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2,1,3-Benzothiadiazole-Modified DNA

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The use of 2,1,3-benzothiadiazole (BTD) as a structural element with advanced electronic properties for DNA hybrids is described. Bis(alkynyl)- and bis(carboxamide)-derived BTD units are shown to support duplex stability through interstrand stacking interactions. Placement of the BTD units opposite to a natural base, however, leads to considerable destabilization. The bis(alkynyl)-derived BTD **W** is strongly fluorescent, and quantum yields of up to 0.20 are observed. Its fluorescence behavior is strongly dependent on the neighboring nucleobases. The quenching effect of the natural bases decreases in the order G >> A ≥ T ≥ C and correlates very well with the free energies for charge separation (ΔG_{CS}) through photoinduced electron transfer, as calculated by the Rehm–Weller equation. Fluorescence of **W** is completely quenched when it is placed against the bis(carboxamide)-derived BTD **V**. The described BTD-based compounds **W** and **V** represent valuable building blocks for the construction of highly ordered, DNA-based materials with special optical and electronic properties.

Introduction

DNA represents a versatile tool for the directed assembly of functional materials, such as chromophores, metal ligands, nanoparticles and proteins.[1–5] The use of functionalized nucleic acids for applications in nanotechnology has emerged as an independent area of research with implications in the fields of diagnostics, electronics, and material sciences.[6–15] Modified nucleic acids containing various types of building blocks have been described towards this end.[16–25] As part of our work aimed at the functional expansion of DNA, we have investigated the structural and spectroscopic effects of non-nucleosidic, polyaromatic building blocks on nucleic acids.[26–32] 2,1,3-Benzothiadiazole (BTD) derivatives are widely used fluorophores with excellent spectroscopic properties, such as high molar absorptivities, high quantum yields, and large Stoke's shifts.[33–35] Due to its electron-accepting properties, BTD is a common component in "donor–acceptor" based π-conjugated polymers.[36–42] Derivatives find applications in photovoltaic devices, as electroluminescent materials, two-photon absorbing materials, and near-IR emitting fluorophores.[43–46] Furthermore, the molecular polarization supports the formation of highly ordered structures with strong π–π interactions.[47–49] Alkynyl-substituted BTD derivatives were described as DNA markers, presumably acting through an intercalative binding mode.[50,51] Because of these structural and electronic properties, BTD presents itself as an interesting building block for DNA-based functional materials. Interactions between fluorescent labels and DNA nucleobases have been extensively investigated, and the effect of nucleobases on fluorescent labels is of particular importance for the use of genetic diagnostic probes.[52–58] The mechanism of fluorescence quenching by DNA nucleobases is best explained by charge transfer processes that occur between the nucleobase and the fluorophore.[59–62] When the nucleobase acts as the electron-donating species, the excited state of the electron-accepting label is quenched by electron transfer, and a charge-separated state is formed. The probability of this pathway is best estimated by the change in free energy for charge separation (ΔG_{CS}) according to the Rehm–Weller equation.[63] Here, we report the synthesis and the electronic and structural properties of BTD-modified DNA. BTD units were connected to the DNA phosphate backbone through triple bonds and amide groups. The influence of the BTD building blocks on the thermal stability and the fluorescence properties are described, and the influence of the four types of nucleobases on BTD-fluorescence is correlated with their oxidation potentials.

Results and Discussion

Preparation of the BTD-based phosphoramidite building blocks is shown in Scheme 1. Starting from 4,7-dibromo-2,1,3-benzothiadiazole **1**, the hexynyl linker chains were introduced by using the Sonogashira cross-coupling reaction to give diol **2**. 4,4′-Dimethoxytrityl (DMT) protection of one hydroxy group yielded the mono-DMT-protected intermediate **3**. Highest yields were obtained by slowly adding a dilute solution of DMT chloride over a period of 4 h.

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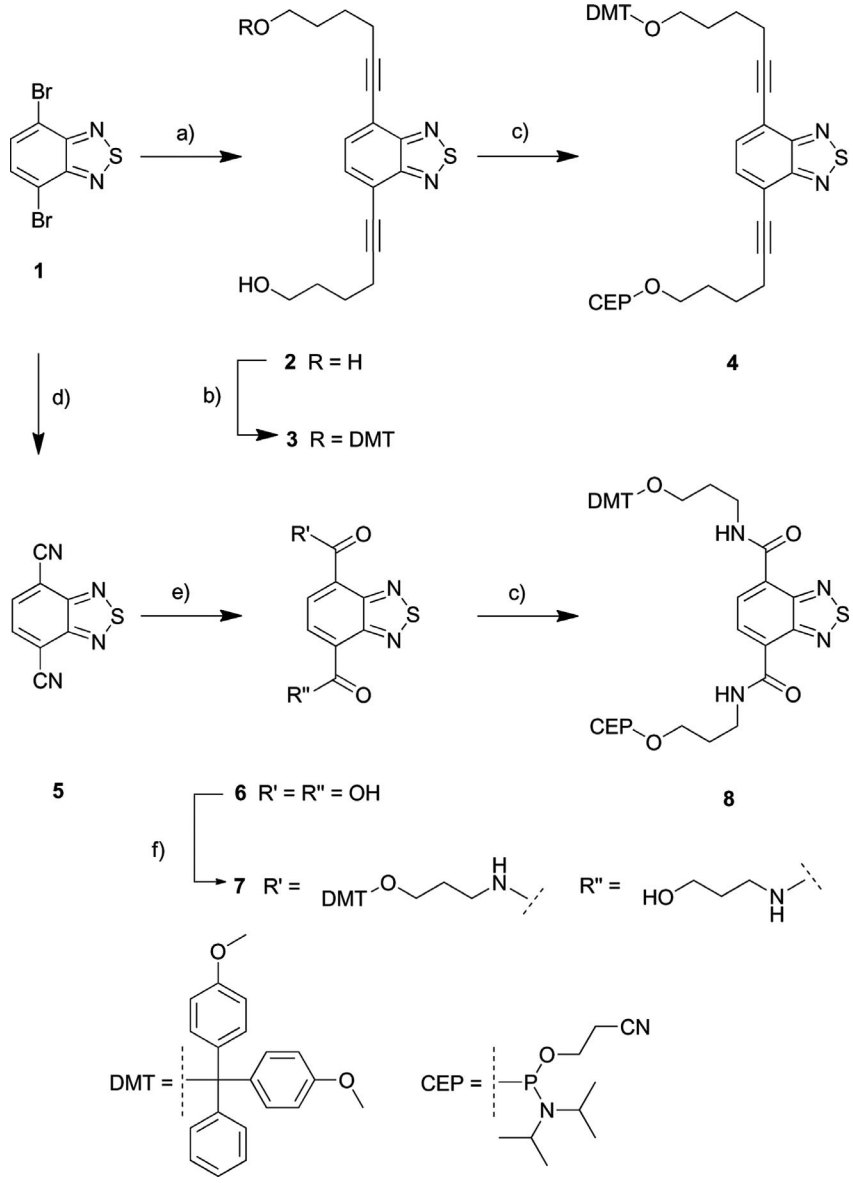


Scheme 1. Reagents and conditions: (a) 5-hexyn-1-ol (2.2 equiv.), CuI (0.06 equiv.), bis(triphenylphosphane)palladium(II) dichloride (0.05 equiv.), Et_3N (4.4 equiv.), dioxane, 60 °C, 1 h (92%); (b) 4,4′-dimethoxytrityl chloride (1.0 equiv.), pyridine, DMAP (0.15 equiv.), CH_2Cl_2, room temp., 4 h (45%); (c) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.1 equiv.), *N*,*N*-diisopropylethylamine (2.0 equiv.), CH_2Cl_2, room temp., 1 h (**4**: 90%; **8**: 75%); (d) CuCN (4 equiv.), pyridine, NaI (0.1 equiv.), DMF, 180 °C, 6 h (80%); (e) 25% NaOH in water, 100 °C, 2 h (85%); (f) 3-hydroxypropylamine/3-(4,4′-dimethoxytrityloxy)propylamine (1:1), HBTU (2.2 equiv.), *N*,*N*-diisopropylethylamine (4.0 equiv.), DMF, room temp., 2 h (33%).

Compound **3** was phosphitylated to 4,7-dihexynyl-BTD-phosphoramidite **4**. For the synthesis of the second building block, amide linker chains were introduced by converting **1** through the Rosenmund–von Braun reaction into BTD-4,7-dicarbonitrile **5**,[64] which was subsequently hydrolyzed to BTD-4,7-dicarboxylic acid **6**.[65] Amide formation involved treatment of **6** with a 1:1 mixture of 3-hydroxypropylamine and 3-(4,4′-dimethoxytrityloxy)propylamine[66] to provide the mono-DMT-protected intermediate **7**. Phosphitylation finally gave the amide-derived BTD-phosphoramidite **8**. The two building blocks **4** and **8** were incorporated into DNA oligonucleotides by using phosphoramidite chemistry (see below).[67]

Compounds **2** and **6** were characterized by UV/Vis absorbance and fluorescence spectroscopy. Figure 1 compares the spectroscopic properties of the bis(alkynyl)- and the bis(carboxylic acid)-derived BTDs **2** and **6**. The absorption spectrum of **2** exhibited a bathochromic shift compared to **6** (approximately 70 nm in the lowest energy bands). Both compounds exhibit a broad unstructured band in the fluorescence spectrum. The emission maximum of compound **2** is again redshifted by approximately 55 nm. In comparison, the alkynyl-substituted derivative **2** showed significantly higher steady-state fluorescence intensity than the bis(carboxylic acid) **6** (quantum yields: 0.23 for **2** and 0.046 for **6**). Similar bathochromic shifts and increased quantum yields

Table 1. Properties of compounds **2** and **6**.

	Compound **2**	Compound **6**
Abs. λ_{max} [nm] (ε [L mol^{-1} cm^{-1}])[a]	240 (16400), 260 (22900), 270 (26100), 310 (8700), 320 (12700), 390 (8000)	230 (12400), 315 (11300)
Emission λ_{max} [nm] (quantum yield)[b]	515 (0.23)	460 (0.05)
Reduction potential [V][c]	–1.12	–0.67

[a] 10 μM in water. [b] Quinine sulfate as fluorescence standard. [c] Versus Ag/AgCl; concentration 1.0 mM (**2**) and 0.5 mM (**6**); 0.1 M tetrabutylammonium hexafluorophosphate in acetonitrile.

were observed with pyrene.[28] Moreover, replacement of the amide linkers by triple bonds in non-nucleosidic aromatic DNA building blocks increased the hydrophobicity of the units, which led to a substantial increase in the stacking interactions.[28,68] In combination with the described intramolecular polarization of BTD, the alkynyl linkers should lead to a preferential hybrid stability in complementary DNA strands containing building block **2**. In addition, the advanced fluorescence properties of this compound are worth further investigation in the context of the DNA environment, especially with regard to their electronic interactions with nucleobases. In general, BTD derivatives are known as strong electron acceptors. The reduction potentials of **2** and **6** were determined by cyclic voltammetry to be –1.12 and –0.67 V, respectively (vs. Ag/AgCl, see the Supporting Information). This and other relevant electronic properties of the two monomeric building blocks are summarized in Table 1.

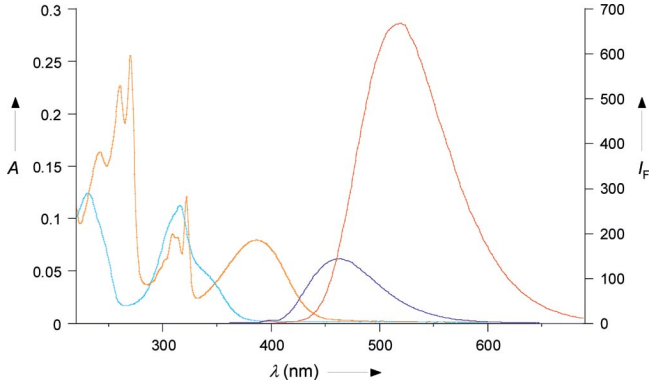


Figure 1. UV/Vis absorbance and fluorescence spectra of compounds **2** (10 μM; orange/red; λ_{ex} = 350 nm) and **6** (10 μM: light-blue/blue; λ_{ex} = 350 nm) in water.

The feasibility of photoinduced electron transfer (PET) between the chromophores and the four canonical DNA nucleobases adenine (A), guanine (G), cytosine (C), and thymine (T) was calculated by using the Rehm–Weller equation[52,63] (see the Supporting Information) and are summarized in Table 2. Based on these calculations, the change in Gibbs free energy of the photoinduced charge separation (ΔG_{CS}) between **2** and the nucleobases is exergonic only for guanine; for the three other bases, ΔG_{CS} is slightly or strongly endergonic. All ΔG_{CS} values for PET between **6** and the four DNA nucleobases were strongly exergonic. These values of ΔG_{CS} helped to delineate the quenching effects of the nucleobases on the BTD building blocks (see below).

Table 2. Change in Gibbs free enthalpies of photoinduced charge separation (ΔG_{CS}) between **2** or **6** and the four DNA nucleobases.

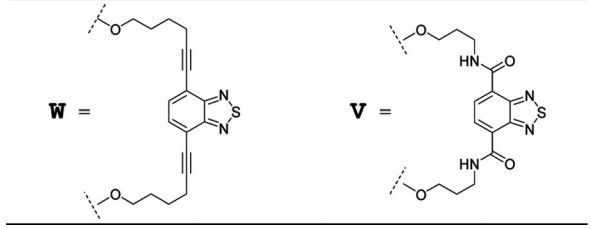
Base	E_{ox} [V vs. SCE][a]	ΔG_{CS}(**2**) [eV][b]	ΔG_{CS}(**6**) [eV][b]
G	1.25	–0.40	–1.16
A	1.72	0.07	–0.69
T	1.87	0.22	–0.54
C	1.90	0.25	–0.51

[a] Values taken from the literature.[53] [b] Calculated according to Rehm and Weller.[63] The values for the excitation energies were 2.80 eV (443 nm; **2**) and 3.10 eV (400 nm; **6**). For more details see Figure 1 and the Supporting Information.

The two building blocks **4** and **8** were incorporated into DNA oligonucleotides by phosphoramidite chemistry.[67] The studied oligonucleotides **9**–**20** are shown in Table 3. Oligonucleotides **9** and **20** contain a 4,7-dihexynyl-derived BTD unit (abbreviated as **W**) in the middle of the sequence. Based on the calculations described above, guanine was expected to have a strong quenching effect. Therefore, the flanking DNA parts were composed exclusively of AT base pairs. Duplex formation with any of the oligonucleotides **10**–**19** leads to a hybrid with one of the natural nucleobases or a 4,7-dicarboxamide-derived BTD unit (abbreviated as **V**) opposite to **W**. This set allows the effect of **W** on hybrid stability as well as the quenching effect of the individual nucleobases on BTD fluorescence to be established.

Table 3. Oligonucleotides **9**–**20** and the structures of BTD-based building blocks **W** and **V**.

Hybrid	Oligomer (B)
5'-TAATAATAAATA **W** ATAAATAATAAT	**9**
3'-ATTATTATTTAT **B** TATTTATTATTA	**10** (A) **13** (G)
	11 (T) **14** (**V**)
	12 (C)
	15 (A) **18** (G)
	16 (T) **19** (**V**)
5'-TAATAATAAATA **B** ATAAATAATAAT	**17** (C)
3'-ATTATTATTTAT **W** TATTTATTATTA	**20**



Melting temperature (T_m) values of the hybrids were determined by conventional thermal denaturation (see the Supporting Information). Introduction of **W** opposite one of the natural bases led to a considerable decrease in the

T_m of the hybrid (10 or 14 °C compared to the hybrid with an AT or a GC; see Table 4 and the Supporting Information). Replacement of an entire base pair with a **W*V** or **W*W** pair, however, resulted in a significantly smaller destabilization. A **W*W** pair (**9*20**) has approximately the same stabilizing effect as an AT base pair (cf. **10*16**). T_m values of hybrids with any of the four DNA bases in the counter strand facing **W** (hybrids with **9** or **20**) were equal within the experimental error. Overall, the T_m values were constant within 1 °C for all combinations with a natural DNA base opposite **W**. This is in agreement with previous studies involving other non-nucleosidic aromatic building blocks and their effects on DNA hybrids,[69] and suggests that there is little interaction between **W** and the nucleobases. Interaction between pairs of **W** or between **W** and **V**, restore the duplex stability nearly completely. The stability of hybrid **9*20** (**W*W**) is an indication that the high polarization in BTD leads to favorable π–π stacking interactions.[49] The combination of **W** and **V** (**9*14** and **19*20**) are also favorable, albeit to a lesser extent. Considering that the aromatic surfaces of **W** and **V** are smaller than those of other non-nucleosidic polyaromatic DNA building blocks, such as pyrenes, phenanthrenes, or perylenebis(imides),[70,71] **W** and **V** efficiently stabilize DNA hybrids by interstrand stacking interactions. The smaller aromatic surface is largely compensated for by the polarity of the BTD core.

Table 4. Quantum yields (Φ) and T_m values of hybrids.

Hybrid	Pair	T_m [°C][a]	ΔT_m [°C][b]	Φ[c]
16*10	**T*A**	41.5	–	–
17*13	**C*G**	44.5	–	–
9*10	**W*A**	32.0	–9.5/–12.5	0.12
9*11	**W*T**	32.0	–9.5/–12.5	0.12
9*12	**W*C**	31.5	–10.0/–13.0	0.12
9*13	**W*G**	32.5	–9.0/–12.0	0.02
9*14	**W*V**	39.5	–2.0/–5.0	<0.01
15*20	**A*W**	32.0	–9.5/–12.5	0.13
16*20	**T*W**	32.5	–9.0/–12.0	0.16
17*20	**C*W**	32.0	–9.5/–12.5	0.20
18*20	**G*W**	32.0	–9.5/–12.5	0.03
19*20	**V*W**	40.0	–1.5/–4.5	<0.01
9*20	**W*W**	41.5	0.0/–3.0	0.11

[a] 1.0 μM single-strand concentration, 100 mM NaCl, 10 mM sodium phosphate buffer, pH = 7.0; experimental error ±1.0 °C (see also the Supporting Information). [b] First value against **16*10**, second against **17*13**. [c] Coumarin 343 was used as fluorescence standard (see the Supporting Information).

UV/Vis absorbance spectra of all hybrids were recorded. The spectra of three different types of hybrids are shown in Figure 2a and are representative for the whole study (see also the Supporting Information). The region below 300 nm is dominated by the absorption bands of the nucleobases, whereas the area between 300 and 470 nm shows only electronic transitions from the BTD derivatives **W** and/or **V**. The band with a maximum at 400 nm is due to the bis(alkynyl)-derived BTD **W**, whereas the 325 nm band represents an overlay of **W** and **V** absorbance. The incorporation of **W** and **V** into DNA strands caused a slight bathochromic shift of the absorbance bands compared to the spectra of **2** and **6** (8 and 9 nm). A hypochromic effect was observed in hybrid **19*20** (**W** and **V**) at 400 nm compared to hybrid **17*20** (**W** next to C), which serves as an indication for aromatic stacking interactions between **W** and **V**. For steady-state fluorescence measurements, **W** was excited at 400 nm. The emission spectra of the same three hybrids discussed above are shown in Figure 2b. Emission curves are identical in shape and position. Considerable differences, however, were observed in their intensities. The fluorescence of the bis(carboxamide)-derived BTD **V** was nearly completely quenched after incorporation into DNA strands, which is not unexpected, because the corresponding monomer **6** also showed a relatively weak fluorescence (Figure 1). Additionally, the ΔG_{CS} values for this building block with all four DNA bases are strongly exergonic (see the Supporting Information), which explains why the two DNA strands containing **V** were nonfluorescent (see the Supporting Information). The quenching effect exerted by the natural bases on the fluorescence of **W** follows the order G >> A ≥ T ≥ C and, thus, closely follows the calculated values of ΔG_{CS} obtained for this series (Table 2). Hybrid **17*20** (**W** against C) has a quantum yield of almost 0.2, which is close to the quantum yield of the monomeric building block **2** in water. This shows that A, T, and C residues in the immediate neighborhood of the BTD fluorophore have very little or no quenching effect.

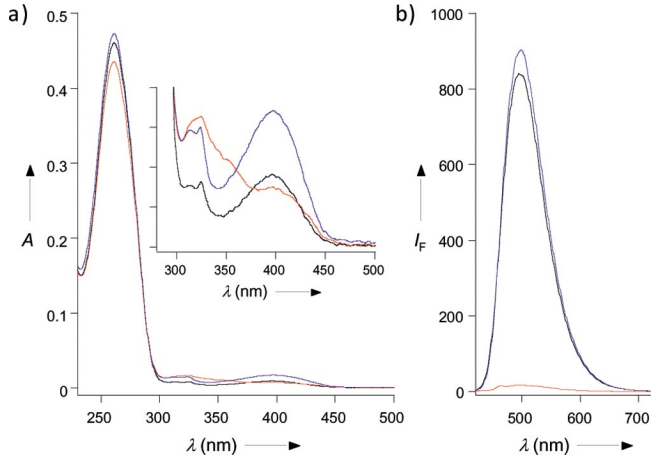


Figure 2. UV/Vis absorbance (a) and fluorescence spectra (b) of hybrids **9*20** (blue), **17*20** (black) and **19*20** (red). Temperature: 15 °C; λ_{ex} = 400 nm.

The combination of **V** and **W** (hybrid **19*20**) results in almost complete quenching of **W** (Φ < 0.01, Table 4) leading to an on/off behavior of the **W** fluorescence depending on the presence or absence of **V**. The emission curves of hybrids **17*20** (**W** opposing C) and **9*20** (**W** opposing **W**) differ only slightly in their intensities but are identical in shape and position. Association of two bis(alkynyl)-derived BTD molecules does not lead to excimer fluorescence. Instead, a certain degree of self-quenching is observed. Self-quenching was reported for *syn*- and *anti*-[2.2](4,7)benzo-

thiadiazolophanes. The quenching effect was much stronger when the thiadiazole units were arranged *syn* to each other.[72] An *anti* arrangement resulted in only partial quenching. Partial quenching was observed in hybrids, which suggests an *anti* arrangement of the two BTD units. An energy-minimized structure of hybrid **9*20**, in which the two bis(alkynyl)-derived BTD units **W** are π-stacked in an interstrand fashion, is shown in Figure 3.

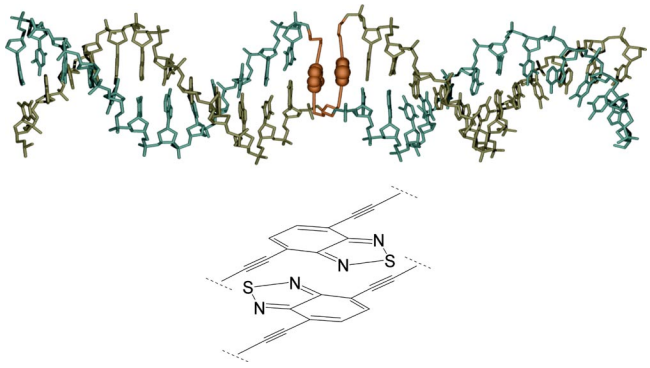


Figure 3. An Amber-minimized HyperChem model of hybrid **9*20** with the two **W** units in orange (top). The *anti* arrangement of π–π-stacked thiadiazole units is illustrated below.

The results show that the fluorescence efficiency of **W** critically depends on the neighboring groups in the direct vicinity. The quantum yields of the hybrids are displayed graphically in Figure 4. White and black bars represent hybrids in which **W** is sandwiched between two adenine or thymidine units, respectively. Generally, the highest quantum yield resulted from combinations with C (**17*20**), whereas the fluorescence was quenched in all combinations with G. The quenching effect of G can be explained by the exergonic ΔG_{CS} value for the **W***G combination. The values

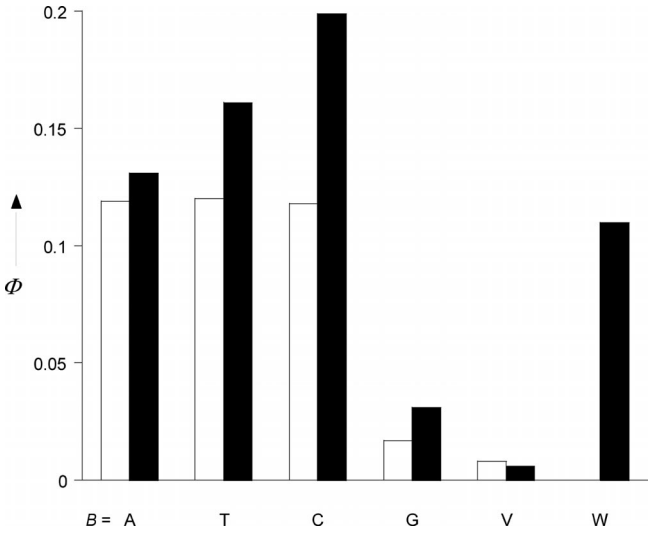


Figure 4. Quantum yields of the hybrids listed in Table 4. White bars: hybrids containing strand **9**; black bars: hybrids containing strand **20**; *B* indicates the partner in the counter strand.

of the experimental fluorescence quantum yields of **W** in hybrids containing **20** followed exactly the order of the oxidation potentials of the DNA bases (Table 2). The same quenching effects were also observed when G was placed directly next to **W** in the same strand (see the Supporting Information).

Conclusions

Two types of 2,1,3-benzothiadiazole-based phosphoramidite building blocks were synthesized. The building blocks were integrated into oligodeoxynucleotides through alkynyl or carboxamide linkers (**W** and **V**). Both building blocks were found to support duplex stability in hybrids containing a BTD unit in each of the strands in opposite positions through interstrand stacking interactions. This stabilization is attributed to the high polarization in the BTD molecules caused by the electron-withdrawing thiadiazole and the favorable π–π stacking interactions resulting therefrom. Placement opposite to a natural base, however, resulted in considerable destabilization. The bis(alkynyl)-derived BTD **W** exhibited a fluorescence behavior that was strongly dependent on the neighboring nucleobases. The quenching effect decreased in the order G >> A ≥ T ≥ C and correlated very well with the calculated free energies for charge separation (ΔG_{CS}) through photoinduced electron transfer. Fluorescence of **W** is essentially completely quenched when it is placed against the bis(carboxamide)-derived BTD **V**. The described BTD-based compounds **W** and **V** represent valuable building blocks for the construction of highly ordered, DNA-based materials with special optical and electronic properties.

Experimental Section

General: Compounds **5** and **6** were synthesized according to protocols described in the literature.[64,65] All other compounds were synthesized as described below and analyzed by NMR spectroscopy (Bruker, 300 MHz) and mass spectrometry [LTQ Orbitrap XL; accurate mass determination with nanospray ionization (NSI) in the positive mode; acetonitrile as solvent]. The natural nucleotide phosphoramidite building blocks were purchased from SAFC Proligo (Hamburg, Germany). Oligonucleotides **9**, **14**, **19**, **20**, **21**, **26**, **31**, and **32** were synthesized with an Applied Biosystems 394-DNA/RNA synthesizer by using standard synthetic procedures ("trityl-off" mode). The coupling time for the artificial building blocks was prolonged to 2 min. The coupling yields for all building blocks per single step were between 95 and 99% ("trityl assay"). Cleavage from the solid support and final deprotection were achieved by treatment with 30% NH_4OH solution (55 °C, 2 h). All oligonucleotides were purified by reverse-phase HPLC (LiChrospher 100 RP-18 5 μm column; 0.1 M triethylammonium acetate at pH = 7.0 and acetonitrile). The fully natural oligonucleotides (**10**–**13**, **15**–**18**, **22**–**25**, and **27**–**30**) were purchased from Microsynth AG (Balgach, Switzerland). All oligonucleotides were characterized with a Shimadzu LCMS-2010EV (Waters XTerra MS C-18 3.5 μm column; 50 mM ammonium formate and acetonitrile) and quantified by UV absorption measurements at 260 nm (molar extinction coefficient

at 260 nm: 20000 $M^{-1}cm^{-1}$ for **W** and 9100 $M^{-1}cm^{-1}$ for **V**). The solutions for all spectroscopic measurements were 1 μM single-strand concentration (2 μM total oligonucleotide concentration), 100 mM sodium chloride, and 10 mM sodium phosphate buffer at pH = 7.0. UV/Vis absorbance and thermal denaturation experiments were carried out with a Varian Cary-100 Bio-UV/Vis spectrophotometer. Melting-temperature values (T_m) were determined from the 1st derivative of the second cooling ramp of a cooling-heating-cooling cycle (temperature range 10–80 °C, temperature gradient 0.5 °C/min, optic path length 10 mm). Fluorescence data were collected with a Varian Cary Eclipse fluorescence spectrophotometer. The instrumental setup was 5 nm slit width (excitation and emission), 800 V detector sensitivity and 15 °C probe temperature (if not mentioned differently). Cyclic voltammetry measurements were carried out with a Metrohm 663 VA Stand with a PGStat 20. The scan speed was 100 mV/s, sample concentration was 1.0 mM in acetonitrile with 0.1 M tetrabutylammonium hexafluorophosphate. Molecular modeling by using HyperChem (HyperCube, version 8.5) was performed as follows: the ideal DNA double strand (template) was disconnected at the corresponding phosphate units, and the W building blocks were introduced. The geometry of the whole resulting duplex was optimized by applying the Amber force field.

4,7-Dihexynyl-Derived BTD Diol (2): A solution of 4,7-dibromo-2,1,3-benzothiazole (700 mg, 2.38 mmol), 5-hexyn-1-ol (580 μL, 5.24 mmol), and Et_3N (1.46 mL, 10.48 mmol) in 1,4-dioxane (15 mL) was degassed for 5 min with a stream of argon directly into the solution. Bis(triphenylphosphane)palladium(II) dichloride (84 mg, 0.12 mmol) and copper(I) iodide (27 mg, 0.14 mmol) were added at once. The suspension was stirred at 60 °C for 1 h. The reaction mixture was diluted with ethyl acetate and washed three times with saturated aqueous ammonium chloride and twice with saturated aqueous sodium chloride. The organic layer was dried with magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/dichloromethane, 1:1). Compound **2** was isolated as an orange solid (721 mg, 92%); R_f = 0.22 (ethyl acetate/dichloromethane, 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 2 H), 3.81–3.71 (m, 4 H), 2.69–2.60 (m, 4 H), 1.85–1.76 (m, 8 H), 1.68–1.55 (m, 2 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 154.8, 132.4, 117.3, 98.7, 77.4, 62.4, 32.1, 25.0, 19.9 ppm. HRMS (NSI): calcd. for $C_{18}H_{20}N_2O_2S$ $[M]^+$ 328.12; found 329.1320 $[M + H]^+$.

DMT-Protected 4,7-Dihexynyl-Derived BTD (3): To a solution of **2** (600 mg, 1.82 mmol), pyridine (180 μL, 2.19 mmol), and 4-(dimethylamino)pyridine (33 mg, 0.27 mmol) in dichloromethane (13 mL), 4,4′-dimethoxytrityl chloride (618 mg, 1.82 mmol) in anhydrous dichloromethane (13 mL) was added dropwise at room temperature over 3 h. The reaction mixture was stirred at room temperature for 1 h, then diluted with ethyl acetate and washed twice with saturated aqueous ammonium chloride, once with saturated aqueous sodium hydrogen carbonate and once with saturated aqueous sodium chloride. The organic layer was dried with magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography [ethyl acetate/hexane, 4:3 with 2% triethylamine]. Compound **3** was isolated as a yellow gel (525 mg, 45%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.58 (m, 2 H), 7.47–7.40 (m, 2 H), 7.37–7.15 (m, 7 H), 6.85–6.77 (m, 4 H), 3.81–3.71 (m, 8 H), 3.13 (t, J = 5.65 Hz, 2 H), 2.69–2.53 (m, 4 H), 1.90–1.72 (m, 8 H), 1.49 (br. s, 1 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 158.5, 154.8, 145.5, 136.8, 132.4, 130.2, 128.4, 127.8, 126.7, 117.5, 117.3, 113.1, 99.0, 98.6, 85.9, 77.4, 77.0, 63.0, 62.5, 55.3, 32.1, 29.6, 25.8, 25.0, 20.0, 19.9 ppm. HRMS (NSI): calcd. for $C_{39}H_{38}N_2O_4S$ $[M]^+$ 630.26; found 653.2447 $[M + Na]^+$.

4,7-Dihexynyl-Derived BTD Phosphoramidite (4): To a solution of **3** (500 mg, 0.79 mmol) and *N*,*N*-diisopropylethylamine (270 μL, 1.59 mmol) in anhydrous dichloromethane (16 mL), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (130 μL, 0.87 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure and the crude product purified by column chromatography [ethyl acetate/hexane, 1:1 with 2% triethylamine]. Compound **4** was isolated as a yellow gel (598 mg, 90%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.66–7.52 (m, 2 H), 7.49–7.40 (m, 2 H), 7.38–7.14 (m, 7 H), 6.87–6.74 (m, 4 H), 3.96–3.50 (m, 12 H), 3.13 (t, J = 5.63 Hz, 2 H), 2.72–2.50 (m, 6 H), 1.96–1.68 (m, 8 H), 1.20 (s, 3 H), 1.19 (s, 3 H), 1.18 (s, 3 H), 1.17 (s, 3 H) ppm. ^{13}C (300 MHz, $CDCl_3$) δ = 158.5, 154.8, 145.5, 136.8, 132.5, 132.4, 130.2, 128.3, 127.8, 126.7, 117.7, 117.5, 117.3, 113.1, 98.9, 98.5, 85.9, 77.4, 77.0, 63.4, 63.2, 63.0, 58.6, 58.3, 55.3, 43.3, 43.1, 30.6, 30.5, 29.6, 25.8, 25.3, 24.83, 24.78, 24.74, 24.68, 20.6, 20.5, 20.0, 19.8 ppm. ^{31}P NMR (300 MHz, $CDCl_3$): δ = 147.6 ppm. HRMS (NSI): calcd. for $C_{48}H_{55}N_4O_5PS$ $[M]^+$ 830.36; found 831.3714 $[M + H]^+$.

DMT-Protected 4,7-Diamido-Derived BTD (7): To a solution of **6** (250 mg, 1.12 mmol) and *N*,*N*-diisopropylethylamine (765 μL, 4.46 mmol) in anhydrous DMF (4 mL) under argon, 3-(4,4′-dimethoxytrityloxy)propylamine[66] (421 mg, 1.12 mmol) and 3-hydroxypropylamine (85 μL, 1.12 mmol) in anhydrous DMF (4 mL) was added. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU; 931 mg, 2.46 mmol) was added at once to the reaction mixture. The mixture was stirred at room temperature for 2 h, then diluted with ethyl acetate and washed once with saturated aqueous ammonium chloride, saturated aqueous sodium hydrogen carbonate, and saturated aqueous sodium chloride. The organic phase was dried with magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography [ethyl acetate with 2% triethylamine, changed to ethyl acetate/methanol (95:5) with 2% triethylamine after the bis(DMT)-protected product was eluted]. Compound **7** was isolated as a brownish foam (240 mg, 33%). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.23 (t, J = 6.22 Hz, 1 H), 9.02 (t, J = 5.37 Hz, 1 H), 8.79–8.66 (m, 2 H), 7.48–7.11 (m, 9 H), 6.78–6.67 (m, 4 H), 3.91–3.60 (m, 12 H), 3.29 (t, J = 5.84 Hz, 2 H), 3.10 (t, J = 6.50 Hz, 1 H), 2.09–1.83 (m, 4 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 163.8, 162.4, 158.5, 152.2, 152.2, 145.1, 136.4, 133.3, 132.9, 130.2, 128.4, 127.9, 127.2, 126.9, 113.1, 86.3, 77.4, 61.6, 59.4, 55.3, 38.3, 36.8, 32.8, 29.8 ppm. HRMS (NSI): calcd. for $C_{35}H_{36}N_4O_6S$ $[M]^+$ 640.24; found 663.2247 $[M + Na]^+$.

4,7-Diamido-Derived BTD Phosphoramidite (8): To a solution of **7** (200 mg, 0.31 mmol) and *N*,*N*-diisopropylethylamine (110 μL, 0.63 mmol) in anhydrous dichloromethane (6 mL), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (51 μL, 0.34 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure and the crude product purified by column chromatography (ethyl acetate with 2% triethylamine). Compound **8** was isolated as a brownish foam (200 mg, 75%). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.16 (t, J = 5.56 Hz, 1 H), 9.06 (t, J = 5.37 Hz, 1 H), 8.79–8.63 (m, 2 H), 7.46–7.05 (m, 9 H), 6.78–6.70 (m, 4 H), 3.96–3.53 (m, 16 H), 3.32 (t, J = 5.84 Hz, 2 H), 2.68 (t, J = 6.31 Hz, 2 H), 2.14–1.90 (m, 4 H), 1.21 (s, 3 H), 1.20 (s, 3 H), 1.19 (s, 3 H), 1.17 (s, 3 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 162.7, 162.5, 158.5, 152.2, 145.1, 136.4, 133.0, 132.9, 130.2, 128.4, 128.1, 127.8, 127.7, 126.9, 117.7, 113.1, 86.3, 77.4, 61.5, 61.3, 58.7, 58.4, 55.3, 43.3, 43.2, 38.2, 37.5, 31.2, 31.1, 29.8, 24.8, 24.7, 20.6, 20.5 ppm. ^{31}P NMR (300 MHz, $CDCl_3$): δ = 147.9 ppm. HRMS (NSI): calcd. for $C_{44}H_{53}N_6O_7PS$ $[M]^+$ 840.34; found 863.3331 $[M + Na]^+$.

Supporting Information (see footnote on the first page of this article): Additional analytical details (MS, NMR), UV/Vis, fluorescence, and CD spectra, cyclic voltammograms.

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