction kinetics were recorded on a Hitachi 150-20 spectrophotometer (Hitachi, Japan). For experimental details of solid-phase synthesis of oligonucleotides, their enzymatic phosphorylation and condensation as well as of electrophoresis see [6].

1b: Colorless crystals (62%); m.p. 163^0C. UV (MeOH): 277 (7.600), 292 nm (7.700). ^{1}H-NMR: 2.26 (m, H_α-2'); 2.58 (m, H_β2'); 3.57 (m, H-5'); 3.86 (m, H-4'); 4.38 (m, H-3'); 5.00 (m, Pac-H and HO-5'); 5.33 (d, J = 4.0, HO-3'); 6.69)t, J = 7.5, H-1'); 6.84 (d, J = 3.8, H-5); 6.95-7.36 (m, aromat. H); 7.71 (d, J = 3.8, H-6); 8.57 (s, H-2); 10.92 (s, NH). Anal. calc. for $C_{19}H_{20}N_4O_5$: C 59.37, H 5.24, N 14.58; found: C 59.57, H 5.34, N 14.62.

1c: Colorless foam (80%); UV (MeOH): 276 nm (9.500). ^{1}H-NMR: 2.33 (m, H_α-2'); 2.60 (m, H_β-2'); 3.71 (m, H-5'and CH_3O); 3.95 (m, H-4'); 4.39 (m, H-3'); 5.02 (s, Pac-H); 5.38 (d, J = 4.4, HO-3'); 6.67 (t, J = 7.2, H-1'); 6.82 (d, J = 3.8, H-5); 6.80-6.99 (m, aromat. H); 7.21-7.35 (m, aromat. H); 7.52 (d, J = 3.8, H-6); 8.54 (s, H-2); 10.94 (s, NH): Anal. calc. for $C_{40}H_{38}N_4O_7$: C 69.92, H 5.54, N 8.16; found: C 70.17, H 5.70, N 8.07.

1d: Amorphous solid (81%); UV (MeOH): 275 nm (11.000). ^{1}H-NMR: 1.16 (t, J = 7.2, $HNEt_3$); 2.29 (m, H_α-2'); 2.71 (, H_β-2'); 3.01 (q, J = 6.3, $HNEt_3$); 3.19 (m, H-5'); 3.71 (s, CH_3O); 4.13 (m, H-4'); 4.83 (m, H-3'); 5.02 (s, Pac-H); 5.48 (d, J = 5.9, H-P); 6.66 (t, J = 6.7, H-1'); 6.85 (d, J = 3.7, H-5); 6.81-7.38 (m, aromat. H); 7.50 (d, J = 3.8, H-6); 8.53 (s, H-2); 10.75 (s, NH^+); 10.99 (s, NH). ^{31}P-NMR: 1.24 (J(P-H) = 580, J(P-H-4') = 7.6). Anal. calc. for $C_{46}H_{54}N_5PO_9$: C 64.82, H 6.34, N 8.22; found: C 64.62, H 6.40, N 8.22.

2b: Colorless crystals (60%) m.p. 150 ^{0}C. UV (MeOH): 272 nm (17.500). ^{1}H-NMR: 2.37 (m, H_α-2'); 2.80 (m, H_β-2'); 3.58 (m, H-5'); 3.91 (m, H-4'); 4.47 (H-3'); 5.06 (m, Pac-H and HO-5'); 5.41 (d, HO-3'); 6.48 (t, J = 6.7, H-1'); 6.83-7.36 (m. aromat. H); 8.70 (s, H-2); 8.73 (s, H-8); 10.92 (s, NH). Anal. calc. for $C_{18}H_{19}N_5O_5$: C 56.10, H 4.97, N 18.17; found: C 56.27, H 4.88, N 18.09.

2c: Colorless foam (77%); UV (MeOH): 234 (22.300), 273 nm (16.700). ^{1}H-NMR: 2.39 (m, H_α-2'); 2.95 (m, H_β-2'); 3.18 (m, H-5'); 3.70 (s, CH_3O); 4.02 (m, H-4'); 4.53 (m, H-3'); 5.04 (s, Pac-H); 5.43 (d, J = 4.5, HO-3'); 6.48 (t, J = 6.4, H-1'); 6.75-7.41 (m, aromat. H); 8.60 (s, H-2); 8.62 (s, H-8); 10.96 (s, NH). Anal. calc. for $C_{39}H_{37}N_5O_7$: C 68.07, H 5.38, N 10.18; found: C 68.01, H 5.47, N 10.14.

2d: Colorless foam (80%); UV (MeOH): 236 (22.800), 273 nm (19.500). ^{1}H-NMR: 1.14 (t, J = 7.3, $HNEt_3$); 2.23 (m, H_α-2'); 2.77 (m, H_β-2'); 2.95 (q, J = 7.2, $HNEt_3$); 3.24 (m, H-5'); 3.71 (s, OCH_3); 4.22 (m, H-4'); 4.85 (m, H-3'); 5.04 (s, Pac-H); 5.49 (d, J = 584, H-P); 6.47 (t, J = 6.7, H-1'); 6.76-7.34 (m, aromat. H); 8.56 (s, H-2); 8.59 (s, H-8); 10.96 (s, NH). ^{31}P-NMR: 1.10 (J(P-H) = 576; J(P-H-4') = 8.3). Anal. calc. for $C_{45}H_{53}N_6PO_9$: C 63.37, H 6.62, N 9.85; found: C 63.21, H 6.44, N 9.87.