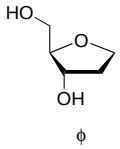
# A Stable DNA Duplex Containing a Non-Hydrogen-Bonding and Non-Shape-Complementary Base Couple: Interstrand Stacking as the Stability Determining Factor

Christine Brotschi, Adrian Häberli, and Christian J. Leumann*

Hydrogen-bonding and stacking interactions between nucleobases are the major components of the noncovalent forces that stabilize the DNA and RNA double helix.[1, 2] The relative contribution of each factor to the stability has been a matter of debate since the discovery of the structure of the double helix. Recent reports on stable duplex formation and selective DNA-polymerase-mediated replication of oligodeoxynucleotides that contain hydrophobic, non-hydrogen-bonding base pairs led to a new twist in this discussion,[3–14] and have triggered a re-evaluation of the importance of aromatic stacking interactions and shape selectivity in the base-pair formation.[15]

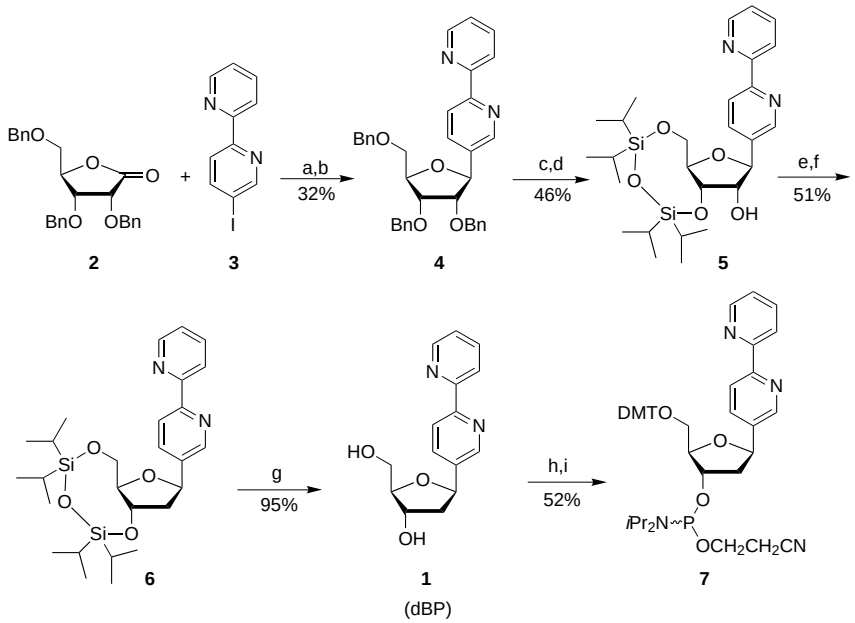
In a research program devoted to the introduction of specific metal-binding sites into DNA,[16–18] we became interested in the synthesis and incorporation into oligonucleotides of the nucleoside analogue **1** (dBP; see Scheme 1), which contains a 2,2′-bipyridyl (BP) unit as a base-surrogate.[19] Herein we report the efficient self-recognition of dBP within a DNA duplex, with high selectivity and stability in the absence of transition metal ions.

The synthesis of the corresponding deoxy-C-nucleoside **1** started from 2,3,5-tri-*O*-benzyl-D-ribonolactone **2**[20] and 4-iodo-2,2′-bipyridine **3**[21, 22](Scheme 1) and followed established routes in C-glycoside synthesis (Scheme 1):[23, 24] Lithiation of **3** followed by addition to lactone **2** resulted in the intermediate formation of the corresponding hemiacetals, which upon reduction with $Et_3SiH$ afforded selectively the $\beta$-isomeric C-glycoside **4** ($^1$H NMR NOE). After a series of typical transformations for the selective removal of the 2′-hydroxy group, C-glycoside **1** was obtained and subsequently converted into the corresponding phosphoramidite building block **7**.



ϕ

The incorporation of **7** into the duplex **8** (Table 1) was effected by standard protocols in automated DNA synthesis.[25] The thermal stability of the duplexes **8** (X, Y = dBP, dA, dG, dC, dT, and the abasic site analogue ϕ) was assessed by determination of the melting temperatures ($T_m$) by using UV spectroscopy (10 mM $NaH_2PO_4$, 150 mM NaCl, pH 7.0; Figure 1a; Table 1). Inspection of the $T_m$ data




Scheme 1. Reagents and conditions: a) **3** (1.0 equiv), *n*BuLi (1.0 equiv), THF, −78 °C, 2 h, then **2** (0.9 equiv), −78 °C → RT, 16 h; b) $Et_3SiH$ (5 equiv), $BF_3 \cdot OEt$ (5 equiv), $CH_2Cl_2$, −78 °C → RT, 16 h; c) $BBr_3$ (3.4 equiv), $CH_2Cl_2$, −78 °C, 4 h; d) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.0 equiv), pyridine, RT, 5 h; e) 1,1′-thiocarbonyl diimidazole (1.2 equiv), $CH_3CN$, RT, 18 h; f) $Bu_3SnH$ (1.6 equiv), AIBN (1.5 equiv), toluene, 80 °C, 2 h; g) $(HF)_3 \cdot NEt_3$ (1.0 equiv), THF, RT, 18 h; h) 4,4′-dimethoxytrityl (DMT) chloride (1.2 equiv), pyridine, RT, 16 h; i) $iPr_2NEt$ (3 equiv), $[(iPr_2N)(NCCH_2CH_2O)P]Cl$ (1.3 equiv), THF, RT, 3 h. AIBN = $\alpha,\alpha'$-azobisisobutyronitrile.

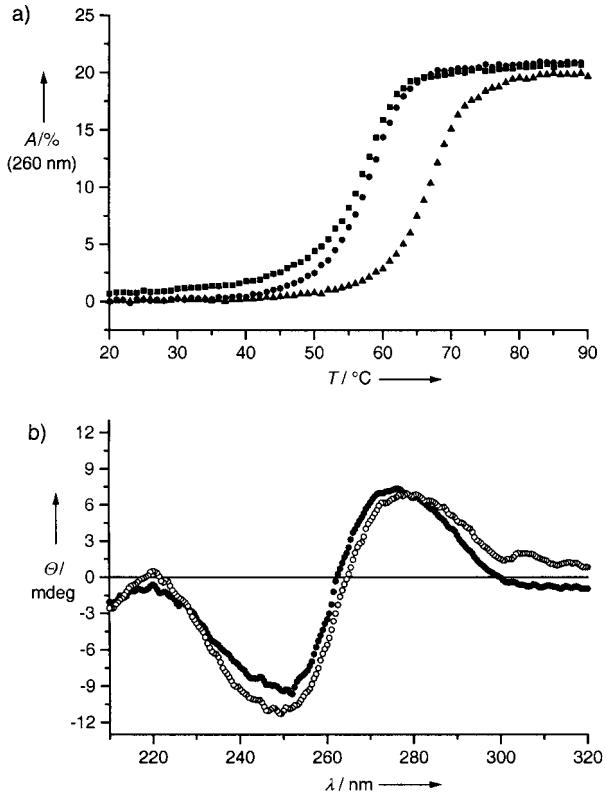



Figure 1. a) UV melting curves of the duplexes **8** with X = Y = dBP (▲), X = dBP, Y = ϕ (●), and X = dBP, Y = dT (■); $A$ = standardized absorption; experimental conditions are described in Table 1; b) CD spectra of duplexes **8** with X = Y = dBP (○) and with X = dA, Y = dT (●); $c$(**8**) = 2.4 μM, buffer described in Table 1).

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

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 1433-7851/01/4016-3012 $ 17.50+.50/0 

Table 1. Sequence of the 19-mer DNA duplex **8** and $T_m$ data for various combinations of X and Y, as extracted from UV melting curves (260 nm, 1.2 μM, in 10 mM $NaH_2PO_4$, 150 mM NaCl, pH 7.0).

| | | | 5′-GATGAC**X**GCTAGCTAGGAC<br>3′-CTACTG**Y**CGATCGATCCTG | | **8** | |
|---|---|---|---|---|---|---|
| Y | X BP | φ | A | G | T | C |
| BP | **67.4** | **58.5** | n.d.[a] | n.d.[a] | n.d.[a] | n.d.[a] |
| T | 57.9 | 50.1 | **64.0** | 60.0 | 58.5 | 55.7 |
| C | 59.7 | 52.0 | 55.6 | **66.7** | 56.1 | 54.6 |
| A | 61.4 | 52.5 | 57.0 | 59.2 | **64.1** | 55.8 |
| G | 62.0 | 54.8 | 59.2 | 58.6 | 60.1 | **66.0** |

[a] Not determined.

reveals that the unnatural base pair dBP:dBP in the given sequence ($T_m = 67.4\,^{\circ}C$) is more stable than a dA:dT base pair by 3.4 K, and of similar stability to a dG–dC base-pair (+0.7 K). The self-recognition of dBP is selective. When each of the natural bases is placed opposite a dBP residue in the duplex, $T_m$ decreases by 5.4–9.5 K, which corresponds to the differences in the free enthalpies of duplex formation ($\Delta\Delta G^{\circ}$) of 1.7–3.6 kcal mol$^{-1}$ (Table 2) relative to the dBP:dBP duplex. Thus the effect of a dBP:dN base-pair on duplex stability is similar to that resulting from a mismatched natural base pair. Importantly, if the dBP residue is located opposite to an abasic site analogue, a decrease in thermal stability of 8.9 K is observed.

Table 2. Two-state model dependent thermodynamic data of duplex formation obtained from curve fitting of the experimental UV melting curve.[27] Estimated error: ±5%.

| Y (X = BP) | $\Delta H$ [kcal mol$^{-1}$] | $\Delta S$ [cal K$^{-1}$ mol$^{-1}$] | $\Delta G^{25\,^{\circ}C}$ [kcal mol$^{-1}$] |
|---|---|---|---|
| A | −115 | −317 | −21.0 |
| T | −106 | −293 | −19.1 |
| G | −108 | −296 | −20.5 |
| C | −112 | −308 | −20.4 |
| BP | −113 | −303 | −22.7 |

To exclude traces of transition metal ions bound to dBP, which would thus interfere with the $T_m$ experiments, EDTA (0.1 M) was included in the buffer in control experiments. No differences in $T_m$ were observed in these cases. Given the low p$K_a$ of the bipyridyl unit (4.3) and the unfavorable geometry of the ring nitrogen atoms for interresidue hydrogen bonding, we also exclude the formation of a protonated dBPH$^+$:dBP base pair at pH 7.0.

A structural model of a DNA duplex with two opposing dBP units clearly shows that the two bases can only be accomodated in the double helix without major backbone distorsion if the distal pyridyl rings are stacked. Indeed, a molecular dynamics simulation of the duplex **8** (X = Y = dBP) with the two bipyridyl bases adjacent to each other in the starting conformation already lead to a partially stacked geometry (Figure 2, top) during initial energy minimization. This arrangement remains stable over the whole period of the 200-ps trajectory of the simulation. As can be seen in Figure 2 (bottom), the stacking of the two dBP residues results in an increase in the distance between the two adjacent dG:dC

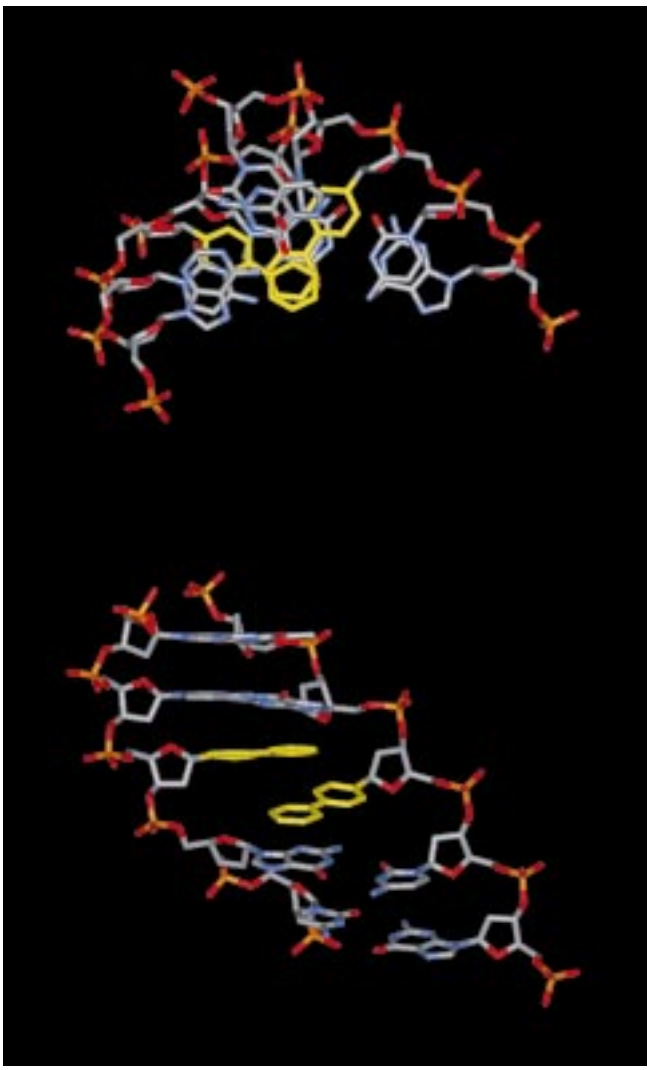


Figure 2. Structure of a section of duplex **8** (X = Y = dBP), which contains the two dBP residues (bases in yellow) as well as the adjacent natural base pairs (two on either side). The structure corresponds to the average of the last 20 ps of an unrestrained molecular dynamics simulation at 300 K on a 200-ps trajectory, using the amber force field incorporated in InsightII/Discover3 V98.0. Instead of explicit solvent molecules, a distance-dependent permittivity of $\varepsilon = 4r$ ($r$ = distance) was used as a screening function. Top: view along the helical axis, showing the almost ideal overlap of the distal pyridine rings of dBP; bottom: view from the major groove side which shows the stacked dBP base couple within the base stack of the DNA.

base-pairs, which is permitted by a rearrangement of the local backbone conformation. The overall B conformation of the double helix remains mostly intact, a fact that is fully supported by the circular dichroism (CD) spectra of the duplexes **8** with X:Y = dA:dT and dBP:dBP (Figure 1b).

The combined experimental and modeling results for the non-hydrogen-bonding dBP-containing oligonucleotide duplex **8** led us to conclude that interstrand stacking of two opposite non-hydrogen-bonded and non-shape-complementary aromatic residues is an integral part of the duplex stabilization. A review of the properties of the recently described duplexes with various hydrophobic base analogues by Romesberg, Schultz and co-workers,[3, 5, 6] and by Kool and co-workers[8, 10, 14] is in perfect agreement with the findings

reported herein. The shape-complementary difluorotoluene:4-methylindole and related base couples,[8, 14] as well as analogous arrangements of substituted phenyl groups with geometries that do not allow interstrand stacking destabilize a DNA duplex.[5] On the other hand, extended aromatic units, for example, the isocarbostyryls (1-hydroxyisoquinolines)[3, 6] or pyrene,[10] which have the potential (at least partially) to stack if they occupy opposite positions in a duplex, show equal or higher stability than natural base pairs. The importance of interstrand stacking to duplex stability has also been pointed out recently in the case of the DNA and RNA analogues p-RNA and homo-DNA.[26] A high-resolution structural analysis of a duplex that contains a dBP base pair is currently in progress.

The reported experimental results provide the basis for the simple rationale that non-hydrogen-bonding non-shape-complementary base-arrangement in a DNA duplex can attain similar or even enhanced stabilities relative to a Watson–Crick base pair only if interstrand base stacking of such opposing units is possible. This rationale may prove useful in further base design for applications in molecular biology or materials science.

Received: March 20, 2001 [Z16817]
Publication delayed at authors' request

[1] C. R. Cantor, P. R. Schimmel in *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (Ed.: P. C. Vapnek), Freeman, New York, **1980**, pp. 311–341.
[2] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, New York, **1984**.
[3] M. Berger, A. K. Ogawa, D. L. McMinn, Y. Wu, P. G. Schultz, F. E. Romesberg, *Angew. Chem.* **2000**, *112*, 3069–3071; *Angew. Chem. Int. Ed.* **2000**, *39*, 2940–2942.
[4] D. L. McMinn, A. K. Ogawa, Y. Wu, J. Liu, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **1999**, *121*, 11585–11586.
[5] A. K. Ogawa, Y. Q. Wu, D. L. McMinn, J. Liu, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2000**, *122*, 3274–3287.
[6] Y. Wu, A. K. Ogawa, M. Berger, D. L. McMinn, R. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2000**, *122*, 7621–7632.
[7] K. M. Guckian, T. R. Krugh, E. T. Kool, *J. Am. Chem. Soc.* **2000**, *122*, 6841–6847.
[8] K. M. Guckian, J. C. Morales, E. T. Kool, *J. Org. Chem.* **1998**, *63*, 9652–9656.
[9] T. J. Matray, E. T. Kool, *Nature* **1999**, *399*, 704–708.
[10] T. J. Matray, E. T. Kool, *J. Am. Chem. Soc.* **1998**, *120*, 6191–6192.
[11] J. C. Morales, E. T. Kool, *Nat. Struct. Biol.* **1998**, *5*, 950–954.
[12] J. C. Morales, E. T. Kool, *J. Am. Chem. Soc.* **2000**, *122*, 1001–1007.
[13] S. Moran, R. X.-F. Ren, E. T. Kool, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10506–10511.
[14] B. A. Schweitzer, E. T. Kool, *J. Am. Chem. Soc.* **1995**, *117*, 1863–1872.
[15] E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem.* **2000**, *112*, 1046–1068; *Angew. Chem. Int. Ed.* **2000**, *39*, 990–1009.
[16] E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg, P. G. Schultz, *J. Am. Chem. Soc.* **2000**, *122*, 10714–10715.
[17] K. Tanaka, M. Shionoya, *J. Org. Chem.* **1999**, *64*, 5002–5003.
[18] K. Wiederholt, L. W. McLaughlin, *Nucleic Acids Res.* **1999**, *27*, 2487–2493.
[19] During the revision of this manuscript, a paper appeared in which two homo-dBP units that contained a methylene group between the bipyridyl unit and the C1′ of the deoxyribose unit were introduced into a DNA duplex at opposite positions. A differential increase in duplex stability in the presence of $Cu^{2+}$ ions was observed (H. Weizman, Y. Tor, *J. Am. Chem. Soc.* **2001**, *123*, 3375–3376).
[20] W. Timpe, K. Dax, N. Wolf, H. Weidmann, *Carbohydr. Res.* **1975**, *39*, 53–60.
[21] K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J. Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182–8183.
[22] B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1997**, *119*, 1676–1681.
[23] G. A. Kraus, M. T. Molina, *J. Org. Chem.* **1988**, *53*, 752–753.
[24] K. Krohn, H. Heins, K. Wielckens, *J. Med. Chem.* **1992**, *35*, 511–517.
[25] The corresponding oligonucleotides of duplex **8** with X = Y = dBP were synthesized and purified as described (I. Pompizi, A. Häberli, C. J. Leumann, *Nucleic Acids Res.* **2000**, *28*, 2702–2708). Building block **7** was incorporated with coupling yields >98%. Analysis by using positive-ion ESI-MS revealed experimental masses ($[M+H]^+$ found 5882.4 and 5784.6) that are in agreement with theory ($[M+H]^+$ calcd 5882.8 and 5784.8, respectively), thus confirming the structural integrity of the oligonucleotides.
[26] R. Micura, R. Kudick, S. Pitsch, A. Eschenmoser, *Angew. Chem.* **1999**, *111*, 715–718; *Angew. Chem. Int. Ed.* **1999**, *38*, 680–683, and references therein.
[27] C. Epple, C. J. Leumann, *Chem. Biol.* **1998**, *5*, 209–216.

## Efficient Photooxidative Degradation of Organic Compounds in the Presence of Iron Tetrasulfophthalocyanine under Visible Light Irradiation**

Xia Tao, Wanhong Ma, Tianyong Zhang, and Jincai Zhao*

The degradation of organic pollutants by photocatalysis[1] and (photo-)Fenton reactions[2] has been described extensively. Meunier and co-workers reported an efficient oxidative degradation of trichlorophenol (TCP) in the presence of iron tetrasulfophthalocyanine ([Fe(PcS)]) and $H_2O_2$ in the dark.[3] In this process the metal–peroxo species [Fe(OOH)(PcS)] is involved as an active oxygen intermediate. A merit of this system is that [Fe(PcS)] is a readily available biomimetic catalyst that can be fixed onto amberlite and therefore does not enter into the environment and cause additional pollution. It should, however, be noted that the solvent employed contains a large amount of acetonitrile. If water is used as the sole solvent, the conversion rate of TCP is greatly reduced.[4]

Here we report that when visible light is introduced to an aqueous system containing test compounds, [Fe(PcS)], and

[*] Prof. J. Zhao,[+] X. Tao, W. Ma, T. Zhang
Laboratory of Photochemistry, Center for Molecular Sciences
Institute of Chemistry, The Chinese Academy of Sciences
Beijing 100080 (China)
Fax: (+86) 10-6487-9375
E-mail: jczhao@ipc.ac.cn
[+] Current address:
Institute of Photographic Chemistry
Chinese Academy of Sciences
Beijing 100101 (China)

[**] This work was supported financially by NSFC (nos. 29877026, 4001161947, 29725715, and 20077027) and CAS. The authors thank Dr. Huiyong Zhu, The University of Queensland, Australia, for his useful discussions.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 1433-7851/01/4016-3014 $ 17.50+.50/0 