Pentafluorophenyl–Phenyl Interactions in Biphenyl-DNA

Alain Zahn, Christine Brotschi, and Christian J. Leumann*[a]

Abstract: We prepared and investigated oligonucleotide duplexes of the sequence d(GATGAC(**X**)$_n$GCTAG)·d(CTAGC(**Y**)$_n$GTCATC), in which **X** and **Y** designate biphenyl- (bph) and pentafluorobiphenyl- (^{5F}bph) *C*-nucleotides, respectively, and n varies from 0–4. These hydrophobic base substitutes are expected to adopt a zipperlike, interstrand stacking motif, in which not only bph/bph or ^{5F}bph/^{5F}bph homo pairs, but also ^{5F}bph/bph mixed pairs can be formed. By performing UV-melting curve analysis we found that incorporation of a single ^{5F}bph/^{5F}bph pair leads to a duplex that is essentially as stable as the unmodified duplex ($n=0$), and 2.4 K more stable than the duplex with the nonfluorinated bph/bph pair. The T_m of the mixed bph/^{5F}bph pair was in between the T_m values of the respective homo pairs. Additional, unnatural aromatic pairs increased the T_m by +3.0–4.4 K/couple, irrespective of the nature of the aromatic residue. A thermodynamic analysis using isothermal titration calorimetry (ITC) of a series of duplexes with $n=3$ revealed lower (less negative) duplex formation enthalpies (ΔH) in the ^{5F}bph/^{5F}bph case than in the bph/bph case, and confirmed the higher thermodynamic stability (ΔG) of the fluorinated duplex, suggesting it to be of entropic origin. Our data are compatible with a model in which the stacking of ^{5F}bph versus bph is dominated by dehydration of the aromatic units upon duplex formation. They do not support a model in which van der Waals dispersive forces (induced dipoles) or electrostatic (quadrupole) interactions play a dominant role.

Keywords: biphenyl derivatives · fluoroarenes · isothermal titration calorimetry · oligonucleotides · stacking interactions

Introduction

Hydrogen bonding and stacking interactions between nucleobases are the major noncovalent forces that stabilize the DNA and RNA double helices.[1,2] The relative contribution of each to stability has been a matter of debate since the discovery of the structure of the double helix. New insight into the importance of base stacking for DNA structure and function was obtained by Kool and co-workers, who investigated shape mimics of complementary natural bases that were devoid of the possibility to form hydrogen bonds.[3–6] Although such isosters destabilize DNA duplexes, they can code for each other with high precision in DNA polymerase-mediated replication.[7,8] This finding triggered an extensive search for hydrophobic, aromatic pairs that are orthogonal to the natural base-pairs in their recognition properties. Such pairs are of interest for the extension of the genetic alphabet.[9–14]

Some insight into the physicochemical nature of stacking interactions has come from studies of small-molecule interactions, mostly in apolar solvents.[15] The stacking of aromatic hydrocarbons on the corresponding fluorohydrocarbons has been especially well investigated. It is well known that benzene and hexafluorobenzene have quadrupole moments of similar magnitudes, but with inverted signs.[16] These two compounds, both liquids at room temperature, form a solid aggregate,[17] which is characterized by not only alternating π stacks, but also lateral alternation between hexafluorobenzene and benzene rings.[18] This stacking arrangement is believed to result from the minimization of electronic repulsion of the π systems and maximization of electrostatic and dispersion forces, and not from charge-transfer interactions.[19–21] Further support for this comes from studies of the rotational barriers in 1,8-diarylnaphthalenes[22] and from recent theoretical studies.[23,24] This knowledge is being applied in the field of crystal engineering.[25,26]

Stacking interactions in water are more complicated, as extensive energetic contributions from solvation/desolvation

[a] Dipl.-Chem. A. Zahn, Dipl.-Chem. C. Brotschi, Prof. C. J. Leumann
Department of Chemistry and Biochemistry, University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
Fax: (+41) 31-631-3422
E-mail: leumann@ioc.unibe.ch

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of the aromatic systems may interfere with the systems' intrinsic attractive and repulsive forces. From analysis of the interactions of cationic or anionic porphyrins with benzoic acid derivatives in water it is known that dispersive mechanisms are dominant over electrostatic or donor–acceptor interactions.[27] In the field of nucleic acids recognition there is evidence for advantageous di- or quadrupolar interactions of aromatic hydrocarbons with fluorohydrocarbons as base replacements. This has been shown for the phenyl/pentafluorophenyl case in DNA[28] and PNA,[29] as well as for isosteric fluorobenzene/benzimidazole base replacements in RNA, in which C–F···H hydrogen bonds were also invoked as stabilizing forces.[30] More recently, the effects of the number and position of fluorine atoms within fluorinated DNA bases on duplex stability were evaluated in the "dangling end" motif.[31] The results were consistent with the notion that dispersive induced dipole attractions between fluorohydrocarbons and natural base-pairs, rather than attractions between permanent dipoles or quadrupolar interactions, are relevant to stability. For a duplex containing an internal aromatic, hydrophobic pair, duplex stability is always higher for fluorinated base surrogates than for the parent nonfluorinated analogues. This reinforces the solvation argument.[32]

We recently proposed a novel, zipperlike, interstrand stacking recognition motif for oligodeoxynucleotide duplexes containing bipyridyl- (bpy) or biphenyl- (bph) *C*-nucleotide pairs, in which the terminal phenyl rings overlap (Figure 1).[33–35] The model is supported by the results of molecular dynamics simulations and by preliminary ^{1}H NMR data. This motif is well suited to a more detailed study of stacking interactions, as it can be extended to at least seven internal, consecutive, aromatic pairs in an oligonucleotide duplex without the break down of the double helix structure.[35] Here we describe the synthesis and incorporation into oligonucleotides of the pentafluorobiphenyl (^{5F}bph) *C*-nucleoside (Figure 1). The thermal stabilities (T_m) of duplexes containing one, three, and four consecutive ^{5F}bph/^{5F}bph, ^{5F}bph/bph, and bph/bph residues were measured by recording UV-melting curves, and for selected cases the thermodynamic data of duplex formation were recorded by performing isothermal titration calorimetry (ITC).

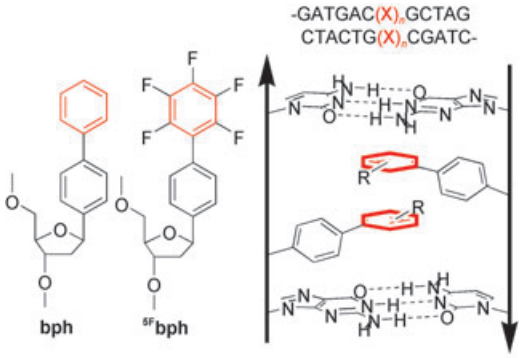
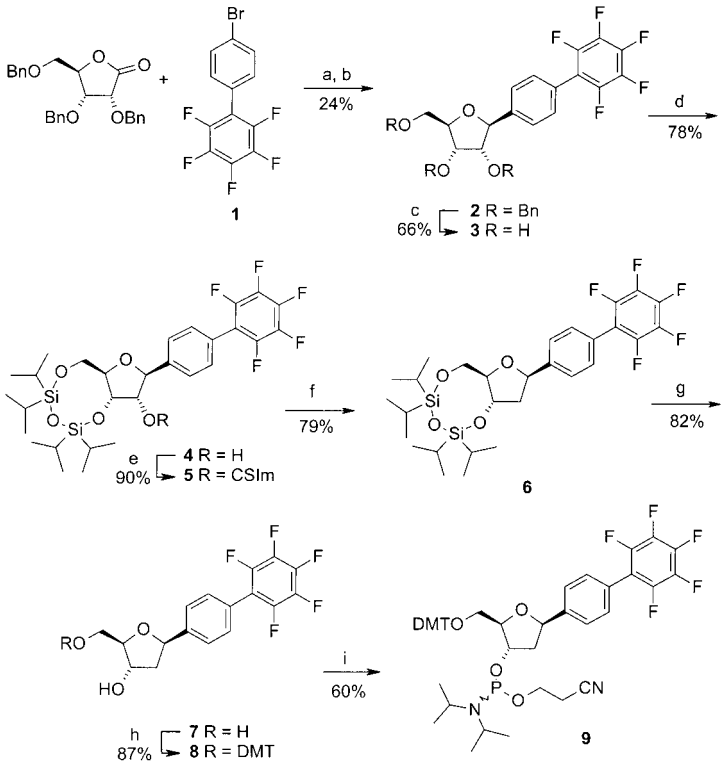


Figure 1. Chemical structures of the biaryl nucleoside analogues investigated, and the proposed interstrand stacking recognition motif of such aromatic units (**X**) in the center of an oligodeoxynucleotide duplex with the indicated sequence.

Results

Synthesis of phosphoramidites: The *C*-nucleoside **7** was synthesized from 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone[36] and 4′-bromo-2,3,4,5,6-pentafluorobiphenyl (**1**),[37] by using established pathways of *C*-nucleoside chemistry (see Scheme 1 and Supporting Information).[38,39] Lithiation of **1** with *n*BuLi, followed by addition to the lactone, resulted in the formation of the corresponding hemiacetal intermediates. These were reduced with Et_3SiH in the presence of a strong Lewis acid ($BF_3{\cdot}Et_2O$) to afford only one anomeric form of the *C*-nucleoside **2** (β-anomer, as determined by performing ^{1}H NMR nuclear Overhauser effect (NOE) experiments, see Supporting Information). Debenzylation with BBr_3 in CH_2Cl_2 (→**3**) followed by selective protection of the 5′- and 3′-hydroxyl groups with 1,3-dichloro-1,1,3,3-tetraisopropyldi-



Scheme 1. Reagents and conditions: a) **1** (1 equiv), *n*BuLi (1 equiv), THF, −78 °C, 1 h, then 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (1 equiv) in THF, −78 °C→RT, 16 h; b) Et_3SiH (5 equiv), $BF_3{\cdot}OEt_2$ (5 equiv), CH_2Cl_2, −78 °C→RT, 16 h; c) BBr_3 (3.5 equiv), CH_2Cl_2, −78 °C, 4 h; d) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.2 equiv), pyridine, RT, 16 h; e) 1,1′-thiocarbonyldiimidazole (1.2 equiv), CH_3CN, RT, 16 h; f) AIBN (0.2 equiv), tris(trimethylsilyl)silane (1.5 equiv), toluene, 85 °C, 30 min; g) $(HF)_3{\cdot}NEt_3$ (10 equiv), THF, RT, 16 h; h) 4,4′-dimethoxytrityl (DMTr) chloride (1.2 equiv), pyridine, RT, 4 h; i) $[(iPr_2N)(NCCH_2CH_2O)P]Cl$ (1.5 equiv), iPr_2NEt (3 equiv), THF, RT, 1.5 h.

siloxane ($TIPDSCl_2$) in pyridine afforded the TIPDS-protected nucleoside **4** in acceptable yield. The 2′-hydroxyl group was then removed by performing Barton–McCombie deoxygenation. For this, **4** was converted to the corresponding thiocarbimidazolide **5** by using 1,1′-thiocarbonyldiimidazole in CH_3CN, followed by treatment with tris(trimethylsilyl)silane (TTMSS) and catalytic amounts of α,α′-azoisobutyronitrile (AIBN) in toluene, to give the TIPDS-protected 2′-deoxynucleoside **6** in high yield. Finally, cleavage of the TIPDS protection group under mild conditions ($(HF)_3{\cdot}NEt_3$ in THF) afforded the 2′-deoxy-*C*-nucleoside **7** in good yield. The *C*-nucleoside **7** was subsequently converted into the corresponding 4,4′-dimethoxytrityl (DMTr)-protected phosphoramidite building block **9** by using standard conditions. Treatment of **7** with 4,4′-dimethoxytritylchloride (DMTrCl) in pyridine (→**8**), followed by addition of $[(iPr_2N)(NCCH_2CH_2O)P]Cl$ under slightly basic conditions (iPr_2NEt) in CH_2Cl_2, yielded phosphoramidite **9** in an overall yield of 5%.

Synthesis of oligonucleotides: The ^{5F}bph-modified oligonucleotides (for sequences see Table 1) were synthesized in the trityl-off mode on a 1 μmol scale by using standard phosphoramidite chemistry. The coupling time was extended to 10 min for the modified units. In the coupling step, tetrazole was replaced by 5-(ethylthio)-1*H*-tetrazole. Coupling yields for the modified units, as judged from detritylation, were within the same range as for nonmodified building blocks (>98%). After detachment from the solid support and deprotection under standard conditions (conc. NH_3, 55°C, 16 h), the oligomers were purified by conducting HPLC, and their structural integrity was verified by performing electrospray ionization mass spectrometry (ESI-MS, see Supporting Information). The bph-modified oligonucleotides were prepared as described previously.[35]

Table 1. T_m data for duplex melting obtained from UV-melting curves (260 nm).

	5′-d(GATGAC(**X**)$_n$GCTAG) 3′-d(CTACTG(**Y**)$_n$CGATC)		
n	**X**	**Y**	T_m [°C][a]
0	–	–	45.0
1	T	A	47.9
1	^{5F}bph	^{5F}bph	44.9
3	^{5F}bph	^{5F}bph	52.1
4	^{5F}bph	^{5F}bph	55.9
1	^{5F}bph	bph	44.5
3	^{5F}bph	bph	51.9
4	^{5F}bph	bph	55.1
1	bph	^{5F}bph	44.0
3	bph	^{5F}bph	51.5
4	bph	^{5F}bph	55.0
1	bph	bph	42.5
3	bph	bph	49.9
4	bph	bph	53.2

[a] $c=1.2$ μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0. Estimated error in $T_m=\pm0.5$°C.

Thermal denaturation studies: Complementary oligodeoxynucleotides were mixed in a 1:1 stoichiometry and subjected to UV-melting curve analysis (Table 1). This data reveals that insertion of one bph/bph pair decreases the T_m of the duplex relative to that of the unmodified duplex by 2.5 K, whereas insertion of a ^{5F}bph/^{5F}bph does not change the T_m. For additionally inserted aromatic couples, an average increase in T_m of 3.0–4.4 K/pair was observed. In all cases, duplexes containing only ^{5F}bph residues are thermally more stable (+2.2 to +2.7 K) than duplexes containing the same number of bph residues. The T_m of mixed bph/^{5F}bph pairs are always in between those of the homo pairs.

Isothermal titration calorimetry: To obtain a picture of the thermodynamic data of duplex formation we performed isothermal titration calorimetry experiments at 301.4 ± 0.1 K with duplexes containing three consecutive, modified, aromatic pairs. The heat versus time signals obtained for a representative case (bph/bph) are shown in Figure 2 (top). Normalization and integration of the heat capacity data gives the enthalpy of duplex formation, which was plotted against the molar ratio of the two single strands (Figure 2, bottom). The association constant K and the entropy change (ΔS) were obtained by applying a nonlinear fit and were used to calculate the free energy (ΔG). The corresponding data for the three cases with bph/bph, bph/^{5F}bph, and ^{5F}bph/^{5F}bph pairs are given in Table 2.

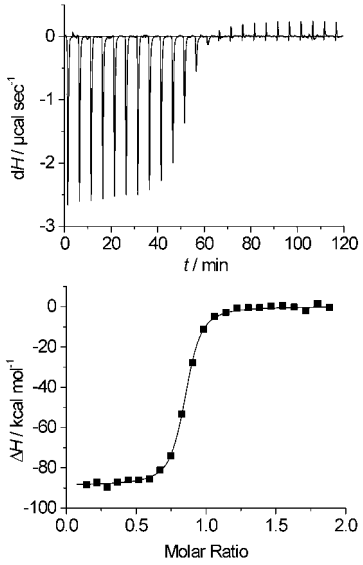


Figure 2. Isothermal titration calorimetry ($T=301.4$ K). Top: heat signal versus time for the titration of d(GATGAC(bph)$_3$GCTAG) ($c=10.0$ μM) with d(CTACTG(bph)$_3$CGATC) ($c=99.8$ μM) in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0; bottom: corresponding normalized heat signal versus molar strand ratio.

The free energy (ΔG) values are in accordance with the thermal melting (T_m) data. The duplex containing only ^{5F}bph residues is 0.9 kcal mol^{-1} more stable than the corresponding duplex containing only bph residues. The stability

Table 2. Thermodynamic data of duplex formation obtained by performing isothermal titration calorimetry (ITC). (For experimental conditions, see legend for Figure 2.)

5′d(GATGAC**XXX**GCTAG) 3′d(CTACTG**YYY**CGATC) **X**	**Y**	$-\Delta H_{ITC}$ [kcal mol^{-1}]	$-\Delta S_{ITC}$ [cal K^{-1} mol^{-1}]	$-\Delta G_{ITC}$[a] [kcal mol^{-1}]
^{5F}bph	^{5F}bph	84.4 ± 0.5	243 ± 2	11.1 ± 0.1
^{5F}bph	bph	82.4 ± 0.4	237 ± 2	11.0 ± 0.1
bph	bph	88.6 ± 0.4	260 ± 2	10.2 ± 0.1

[a] Calculated for $T = 301.4$ K.

of the mixed duplex lies within this range. Interestingly, the enthalpy of duplex formation (ΔH) is 4.2 kcal mol^{-1} more favorable in the bph duplex than in the ^{5F}bph duplex. Thus, the higher thermodynamic stability of the ^{5F}bph duplex is of entropic and not enthalpic origin.

CD spectroscopy: To follow structural changes as the number n of ^{5F}bph pairs increases, and to support the interstrand stacking model, we recorded CD spectra of the corresponding duplexes (Figure 3). The CD spectra are reminiscent of those for B-DNA. It is very likely that the gradual

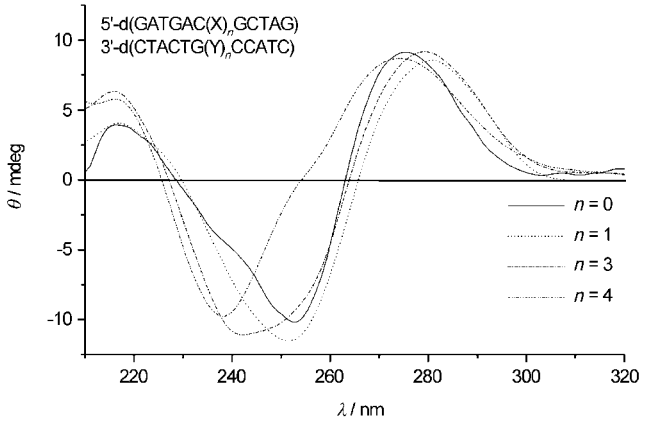


Figure 3. CD spectra of duplexes containing 0–4 ^{5F}bph base-pairs (**X** = **Y** = ^{5F}bph); $c = 3.6$ μM in NaH_2PO_4 (10 mM), NaCl (150 mM), pH 7.0, $T = 20$ °C.

red-shift of the negative maximum from 254 nm to 240 nm for increasing numbers of ^{5F}bph residues reflects the increasing contribution of the ^{5F}bph chromophore to the CD, and not a change in the general structural motif. Furthermore, the CD spectra are very similar to those of the bph duplexes.[35] The CD spectra are thus in accordance with a structure containing highly ordered, aromatic units.

Discussion and Conclusions

The thermal melting analysis of the duplexes containing one single ^{5F}bph/^{5F}bph or bph/bph pair shows an increased thermal stability of 2.5 K for the former duplex. Interestingly, this indicates that coplanarity of the two phenyl rings in the biarylic unit is not necessary for the stability of this motif. Indeed, both systems are expected to have nonplanar arrangements of the two phenyl rings, although the nonplanarity is much more pronounced in the case of the ^{5F}bph, as discernible from the rotation barrier in the gas phase (approximately 11 kcal mol^{-1} for ^{5F}bph and 2 kcal mol^{-1} for bph).[41] Thus, the higher stability achieved by the ^{5F}bph pair may be explained by superior stacking interactions with the neighboring natural base-pairs rather than by structural factors.

One striking observation from the T_m data is that a duplex containing a single ^{5F}bph or bph base-pair has a level of thermal stability similar to that of the corresponding unmodified duplex, and that the stability increases as each subsequent hydrophobic base-pair is added. This is not the case for duplexes containing edge-to-edge arrangements of hydrophobic fluorinated or nonfluorinated shape mimics of natural base-pairs,[32] and demonstrates the importance of interstrand as opposed to intrastrand stacking interactions for duplex stability.

Although a high resolution structure of this zipper motif has not yet been characterized, experimental evidence for it has been obtained from the results of molecular modeling,[33] ^{1}H NMR analysis, and from T_m and CD data in various sequence contexts.[35] Therefore, the data described in this paper are relevant to the discussion of the general contribution of energy to stacking in an aqueous environment, and in particular, to fluorinated versus nonfluorinated aromatic hydrocarbons. In this context, the zipper motif can be regarded as an alternative to the "dangling end" model that has been used by Kool and co-workers to describe base stacking interactions in DNA.[40] Moreover, it raises the possibility of studying multiple aromatic residues in the center of the helix that have stacking contacts to either aromatic residues in the counter strand, or to natural adjacent base-pairs on both faces.

Extension of n to three and four in the duplex sequence results in an alternating stack of aromatic residues from each strand. If quadrupolar effects were energy determining, then the duplex with the mixed (^{5F}bph/bph) stack should show the highest stability. Considering the calorimetric data of Table 2 it becomes clear that this is not the case. The stability of the bph/^{5F}bph system is similar to that of the ^{5F}bph/^{5F}bph stack and only 0.9 kcal mol^{-1} greater than the bph/bph stack. The thermal melting (T_m) data (Table 1) also reflect this behavior. The insertion of three and four aromatic pairs results in parallel T_m increases regardless of the nature of the aromatic unit. Therefore, the difference between T_m values for duplexes with four ^{5F}bph and four bph pairs, respectively, (2.7 K) is essentially the same as the difference for duplexes with only one ^{5F}bph or bph pair, respectively, (2.5 K). We can conclude that quadrupolar effects of pentafluorophenyl/phenyl interactions do not contribute measurably to the stacking energy in this case. This is in contrast to aryl/fluoroaryl systems in both the solid state, in which quadrupolar effects generally dictate structure,[17,18,26,42] and in organic solvents, in which electrostatic models also apply.[22b,43]

Small, but statistically significant, differences in the enthalpies of duplex formation were revealed by ITC analysis,

in which bph pairing was more exothermic than ^{5F}bph pairing (Table 2). Assuming that there is no significant difference in single-strand energies at the temperature used in the experiment, the higher thermodynamic stability of the ^{5F}bph duplex is of entropic origin. This fact does not support the dominance of attractive van der Waals or electrostatic interactions between the hydrophobic residues. It is, however, in accordance with an enhanced hydrophobic effect for ^{5F}bph, arising from desolvation. This is supported by the generally higher values for log *P* and the larger surface areas of the fluoroaryls.[32]

In conclusion, our data are compatible with a model in which the energies of the ^{5F}bph versus bph interactions are dominated by dehydration of the aromatic units during single-strand to duplex transition. They do not support a model in which van der Waals dispersive forces (induced dipoles) or electrostatic (quadrupole) interactions play a dominant role.

Experimental Section

Experimental details for the reactions and products of Scheme 1, for oligonucleotides containing ^{5F}bph residues, as well as for UV-melting curve analysis, CD spectroscopy, and isothermal titration calorimetry can be found in the Supporting Information.

Acknowledgements

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