Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 3434–3439

Helical self-assembled chromophore clusters based on DNA-like architecture

Elke Mayer-Enthart, Clemens Wagner, Janez Barbaric and Hans-Achim Wagenknecht*

University of Regensburg, Institute for Organic Chemistry, Universitätstr. 31, D-93053 Regensburg, Germany

Received 31 May 2006; revised 18 December 2006; accepted 18 December 2006
Available online 3 February 2007

Abstract—DNA duplexes were functionalized covalently by clusters of five adjacent chromophores consisting of 5-(pyren-1-yl)-2′-deoxyuridine (Py-U) and 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine (Pz-U). The chromophores form a regular helical π-array along the major groove of duplex DNA when the 5-fold chromophore-modified oligonucleotides are hybridized with an unmodified counter strand. As a result, these chromophores interact significantly and their fluorescence and absorption properties can be modulated by the sequence within the π-array. The 5-fold Py-U stack shows a strongly enhanced emission. The presence of intervening Pz-U groups quenches the fluorescence of the Py-U chromophores. Such modulation of the optical properties within a chromophore stack is potentially useful for optical nanodevices and as nucleic acid sensors for molecular diagnostics. The duplex architecture of DNA is suitable to provide the supramolecular structural scaffold for a directed arrangement of chromophores.
© 2007 Published by Elsevier Ltd.

1. Introduction

The central problem for advances in bio- and nanotechnology is the miniaturization, which drives the search for application of molecular wires and devices on the nanometer scale.[1–7] Conceptually, such organic nanomaterials can be realized by using the so-called bottom–up approach in which the system is composed of small synthetic building blocks with recognition, structuring, and most importantly, self-assembling properties, preferably by hydrogen-bonding.[8–11] A clear structural scaffold is required for such molecular π-systems since the properties depend on the relative orientation and the resulting photophysical interactions of each of the molecular components with the others. For this approach, oligonucleotides play an especially important role[9–15] since their unique properties provide a suitable basis in order to use duplex DNA-like architectures as a structural scaffold.

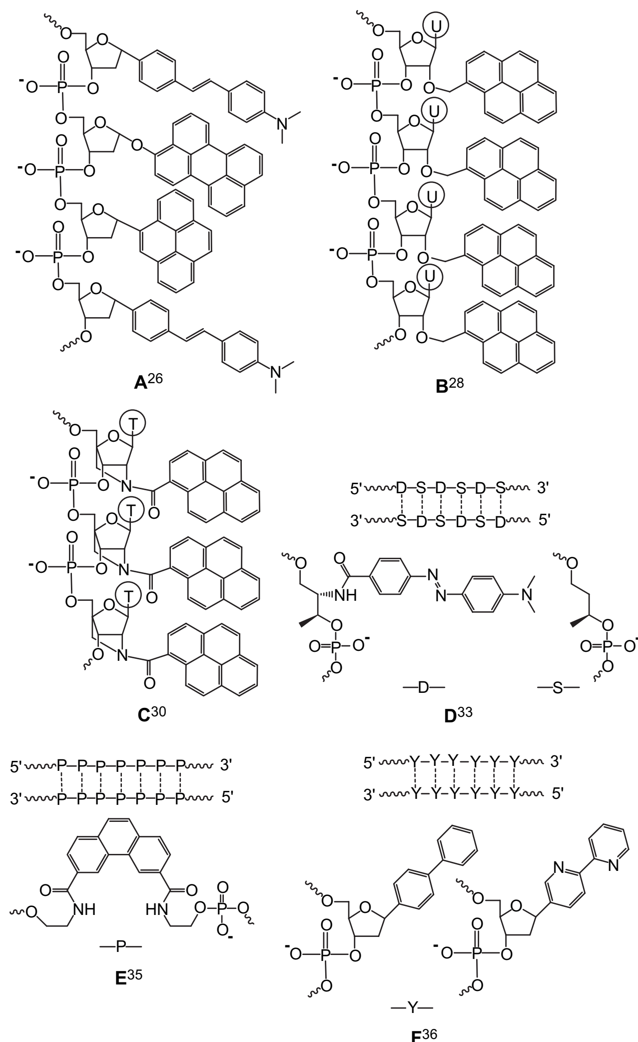
1. Self-assembly: two oligonucleotides spontaneously organize themselves into a duplex structure as encoded by the DNA base sequence.
2. Regular helical structure as a predictable topology: in B-DNA, the base-pair distance along the helical axis is 3.4 Å providing the ideal basis for photophysical interactions.
3. Synthetic accessibility: automated oligonucleotide chemistry makes DNA available in any desired base sequence and also in large quantities.
4. Functional bioorganic π-system: a building block strategy can be applied for synthetic functionalization on the way to new DNA-inspired structures.
5. Molecular recognition and hierarchical organization: based on the DNA helix as a regular secondary structure, sequence-specific recognition by DNA-binding proteins and even complex tertiary structures can be envisioned, e.g. four-way junctions.[16,17]

The generation of defined molecular structures based on functionalized nucleic acids is a research topic of increasing interest with important applications in nanobiotechnology.[9,10,12–14] In particular, π-arrays and clusters of organic chromophores have drawn considerable interest because of their properties that significantly differ from the monomeric state and that find potential applicability in molecular devices based on multistep electronic exciton migration.[18,19] The functionalization of RNA and DNA by single or dual fluorescent labeling is routinely applied for molecular diagnostics.[20–22] Furthermore, DNA has been applied as a template for the helical assembly of non-covalently bound chromophores, e.g. cyanine dyes, in the minor groove.[23,24] But in principle, it should be possible to apply the self-assembled and regular structure of duplex DNA as a supramolecular scaffold to allow that more than two organic chromophores interact with each other photophysically (representative examples A–F summarized in Scheme 1). Organic chromophores have been synthetically incorporated as C-nucleosidic base surrogates (A).[25] A combinatorial approach yielded astonishing new fluorescence properties when four different chromophores have been applied as part of an artificial DNA base stack.[26,27] Alternatively, organic chromophores such as

* Corresponding author. E-mail: achim.wagenknecht@chemie.uni-regensburg.de

0040–4020/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tet.2006.12.097

pyrenes can be attached to the 2′-position of the ribofuranoside moiety of RNA (B). Such a helical pyrene-array along the outside of duplex RNA was described recently exhibiting a significant pyrene excimer fluorescence enhancement.[28] In a structurally similar fashion LNA has been functionalized by multiple pyrene chromophores (C).[13,29,30] Interestingly, these LNA systems exhibit promising optical properties, e.g. signaling of full complementarity.[31] Several methyl red dyes can be incorporated as nucleoside analogs into DNA that form ordered clusters inside the helical duplex (D).[32,33] In a similar approach, stacked clusters of phenanthrenes have been observed in duplex DNA with the corresponding non-nucleosidic base surrogates (E).[34,35] The interstrand aromatic stacking of bipyridine and biphenyl as C-nucleosides in DNA can be applied as a recognition motif (F).[36]



Scheme 1. Representative examples A–F for chromophores that have been used for clusters covalently attached to DNA.

Recently, we introduced 5-(pyren-1-yl)-2′-deoxyuridine (Py-U),[37,38] 8-(pyren-1-yl)-2′-deoxyguanosine,[39] 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine (Pz-U),[40] ethidium,[41–43] and 1-ethynylpyrene[44,45] as fluorescent probes in DNA. With respect to potential applications in DNA analytics, it may be possible to enhance and modulate the fluorescence properties by the incorporation of several adjacent fluorophores into DNA. One suitable and important way to do this is to attach chromophores covalently to natural DNA bases. Recently, we showed that a helical and regularly structured π-array of 1-ethynylpyrene groups that have been covalently attached to the 5-position of uridines can only be formed if more than three chromophores are placed adjacent to each other.[46] Similar DNA systems with five adjacent Py-U units showed a remarkably strong fluorescence enhancement that is sensitive to DNA base mismatches and thermal denaturation of the duplex.[47] Herein, we want to present our subsequent investigations to characterize the optical properties of DNA duplexes that have been functionalized by a helical stack consisting of five adjacent chromophores with mixed Py-U or Pz-U sequences.

2. Results and discussion

Using our previously described protocols for the synthesis of Py-U[48] and Pz-U[40] and their incorporation into oligonucleotides[37,40] we prepared the following sets of DNA duplexes. **DNA1** and **DNA4** bear a row of five identical adjacent modifications, either Py-U or Pz-U chromophores, respectively. Additionally, we wanted to study the modulation of the fluorescence properties by the incorporation of mixed fluorophores. Hence **DNA2** and **DNA3** contain mixed sequences of five adjacent Py-U and Pz-U chromophores. Another important issue is the question if the sequence-selectivity of the unmodified complementary oligonucleotides is maintained in order to apply them as probes in molecular diagnostics with DNA. Thus in addition to the ‘right’ complementary strands in **DNA1–DNA4**, we prepared the duplexes **DNA1a/DNA1b**, **DNA2a/DNA2b**, and **DNA3a/DNA3b** in which either one adenine in the middle (a) or all five adenines (b) have been replaced by guanines (Scheme 2).

2.1. Thermal dehybridization

First, we recorded the melting temperatures at 260 nm (Table 1). We know from previous studies that the incorporation of a single Py-U or Pz-U group destabilizes the thermal stability of the DNA duplex by a few degree Celsius.[37,39,46] Nevertheless it was interesting to observe that the incorporation of five pyrene or 1-ethynylpyrene chromophore units does not destabilize the duplex additionally.[46,47] It seems that some amount of destabilization can be regained by the stabilizing effect of the hydrophobic interactions between the aromatic moieties of the pyrene or phenothiazine chromophores. This interpretation is supported by comparison of the melting temperatures of the duplexes **DNA1–DNA4**. In fact, those T_m values are all very similar. Additionally, the replacement of all five counter adenines by guanines in **DNA2b** and **DNA3b** results in more stable duplexes than **DNA2** and **DNA3**. The latter results could be explained by the assumption that the introduction of a row of the wrong counterbase guanine introduces more conformational flexibility into the DNA helix that allows the chromophores to gain hydrophobic stabilization.

2.2. Absorption spectroscopy

The optical properties of the 5-fold modified duplexes **DNA1–DNA4** were investigated by UV–vis absorption, steady-state fluorescence, and CD spectroscopy. In

Scheme 2. Sequences of duplexes **DNA1–DNA1b**, **DNA2–DNA2b**, **DNA3–DNA3b**, and **DNA4**.

Table 1. Melting temperatures of **DNA1–DNA4** (2.5 μM) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7

Duplex	T_m up (°C)	T_m down (°C)
DNA1[47]	56	50
DNA1a[47]	61	50
DNA1b[47]	—[a]	—[a]
DNA2	57	55
DNA2a	55	53
DNA2b	62	62
DNA3	55	52
DNA3a	56	52
DNA3b	67	63
DNA4	56	53

[a] No cooperative melting transition was observed.

comparison to DNA duplexes that have been modified with a single Py-U label,[37,38,47] **DNA1** shows a small bathochromic shift together with a significant intensification of the absorption shoulder at ca. 380 nm (Fig. 1). Both observations represent the result of ground-state interactions between the Py-U chromophores. In contrast, **DNA2** and **DNA3** show an absorption spectrum comparable to DNA that bears only one Py-U modification.[37,38,47] This result shows that the Py-U chromophores are not interacting with each other in these two duplexes since they are separated by the intervening Pz-U chromophores. **DNA4** has the typical absorption of the Pz-U modification since a broad band can be found between 300 nm and 400 nm.

2.3. Fluorescence spectroscopy

With respect to the well-known optical properties of pyrene and phenothiazine, especially their emission, it is important

Figure 1. UV–vis absorption spectra of the duplexes **DNA1–DNA4** (2.5 μM) in Na-P_i buffer (10 mM, pH 7). The dashed line represents the absorption of a single Py-U-modified DNA as a reference.[47]

to note here that in Py-U and Pz-U, the chromophores are linked covalently to the nucleoside by a single C–C bond yielding a strong electronic coupling.[48–51] Thus, the photoinduced intramolecular exciplexes of these modified nucleosides that we characterized over the last few years contain both excited state and charge-separated state character. The exciplex character in Py-U yields intense, unstructured fluorescence bands with solvent-dependent maxima. The Pz-U chromophore exhibits nearly complete fluorescence quenching due to an efficient electron transfer from the phenothiazine moiety to the uridine group that can be used to study DNA-mediated electron transfer processes.[39]

The fluorescence properties of **DNA1** and **DNA4** bearing five identical adjacent fluorophores were compared when excited at the same wavelength (360 nm, Fig. 2). As described recently, **DNA1** exhibits a significant fluorescence enhancement accompanied by a shift of the maximum emission to 445 nm.[47] It is remarkable that the incorporation of mismatches by replacement of adenines through guanines results in a significant loss of emission quantity and a characteristic hypsochromic shift of the emission maximum to 465 nm (**DNA1b**) compared to 475 nm in single Py-U-modified DNA,[38] indicating an electronically decoupled array of five single PydU chromophores. The loss of emission intensity is not due to fluorescence quenching by charge transfer to the guanines. CD spectra of 'mismatched' 5-fold Py-U-modified DNA duplexes showed clearly that the right-handed helical stack of pyrene chromophores cannot be formed.[47]

The experimental observations for the 5-fold Pz-U-modified duplex **DNA4** are quite different. In comparison with single Pz-U-labeled **DNA** (∼440 nm)[39] the emission maximum of **DNA4** is red-shifted to ∼550 nm. These data reveal a significant interaction between the Pz-U groups in the duplex and the excitation becomes delocalized on a lower exciton state. A similar effect was observed in duplex DNA modified with 2-aminopurine as fluorescent base surrogate for adenine.[52]

The DNA duplexes with the mixed sequence, **DNA2** and **DNA3**, exhibit a number of interesting fluorescence

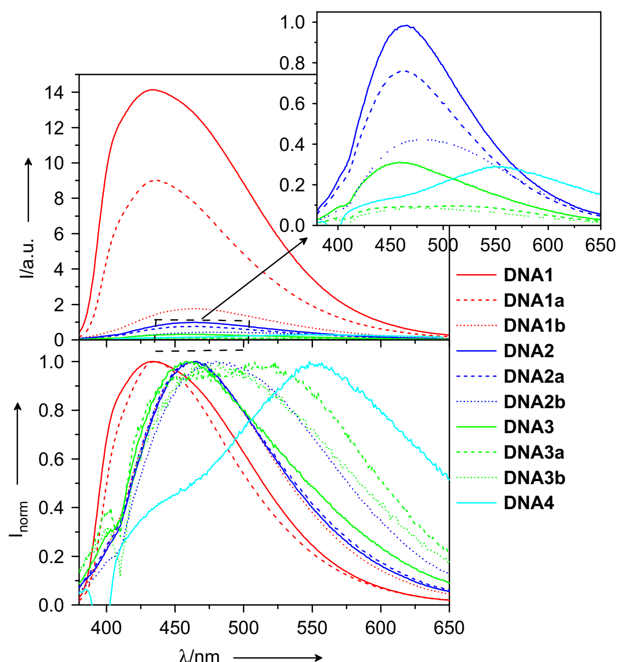


Figure 2. Fluorescence spectra (top) and normalized fluorescence spectra (bottom) of duplexes **DNA1–DNA1b**, **DNA2–DNA2b**, **DNA3–DNA3b**, and **DNA4** (2.5 μM) in Na-P_i buffer (10 mM, pH 7), λ_{exc} 360 nm. The inset shows an enlarged part of the top spectrum.

properties. Firstly, the emission intensity is quenched with the increasing number of Pz-U chromophores as part of the duplex. In fact, **DNA3** shows nearly quantitative fluorescence quenching. Secondly, the incorporation of guanines instead of adenines into the counter strand (**DNA2a/DNA2b** and **DNA3a/DNA3b**) results in an additional loss of fluorescence. Together with the previously described results this effect indicates that the pyrene- and phenothiazine-modified uridines are able to sense their complementary DNA base when they are part of a stack of several adjacent chromophores. Thirdly, the fluorescence properties of **DNA3a** and **DNA3b** exhibit partially the emission of interacting Pz-U chromophores, similar to **DNA4**. Obviously, the conformational flexibility in **DNA3a** and **DNA3b** allows a chromophore interaction in such a way that the stacked emission of the Pz-U group can be found.

2.4. CD spectroscopy

CD spectroscopy was performed to explore more information about the conformation of the modified duplexes **DNA1–DNA4** (Fig. 3). The corresponding spectra exhibit clearly the shape in the absorption range between 250 nm and 300 nm that is typical for the B-type conformation of these DNA duplexes. The reduced melting temperatures compared to the corresponding unmodified duplex[47] indicated a conformational perturbation along the chromophore stack in **DNA1–DNA4**. The results from CD spectroscopy, however, show that this structural perturbation is local and does not change the global B-DNA conformation. More remarkable is the observation that in all four modified DNA duplexes a significant exciton coupled CD signal is observed in the absorption range of the Py-U chromophore or Pz-U chromophore. It is important to note that these signals are not present in the CD spectrum of single Py-U-modified

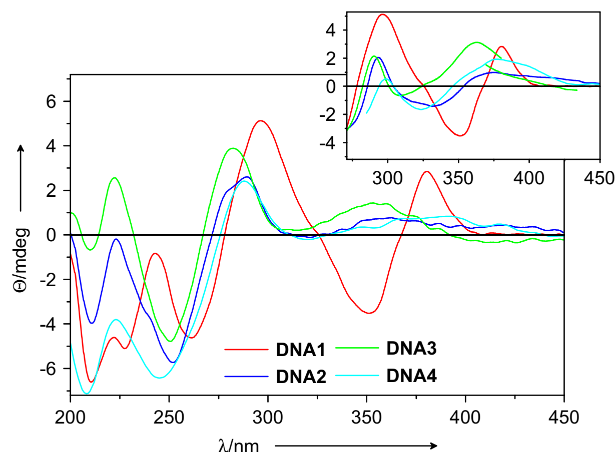


Figure 3. CD spectra of duplexes **DNA1–DNA4** (2.5 μM) in Na-P_i buffer (10 mM). Inset: the CD signals at higher concentrations show more clearly the excitonic signals of the chromophores: **DNA1** (2.5 μM), **DNA2** (5.0 μM), **DNA3** (5.0 μM), **DNA4** (12.5 μM).

DNA[37,38,47] and not in single Pz-U-labeled DNA.[39] Interestingly, all these CD spectra have a sequence of a negative band followed by a positive band between 300 nm and 400 nm. This observation supports clearly the structural idea of a right-handed helical arrangement of the five adjacent chromophores that is based on the DNA architecture and exists in all four duplexes along the DNA-like duplex scaffold.

If the helical arrangement of chromophores depends on the presence of the duplex architecture the corresponding optical properties should change above the melting temperature. Representatively we measured the CD spectra of **DNA1**[47] and **DNA4** in a temperature-dependent way (Fig. 4). It is remarkable to observe that the excitonic CD bands between 300 nm and 450 nm exhibit the DNA-typical melting behavior. The corresponding CD signals of **DNA1** and **DNA4** flatten above 50 °C. The CD spectra of **DNA1** and **DNA4** after dehybridization look very similar to that of single Py-U-labeled duplexes[37,38,47] or single Pz-U-modified DNA[39] at rt (below the corresponding T_m). This means that the helically arranged conformation of the five adjacent chromophores in **DNA1** and **DNA4** clearly depends on the DNA

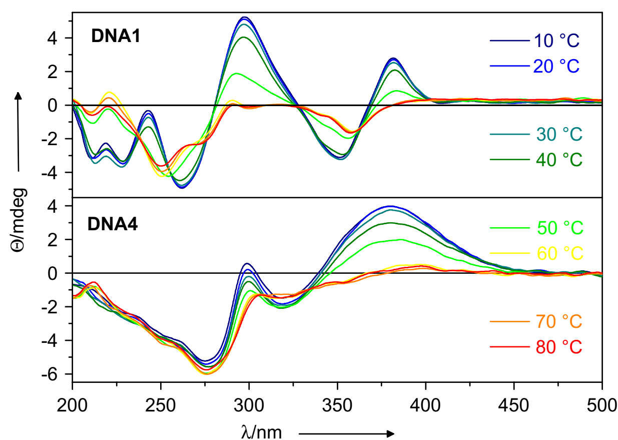


Figure 4. Temperature-dependent CD spectra of duplexes **DNA1** (2.5 μM) and **DNA4** (12.5 μM) in Na-P_i buffer (10 mM, 250 mM NaCl, pH 7).

architecture and breaks down by the thermally induced denaturation of the duplex scaffold.

3. Conclusions

DNA duplexes were functionalized covalently by a cluster of five adjacent chromophores consisting of 5-(pyren-1-yl)-2′-deoxyuridine (Py-U) and 5-(10-methyl-phenothiazin-3-yl)-2′-deoxyuridine (Pz-U). These chromophores interact significantly with each other and form a self-assembled helical π-stacked array of chromophores that is based on the DNA architecture and exhibits DNA-typical melting behavior. The fluorescence and absorption properties can be modulated by the sequence of fluorophores within the π-array. The presence of the Pz-U group quenches the fluorescence of the Py-U chromophores. Work is in progress to clarify that this is due to an electron or energy transfer process. Nevertheless, the modulation of the optical properties in such a helical fluorophore stack is potentially useful for optical nanodevices and as nucleic acid sensors for molecular diagnostics. The duplex architecture of DNA is suitable to provide the supramolecular structural scaffold for a directed arrangement of chromophores.

4. Experimental

4.1. Materials and methods

MALDI-TOF analysis was performed in the analytical facility of the department on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid in aq ammonium citrate as the matrix. C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm) and using Na-P_i buffer (10 mM). The melting temperatures (260 nm, 10–80 °C, interval 1 °C, scan speed 0.7 °C/min) were recorded on a Varian Cary Bio 100 spectrometer. The fluorescence spectra were measured on a Fluoromax-3 fluorimeter (Jobin–Yvon) and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a bandpass of 5 nm for both excitation and emission and are intensity corrected.

4.2. Preparation and characterization of oligonucleotides (general procedure)

The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 µmol) and chemicals from ABI and Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concd NH_4OH at 55 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A=NH_4OAc buffer (50 mM), pH=6.5; B=MeCN; gradient=0–15% B over 50 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm on a Varian Cary Bio 100 spectrometer.[53] Duplexes were formed by heating of modified oligonucleotides in the presence of 1 equiv unmodified complementary strand to 80 °C, followed by slow cooling.

4.2.1. Preparation and characterization of Py-U- and Pz-U-modified oligonucleotides. The phosphoramidites of Py-U and Pz-U as DNA buildings were synthesized according to published procedures.[37,39,48] The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 µmol) and chemicals from ABI and Glen Research. The coupling time for the Py-U or Pz-dU phosphoramidite had to be extended to 15 min. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concd NH_4OH at 55 °C for 10 h, protected from light. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A=NH_4OAc buffer (50 mM), pH=6.5; B=MeCN; gradient=0–40% B over 50 min for **DNA1**–**DNA3** and 0–30% B over 45 min for **DNA4**. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm[53] on a Varian Cary Bio 100 spectrometer, using ε (260 nm)=14.600 $M^{-1}\,cm^{-1}$ for Py-U[37] and ε (260 nm)=53.200 $M^{-1}\,cm^{-1}$ for Pz-U.[39] The modified oligonucleotides were identified by MS (MALDI-TOF): ss**DNA1** *m*/*z* (calcd) 6724, *m*/*z* (exp.) 6727; ss**DNA2** *m*/*z* (calcd) 6756, *m*/*z* (exp.) 6757; ss**DNA3** *m*/*z* (calcd) 6745, *m*/*z* (exp.) 6745; ss**DNA4** *m*/*z* (calcd) 5351, *m*/*z* (exp.) 5349.

Acknowledgements

We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Dr.-Ing.-Leonhard-Lorenz-Stiftung at the Technical University of Munich. C.W. thanks the Technical University of Munich for a Ph.D. fellowship from 2003 until 2005.

References and notes

1. Tour, J. M. *Acc. Chem. Res.* **2000**, *33*, 791–804.
2. James, D. K.; Tour, J. M. *Top. Curr. Chem.* **2005**, *257*, 33–62.
3. Aviram, A.; Ratner, M. A. *Chem. Phys. Lett.* **1974**, *29*, 277–283.
4. Metzger, R. M. *Acc. Chem. Res.* **1999**, *32*, 950–957.
5. Mujica, V.; Ratner, M. A.; Nitzan, A. *Chem. Phys.* **2002**, *281*, 147–150.
6. Mayor, M.; Weber, H. B. *Nanoelectronics and Information Technology*; Waser, R., Ed.; Wiley-VCH: Weinheim, 2003; pp 501–525.
7. Wassel, R. A.; Gorman, C. B. *Angew. Chem., Int. Ed.* **2004**, *43*, 5120–5123.
8. Hofmeier, H.; Schubert, U. S. *Chem. Commun.* **2005**, 2423–2432.
9. Hoeben, F. J. M.; Jonkheijm, P.; Meijer, E. W.; Schenning, A. P. H. J. *Chem. Rev.* **2005**, *105*, 1491–1546.
10. Ajayaghosh, A.; George, S. J.; Schenning, A. P. H. J. *Top. Curr. Chem.* **2005**, *258*, 83–118.
11. Würthner, F. *Chem. Commun.* **2004**, 1564–1579.
12. Carell, T.; Behrens, C.; Gierlich, J. *Org. Biomol. Chem.* **2003**, *1*, 2221–2228.
13. Wengel, J. *Org. Biomol. Chem.* **2004**, *2*, 277–280.
14. Sessler, J. L.; Jayawickramarajah, J. *Chem. Commun.* **2005**, 1939–1949.
15. Bäuerle, P.; Emge, A. *Adv. Mater.* **1998**, *3*, 324–330.

16. Seeman, N. C. *Angew. Chem., Int. Ed.* **1998**, *37*, 3220–3228.
17. Seeman, N. C. *Chem. Biol.* **2003**, *10*, 1151–1159.
18. Tinnefeld, P.; Heilemann, M.; Sauer, M. *ChemPhysChem* **2005**, *6*, 217–222.
19. Kim, D.; Osuka, A. *Acc. Chem. Res.* **2004**, *37*, 735–745.
20. Wojczeweski, C.; Stolze, K.; Engels, J. W. *Synthesis* **1999**, 1667–1678.
21. Johansson, M. K.; Cook, R. M. *Chem.—Eur. J.* **2003**, *9*, 3466–3471.
22. Tan, W.; Wang, K.; Drake, T. J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 547–553.
23. Armitage, B. *Top. Curr. Chem.* **2005**, *253*, 55–76.
24. Hannah, K. C.; Armitage, B. *Acc. Chem. Res.* **2004**, *37*, 845–853.
25. Cuppoletti, A.; Cho, Y.; Park, J.-S.; Strässler, C.; Kool, E. T. *Bioconjugate Chem.* **2005**, *16*, 528–534.
26. Gao, J.; Strässler, C.; Tahmassebi, D.; Kool, E. T. *J. Am. Chem. Soc.* **2002**, *124*, 11590–11591.
27. Gao, J.; Watanabe, S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 12748–12749.
28. Nakamura, M.; Ohtoshi, Y.; Yamana, K. *Chem. Commun.* **2005**, 5163–5165.
29. Sorensen, M.; Petersen, M.; Wengel, J. *Chem. Commun.* **2003**, 2130–2131.
30. Hrdlicka, P. J.; Babu, B. R.; Sorensen, M. D.; Harrit, N.; Wengel, J. *J. Am. Chem. Soc.* **2005**, *127*, 13293–13299.
31. Hrdlicka, P. J.; Babu, B. R.; Sorensen, M. D.; Wengel, J. *Chem. Commun.* **2004**, 1478–1479.
32. Kashida, H.; Asanuma, H.; Komiyama, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 6522–6525.
33. Kashida, H.; Tanaka, M.; Baba, S.; Sakamoto, T.; Kawai, G.; Asanuma, H.; Komiyama, M. *Chem.—Eur. J.* **2006**, *12*, 777–784.
34. Ackermann, D.; Häner, R. *Helv. Chim. Acta* **2004**, *87*, 2790–2804.
35. Langenegger, S. M.; Häner, R. *ChemBioChem* **2005**, *6*, 2149–2152.
36. Brotschi, C.; Mathids, G.; Leumann, C. J. *Chem.—Eur. J.* **2005**, *11*, 1911–1923.
37. Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Chem.—Eur. J.* **2002**, *8*, 4877–4883.
38. Kaden, P.; Mayer-Enthart, E.; Trifonov, A.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1636–1639.
39. Mayer-Enthart, E.; Valis, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3184–3187.
40. Wagner, C.; Wagenknecht, H.-A. *Chem.—Eur. J.* **2005**, *11*, 1871–1876.
41. Huber, R.; Amann, N.; Wagenknecht, H.-A. *J. Org. Chem.* **2004**, *69*, 744–751.
42. Amann, N.; Huber, R.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1845–1847.
43. Valis, L.; Amann, N.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2005**, *3*, 36–38.
44. Mayer, E.; Valis, L.; Wagner, C.; Rist, M.; Amann, N.; Wagenknecht, H.-A. *ChemBioChem* **2004**, *5*, 865–868.
45. Wagner, C.; Mayer-Enthart, E.; Rist, M.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2005**, *3*, 2062–2063.
46. Barbaric, J.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2006**, *4*, 2088–2090.
47. Mayer-Enthart, E.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2006**, *45*, 3372–3375.
48. Mayer, E.; Valis, L.; Huber, R.; Amann, N.; Wagenknecht, H.-A. *Synthesis* **2003**, 2335–2340.
49. Grabowski, Z. R.; Rotkiewicz, K.; Rettig, W. *Chem. Rev.* **2003**, *103*, 3899–4031.
50. For an example see: Fiebig, T.; Stock, K.; Lochbrunner, S.; Riedle, E. *Chem. Phys. Lett.* **2001**, *345*, 81–88.
51. Trifonov, A.; Buchvarov, I.; Wagenknecht, H.-A.; Fiebig, T. *Chem. Phys. Lett.* **2005**, *409*, 277–280.
52. Rist, M.; Wagenknecht, H.-A. *ChemPhysChem* **2002**, *3*, 704–707.
53. Puglisi, J. D.; Tinoco, I. *Methods Enzymol.* **1989**, *180*, 304–325.