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A convenient 'click chemistry' approach to perylene diimide–oligonucleotide conjugates

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Abstract

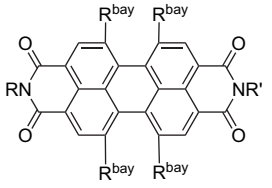
Terminal alkyne-containing phosphoramidite and solid support were prepared and used for the synthesis of 5′- and 3′-alkyne-modified oligonucleotides. The copper-catalyzed Huisgen [3+2] cycloaddition reaction in water–DMSO was used for the efficient preparation of perylene diimide–oligonucleotide conjugates suitable for constructing various types of PDI-modified DNA duplexes.

Keywords: Modified oligonucleotides; Perylene diimide; Alkynes; Azides; Catalytic Huisgen [3+2] cycloaddition

1. Introduction

The impressive breakthrough in self-assembly of DNA nanostructures[1] together with recent developments in DNA metallization[2] could pave the road for DNA-based nanoelectronics. Construction of nanometer-sized elements and their subsequent connection to macroscopic leads fabricated by routine microphotolithography is an obvious method for one-order reduction of linear dimensions of integrated circuits. The crucial issue to be addressed is the introduction of molecular semiconducting components into a DNA nanostructure. We believe that modifications, such as molecular transistors, molecular diodes, and molecules capable of gating the current upon lighting, can be introduced into oligonucleotides. The latter could be used for circuit self-assembly followed by optimized metallization, and semiconducting molecules locked by binding to DNA will be kept in gaps between metallized connectors. Our primary goal is to develop convenient and reliable methods for introduction of molecular semiconductors into DNA duplexes.

Perylene diimide (PDI) derivatives (**1**), well-known dyes possessing high fluorescence quantum yields,[3] are prospective compounds for material sciences,[4] e.g., organic field-effect transistors (OFET).[5] FETs are the essential devices for large-scale integrated circuits. Single molecule OFETs were built using *N*,*N*′-substituted PDIs.[6]



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The remarkable chemical- and photostability of PDIs also makes them promising candidates for labeling of biomolecules. There are several examples of introduction of PDIs into oligonucleotides.[7] Unfortunately, PDIs are powerful electron acceptors capable of photooxidation of nucleobases. Therefore, the fluorescence of PDIs in oligonucleotide conjugates is quenched, and emission spectra were reported for concentrated (2.5 and 10 μM) solutions of conjugates.[7g] Nevertheless, PDIs on oligonucleotides were used in other useful applications—as acceptors in charge separation studies,[7d] for pronounced triplex stabilization,[7a,b,f] and in PDI–PDI-stacking driven formation

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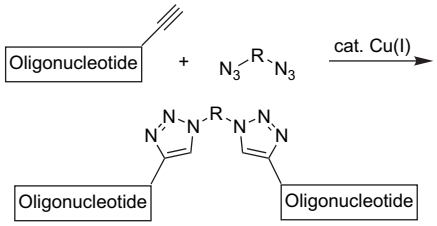
of DNA nanostructures.[7c,f,g] PDI–oligonucleotide conjugates are good building blocks for nanoassemblies of different complexity.[8] Interestingly, tertiary amine derivatives of PDI are prone to non-covalent interactions with DNA and were used for DNA precipitation,[9] and as triplex[10] and G-quadruplex[11] selective reagents.

Unfortunately, low solubility of these substances is a major problem that hinders the preparation of PDI derivatives. Usually, phosphoramidite reagents containing a dimethoxytrityl group that facilitate dissolution of the material were used to introduce PDIs into oligonucleotides.[7a–d,f,g] The use of derivatives bearing several bulky hydrocarbon moieties in the bay region (**1**, R^{bay}=alkyl, aryl) is another method that allows post-synthetic labeling of amino-modified oligonucleotide with PDI bis-succinimide esters.[7e]

Herein we present a convenient method for preparation of 5′- and 3′-oligonucleotide conjugates with sparingly soluble PDIs bearing no bay region substituents (R^{bay}=H), by means of copper-catalyzed[12] Huisgen cycloaddition reaction of alkyne and azide (so-called ‘click chemistry’, Scheme 1), carried out in aqueous environment.

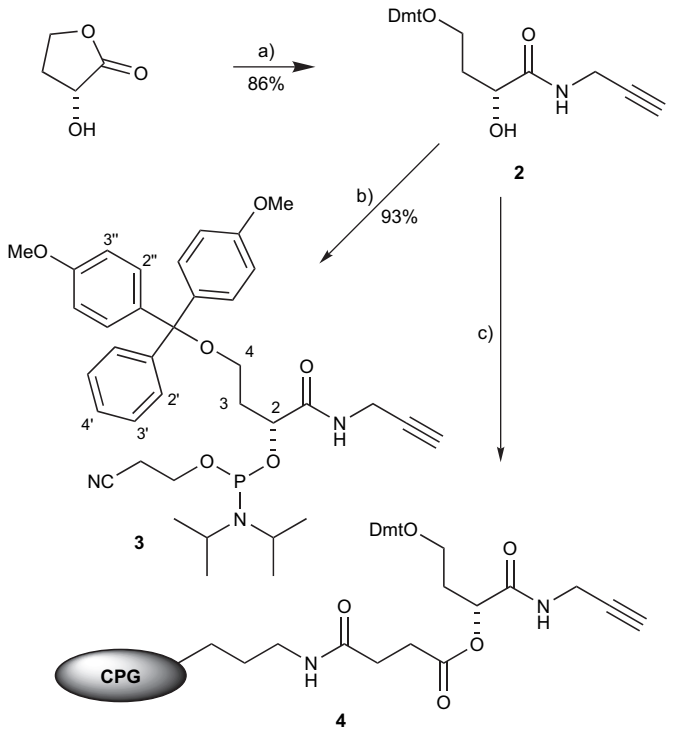


Scheme 1. Oligonucleotide conjugate assembly using [3+2] cycloaddition.

2. Results and discussion

An introduction of a reactive group (either azido or terminal alkyne) into the oligonucleotide is obviously mandatory for the conjugation to take place. The azido group is known to undergo Staudinger reaction[13] with phosphorus(III) compounds used in standard phosphoramidite solid-phase oligonucleotide synthesis (ONS). Unlike azides, terminal acetylenes are known to be stable in ONS.[14] We have used the 2,4-dihydroxybutyramide[15] derivative **2** (Scheme 2) as a non-nucleoside modifying reagent bearing an acetylenic group. This compound can be easily prepared by reaction of commercially available (*R*)-(+)-α-hydroxy-γ-butyrolactone with propargylamine followed by protection of the primary hydroxyl with the 4,4′-dimethoxytrityl group. Phosphoramidite **3** and solid support **4** prepared from **2** can be employed in ONS for the preparation of oligonucleotides containing modification in any position. The 5′- and 3′-alkyne-modified deoxyoligonucleotides prepared from **3** and **4** and their MALDI-TOF analysis results are listed in Table 1.

It should be noticed that oligonucleotides containing 5′-terminal modification should be synthesized in ‘Dmt On’ mode.[15c] Otherwise, a transamidation of propargylamide with ammonia activated by intramolecular assistance of free



Scheme 2. Reagents and conditions: (a) propargylamine, MeOH, 60 °C, 48 h, then DmtCl, Py, overnight; (b) bis(diisopropylamino)-2-cyanoethoxyphosphine, diisopropylammonium tetrazolide, CH_2Cl_2, rt, overnight; (c) succinoyl-aminopropyl-CPG (controlled pore glass), (*i*-PrN=)$_2$C, DMAP, Py/DMF, rt, 48 h.

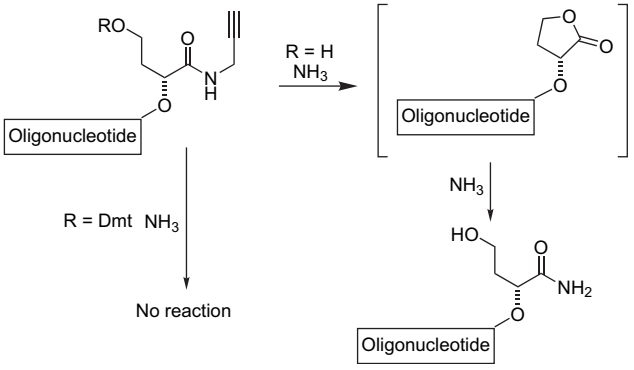
Table 1
Alkyne derivatives of oligonucleotides; **X**—alkyne modification from reagent **3** (5′) or **4** (3′)

#	Sequence (5′→3′)	MALDI-TOF mass (found/calculated[a])
ON1	**X**CA-TTA-CAT-CCA-GAC	4409.4/4409.8
ON2	GTC-TGG-ATG-TAA-TG**X**	4551.0/4551.8
ON3	**X**GT-CTG-GAT-GTA-ATG	4551.3/4551.3

[a] Monoisotopic mass.

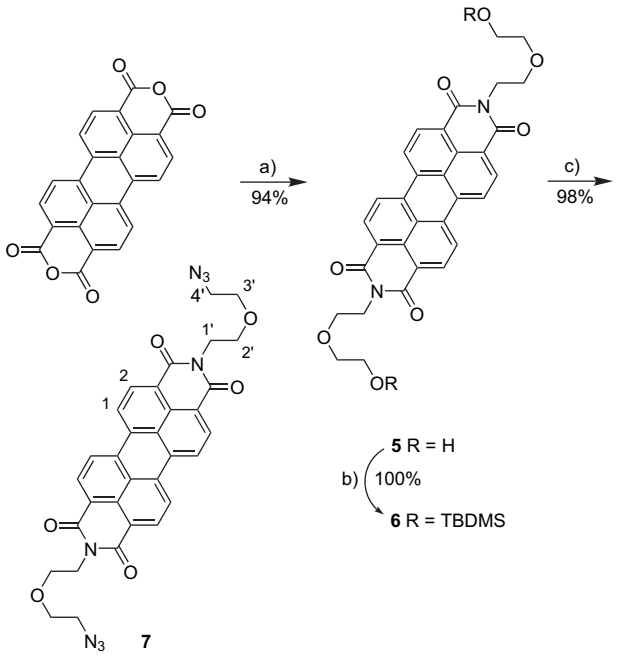
OH group takes place (Scheme 3) during ammonolysis (a step required for oligonucleotide cleavage from solid support).

The structure of the resulting amide was revealed by MALDI-TOF analysis of the ammonolyzed product (mass found/calculated 4370.8/4371.8 in case of **ON1**).



Scheme 3. Hydrolysis of oligonucleotide-5′-oxybutyramides.

An azido counterpart was prepared from perylene-3,4,9,10-tetracarboxylic acid dianhydride (Scheme 4). The starting compound was heated with 2-(2-hydroxyethoxy)ethylamine to give diol **5**, which was not soluble enough in common organic solvents even to obtain an NMR spectrum. In order to characterize it, we have converted it into the known[15] soluble bis-TBDMS ether **6** by treating with corresponding silyl chloride in pyridine. We tried to prepare an azide from **5** via a sulfonate ester; however, all attempts to react diol **5** with either mesyl or tosyl chloride in presence of bases were unsuccessful. Obviously, the reason for this is the poor solubility of the diol.



Scheme 4. Preparation of diazide derivative of PDI. Reagents and conditions: (a) 2-(2-hydroxyethoxy)ethylamine, 130 °C, 3 h; (b) dimethyl-*tert*-butylchlorosilane, Py, 75 °C, 2 h; (c) Ph_3P, DPPA, DEAD, THF, rt, overnight.

Finally, we have revealed that target diazide **7** can be prepared from **5** in a single-step Mitsunobu reaction. Diphenylphosphoryl azide (DPPA) can serve as an azide residue source.[13] The low solubility of compound **7** in $CDCl_3$ and other common solvents allows a ^1H but not a ^{13}C NMR spectrum to be obtained.

The conjugation of diazide **7** with acetylenic oligonucleotides **ON1**–**ON3** (Scheme 1) proceeded in 50% aq DMSO. Noteworthily, only mid-micromolar concentrations of azide can be obtained in pure DMSO, and its solubility in aq DMSO is even lower. Heating of the reaction mixture to 100 °C led to approximately a 2-fold increase in conjugation yield (presumably due to better azide solubility). Thus, the high concentration of DMSO is the crucial prerequisite for the cycloaddition reaction employing azide **7**.

As a catalyst, we have used the copper(I) species obtained by reduction of $CuSO_4$–tris(1-benzyl-1,2,3-triazol-4-ylmethyl)amine (TBTA)[16] complex prepared in situ with either ascorbic acid or tris(2-carboxyethyl)phosphine (TCEP). An excess of TCEP leads to azide degradation due to a Staudinger reaction, thus ascorbic acid appeared preferable. The concentration of Cu in the reaction mixture was 0.5 mM.

Although TBTA did substantially decrease the yield of products resulting from Cu(I)/Cu(II)-catalyzed oxidative destruction of oligonucleotides, oxygen-free inert atmosphere has proved to be essential for the conjugation. Although acceleration of the cycloaddition of azide with acetylene was reported in presence of TBTA,[17] we did not observe the effect. Probably, azide dissolution rather than cycloaddition is the rate-limiting step in the case discussed.

The products of the reaction can be isolated by polyacrylamide gel electrophoresis (PAGE). The electropherograms feature mono-, diconjugates, and small amounts of minor products attributed to monoconjugate azido group thermal degradation products (Fig. 1). The isolated yields of adducts are presented in Table 2.

Oligonucleotide **ON2** possessing 3′-terminal modification afforded low yield of diadduct compared to 5′-labeled counterparts. MALDI-TOF analysis of adducts revealed a very

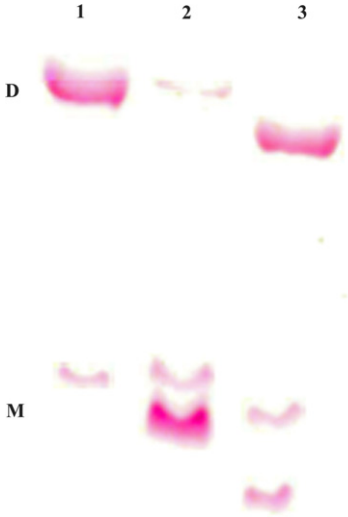


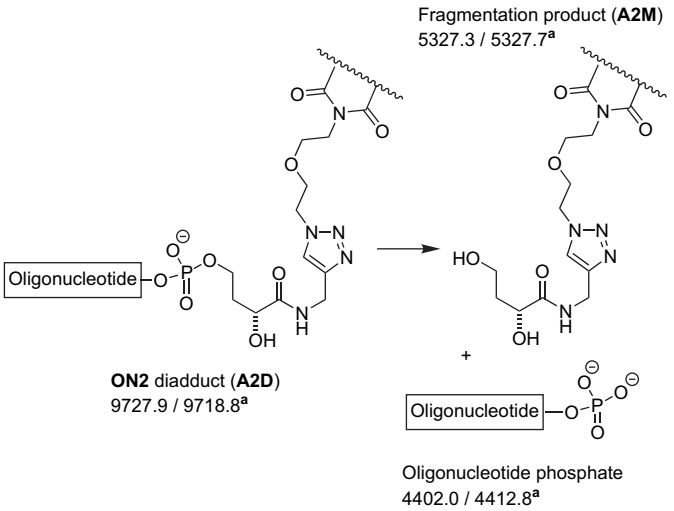
Figure 1. PAGE separation of click reactions of oligonucleotides **ON1** (lane 1), **ON2** (lane 2), and **ON3** (lane 3) with azide **7**; **ON/7** ratio is 2:1. **D**—diadducts **A1D** (lane 1), **A2D** (lane 2), and **A3D** (lane 3), **M**—monoadducts of unidentified nature, the major band in lane 2 is fragmentation product **A2M** (see Scheme 5).

Table 2
Products isolated from reactions of PDI diazide **7** with 5′- and 3′-alkynyl oligonucleotides

Starting oligonucleotide	Azide/oligonucleotide ratio	Monoadduct		Diadduct	
		#	Yield (%)	#	Yield (%)
ON1	1:2	**A1M**	0	**A1D**	41
ON2	1:2	**A2M**[a]	29	**A2D**	9
ON3	1:2	**A3M**	0	**A3D**	59
ON1	1:1	**A1M**	36	**A1D**	42
ON2	1:1	**A2M**[a]	40	**A2D**	11
ON3	1:1	**A3M**	35	**A3D**	35

[a] **A2M** refers to fragmentation product (Scheme 5) rather than to monoadduct of regular structure.

uncommon process of phosphodiester bond hydrolysis in case of **ON2** diadduct (Scheme 5). No monoadduct of regular structure was observed in this case. It is especially unexpected that only the diadduct **A2D** undergoes a fragmentation, taking into account the stability of both fragmentation product **A2M** and starting acetylenic oligonucleotide **ON2** in reaction conditions. The reasons for this are yet unclear. The structures of adducts were confirmed by MALDI-TOF (Table 3).



Scheme 5. Hydrolysis of diadduct **A2D**; [a]MALDI-TOF mass, found/calculated.

Table 3
MALDI-TOF characterization of isolated conjugates

Adduct	MALDI-TOF mass (found/calculated)
A1M	5029.8/5028.5[a]
A1D	9444.7/9439.4[b]
A2M	5327.3/5327.7[a]
A2D	9727.9/9718.8[b]
A3M	5171.1/5170.6[a]
A3D	9729.2/9723.6[b]

[a] Monoisotopic mass.
[b] Average mass.

The fluorescence intensity of perylene diimide in both monoconjugates (Fig. 2) and diconjugates (Fig. 3) displays significant dependence on the neighboring base. In case of neighboring cytosine (as in **A1M** and **A1D**) the conjugates exhibit a fluorescence maximum at approx. 550 nm. In the case of guanine (as in **A2M**, **A2D**, **A3M**, and **A3D**), the fluorescence is almost completely quenched.

Using mono- and diadducts (Table 2) duplexes of various geometry were prepared (Fig. 4). This illustrates the flexibility and usefulness of the method presented for the synthesis of PDI-containing DNA duplexes. The latter will be used for DNA metallization studies. The fluorescence spectra of the duplexes were recorded. The emission of **A1M** and **A1D** is effectively quenched upon hybridization with complementary conjugates possessing guanine vicinal to the dye (Fig. 5).

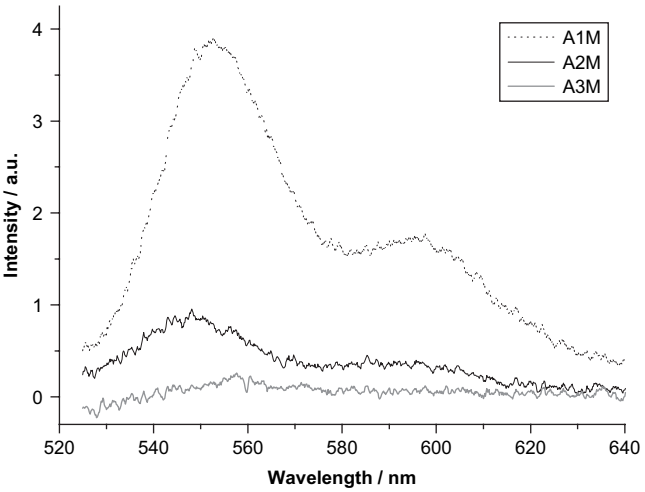


Figure 2. Emission spectra of PDI–oligonucleotide monoadducts.

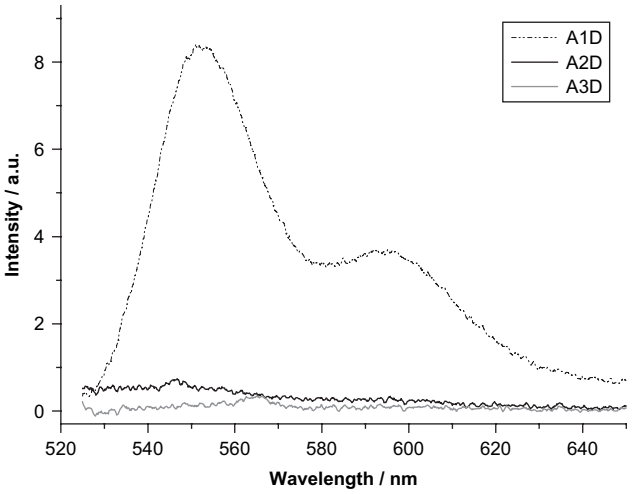


Figure 3. Emission spectra of PDI–oligonucleotide diadducts.

Although the conjugates prepared did not display interesting fluorescent properties, PDI residues can be probably used as quenchers for other fluorophores on DNA. The study of perylene diimide derivatives of oligonucleotides will be continued to evaluate further their potential as building blocks for DNA nanostructures and nanoelectronic devices.

3. Conclusions

In summary, we developed reagents for the automated synthesis of 5′- and 3′-alkyne-modified oligonucleotides and studied their [3+2] cycloaddition reaction in 50% aq DMSO with bis-azido derivative of PDI. Mono- and diconjugates were isolated and characterized. The weak fluorescence of PDI–oligonucleotide conjugates is additionally quenched in the presence of adjacent guanine or after guanine approach from a second strand in the course of hybridization. The process described can be used for preparation of oligonucleotide conjugates with hydrophobic organic molecules.

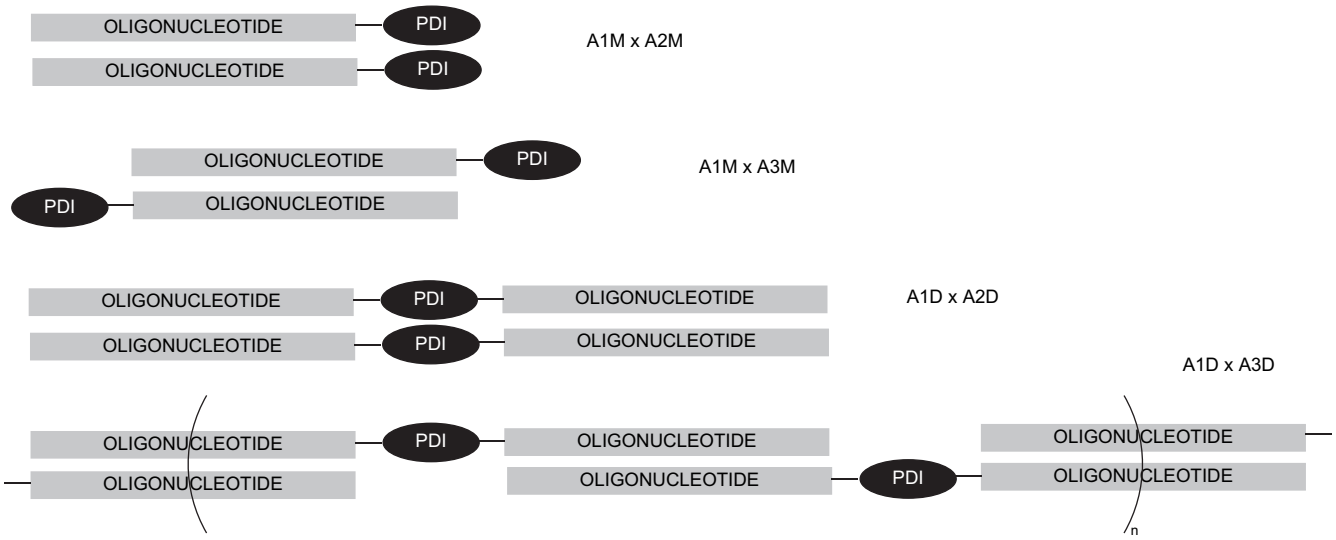


Figure 4. DNA duplexes prepared from PDI–oligonucleotide conjugates.

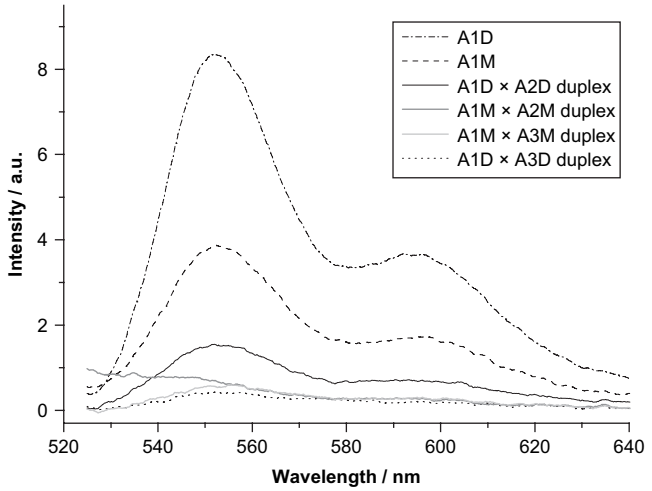


Figure 5. Emission spectra of fluorescent conjugates **A1M**, **A1D**, and their duplexes with complementary PDI conjugates.

4. Experimental section

4.1. General

500 MHz ^{1}H, 125.7 MHz ^{13}C, and 202.4 MHz ^{31}P NMR spectra were recorded on a Bruker DRX-500 spectrometer and referenced to DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C), MeCN-d_3 (1.93 ppm for ^{1}H and 1.30 ppm for ^{13}C), $CDCl_3$ (7.26 ppm for ^{1}H), and 85% aq H_3PO_4 (0.00 ppm for ^{31}P). ^{1}H NMR coupling constants are reported in hertz and refer to apparent multiplicities. Elemental analysis was performed on Thermo Finnigan CHNS-analyser EA1112. High resolution mass spectra were recorded in positive ion mode using IonSpec FT ICR mass spectrometer (MALDI) or PE SCIEX QSTAR pulsar mass spectrometer (ESI). IR spectra were obtained using SPECORD M82 spectrometer. MALDI-TOF mass spectra of oligonucleotides were recorded on Bruker Ultraflex mass spectrometer in positive ion mode; a mixture (1:1 v/v) of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and diammonium hydrogen citrate (80 mg/mL in water) was used as matrix. Theoretical masses of oligonucleotide conjugates were calculated using the monoisotopic element masses for masses up to 8000 and using the average element masses for masses above 8000. Analytical thin-layer chromatography was performed on Kieselgel 60 F_{254} precoated aluminum plates (Merck), spots were visualized under UV light (254 nm). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm. Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. TBTA,[16] diisopropylammonium tetrazolide,[18] bis(diisopropylamino)-2-cyanoethoxyphosphine,[19] and succinoyl-CPG[20] were prepared as described. Pyridine and DMF were distilled over CaH_2. DCM was used freshly distilled from CaH_2. Other solvents were used as received. Oligonucleotides were synthesized using Biosset ASM-700 DNA/RNA synthesizer and purified by HPLC. Fluorescence spectra were recorded on Varian Cary spectrofluorimeter (c=0.3 μM, hybridization buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0, excitation wavelength 505 nm). The duplexes were prepared by mixing oligonucleotides in hybridization buffer, heating at 95 °C for 5 min, and gradually cooling to ambient temperature.

*4.2. (2R)-O^4-(4,4′-Dimethoxytrityl)-N-propargyl-2,4-dihydroxybutyramide (**2**)*

Propargylamine (0.69 mL, 10.1 mmol) and (*R*)-(+)-α-hydroxy-γ-butyrolactone (979 mg, 9.59 mmol) were mixed in MeOH (3 mL) and kept at 60 °C for 48 h. The mixture was evaporated and co-evaporated with toluene (30 mL) and pyridine (3×30 mL). The residue was dissolved in pyridine (30 mL), cooled to 0 °C, and solid 4,4′-dimethoxytrityl chloride (3.41 g, 10.1 mmol) was added. The mixture was kept overnight at rt, diluted with EtOAc (150 mL), washed with

H_2O (3×50 mL), and brine (50 mL), dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (5→10% Me_2CO and 1% Et_3N in PhMe). Yellowish foam; yield 3.80 g (86%); R_f 0.31 (15% Me_2CO and 1% Et_3N in PhMe); HRMS (MALDI+): *m/z* $[M+Na]^+$ calcd for $C_{28}H_{29}NNaO_5^+$: 482.1938, found: 482.1915; ν_{max} (KBr) 3404, 3292, 2928, 2048, 1656, 828 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 8.11 (1H, t, *J* 5.9 Hz, N*H*), 7.37 (2H, d, *J* 7.6 Hz, H-2′), 7.30 (2H, t, *J* 7.6 Hz, H-3′), 7.26–7.18 (5H, m, H-4′ and H-2″), 6.88 (4H, d, *J* 8.7 Hz, H-3″), 5.45 (1H, d, *J* 5.8 Hz, O*H*), 4.02 (1H, m, H-2), 3.89–3.83 (1H, m, ^2J 17.2, J_{CHNH} 5.9, ^4J 2.2 Hz, CH*H*N), 3.82–3.76 (1H, m, ^2J 17.2, J_{CHNH} 5.9, ^4J 2.4 Hz, C*H*HN), 3.74 (6H, s, O*Me*), 3.02–3.12 (2H, m, H-4), 3.01 (1H, m, ≡C*H*), 1.96 (1H, m, H-3b), 1.70 (1H, m, H-3a); δ_C (125.7 MHz, DMSO-d_6) 173.8 (*C*O), 158.0 (2C, C4″), 145.3 (C1′), 136.1 (2C, C1″), 129.7 (4C, C2″), 127.7 (2C), 127.8 (2C, C2′ and C3′), 126.6 (C4′), 113.2 (4C, C3″), 85.4 (Ar_3C), 81.4 (*C*≡CH), 72.5 (≡*C*H), 68.5 (C2), 59.7 (C4), 55.1 (2C, O*Me*), 34.7 (C3), 27.7 (N*C*H_2).

4.3. (2R)-O^2-(2-Cyanoethoxy-N,N-diisopropylamino-phosphinyl)-O^4-(4,4′-dimethoxytrityl)-N-propargyl-2,4-dihydroxybutyramide (**3**)

Compound **2** (681 mg, 1.48 mmol) was co-evaporated with CH_2Cl_2 (3×30 mL) and dissolved in CH_2Cl_2 (25 mL). Diisopropylammonium tetrazolide (318 mg, 1.86 mmol) followed by bis(diisopropylamino)-2-cyanoethoxyphosphine (670 mg, 2.22 mmol) were added under argon. The mixture was stirred under argon overnight, then diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ (2×50 mL), water (2×50 mL), and brine (50 mL), dried over Na_2SO_4, and evaporated. The residue was quickly purified by column chromatography (7% Me_2CO and 2% Et_3N in PhMe) to give the title compound as a mixture of diastereomers. White foam; yield 912 mg (93%); R_f 0.55 and 0.65 (10% Me_2CO and 2% Et_3N in PhMe); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{37}H_{46}N_3NaO_6P^+$: 682.3016, found: 682.2984; ν_{max} (KBr) 3436, 3292, 3060, 2256, 2048, 1680, 828 cm^{-1}; δ_H (500 MHz, MeCN-d_3) 7.44 (2H, m, H-3′), 7.36–7.28 (6H, m, H-2′, H-2″), 7.24 (1H, m, H-4′), 7.05 (0.45H, m), 6.93 (0.55H, m) (N*H*), 6.88 (4H, m, H-3″), 4.38 (0.45H, m), 4.30 (0.55H, m) (H-2), 4.04–3.52 (12H, m, NC*H*Me_2, N*C*H_2, POCH_2, OCH_3), 3.18 (2H, m, H-4), 2.66 (0.90H, t, *J* 6.0 Hz), 2.53 (1.10H, t, *J* 6.0 Hz) (*C*H_2CN), 2.43 (0.45H, m), 2.38 (0.55H, m, ≡C*H*), 2.02–2.15 (2H, m, H-3), 1.16 (3H, d, *J* 6.7 Hz), 1.15 (3H, d, *J* 6.6 Hz), 1.11 (3H, d, *J* 6.9 Hz), 1.01 (3H, d, *J* 6.7 Hz, NCHCH_3); δ_C (125.7 MHz, MeCN-d_3) 172.5, 172.3 (*C*O), 159.5 (C4″), 146.4 (C1′), 137.3 (C1″), 130.9 (C2″), 129.0, 128.9, 128.7, 127.6, 127.2 (C2′ and C3′), 119.4, 119.3 (*C*N), 113.9 (C3″), 86.9 (Ar_3C), 81.2, 81.0 (*C*≡CH), 73.8, 73.6, 72.1, 71.9 (C2), 71.9, 71.8 (≡*C*H), 60.3, 60.0 (C4), 59.8, 59.6, 59.4, 59.2 (POCH_2), 55.8 (O*Me*), 44.1, 44.0, 43.9 (*C*HCH$_3$), 35.1 (C3), 29.1, 28.9 (NCH_2), 24.9, 24.8, 24.7, 24.6 (CH*C*H_3), 20.9, 20.8 (*C*H_2CN); δ_P (202.4 MHz, MeCN-d_3) 151.3, 148.9 (diastereomers).

4.4. Solid support **4**

Compound **2** (391 mg, 0.85 mmol) was dissolved in the mixture of Py (12 mL) and DMF (12 mL). Diisopropylcarbodiimide (0.93 mL, 6 mmol), DMAP (70 mg, 0.57 mmol), and succinoyl-CPG-700 Å (1 g, carboxyl loading 35 μmol/g) were added. The mixture was kept for 48 h at rt and agitated periodically. The unreacted carboxyls were capped by conversion into pyrrolidine amides: the support was suspended in a solution of pentafluorophenol (300 mg, 1.63 mmol) in Py (3 mL), kept for 12 h at rt, and the CPG was filtered off. A solution of pyrrolidine (0.5 mL) in Py (10 mL) was added. After 5 min at rt, the glass was filtered off again and washed with $CHCl_3$ (3×30 mL), MeOH (3×30 mL), *tert*-BuOMe (3×30 mL), and dried in vacuo. The loading of 11.4 μmol/g was determined as described.[20]

4.5. N,N′-Bis(2-[2-hydroxyethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (**5**)

Perylene-3,4,9,10-tetracarboxylic acid dianhydride (785 mg, 2 mmol) was heated with 2-(2-hydroxyethoxy)ethylamine (1.5 mL) under argon at 130 °C for 3 h. The mixture was then cooled to rt and diluted with MeOH (20 mL). The suspension thus obtained was placed into twelve 2-mL centrifuge tubes, and the precipitate separated by centrifugation was additionally washed with MeOH (4×20 mL), combined, and dried in vacuum. Dark red powder; yield 531 mg (94%); HRMS (MALDI+): *m/z* $[M+Na]^+$ calcd for $C_{32}H_{26}N_2NaO_8^+$: 589.1581, found: 589.1561; ν_{max} (KBr) 3476, 1692, 1652, 1596 cm^{-1}. Anal. Calcd for $C_{32}H_{26}N_2O_8$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.70; H, 4.81; N, 4.98.

4.6. N,N′-Bis(2-[2-dimethyl-tert-butylsilyloxyethoxy]-ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (**6**)[7b]

N,*N*′-Bis(2-[2-hydroxyethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (57 mg, 0.1 mmol) was suspended in dry pyridine (7 mL) and dimethyl-*tert*-butylchlorosilane (76 mg, 0.25 mmol) was added. The mixture was heated at 75 °C for 2 h under argon, then cooled to rt, evaporated, and the residue was purified by column chromatography (10% Me_2CO in PhMe). Dark red solid; yield 80 mg (100%); R_f 0.35 (10% Me_2CO in PhMe); HRMS (MALDI+): *m/z* $[M+Na]^+$ calcd for $C_{44}H_{54}N_2NaO_8Si_2^+$: 817.3311, found: 817.3302; δ_H (500 MHz, $CDCl_3$) 8.45 (4H, d, *J* 8.1 Hz), 8.24 (4H, t, *J* 8.1 Hz, H-1, H-2), 4.43 (4H, t, *J* 6.0 Hz, H-1′), 3.87 (4H, t, *J* 6.0 Hz, H-2′), 3.76 (4H, t, *J* 5.3 Hz, H-3′), 3.63 (4H, t, *J* 5.3 Hz, H-4′), 0.84 (6H, s, *Me*), 0.02 (9H, s, *tert-Bu*).

4.7. N,N′-Bis(2-[2-azidoethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (**7**)

Triphenylphosphine (87 mg, 0.33 mmol) was dissolved in THF (3 mL) and *N*,*N*′-bis(2-[2-hydroxyethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (84 mg, 0.15 mmol) was added. Diphenylphosphoryl azide (33 mg, 0.36 mmol)

and diethyl azodicarboxylate (54 μL, 0.35 mmol) were added as a mixture. The resulting suspension was kept overnight, evaporated, and the residue was purified by column chromatography (5% Me_2CO in $CHCl_3$). The fractions containing title compound were evaporated to give the residue that was washed with CH_2Cl_2 (2×1 mL) to remove traces of soluble impurities, and dried. Dark red solid; yield 91 mg (98%); R_f 0.67 (30% Me_2CO in $CHCl_3$); HRMS (MALDI+): *m/z* $[M+Na]^+$ calcd for $C_{32}H_{24}N_8NaO_6^+$: 639.1717, found: 639.1730; ν_{max} (KBr) 2136, 2104, 1692, 1652, 1596 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.70 (4H, d, *J* 8.0 Hz), 8.63 (4H,t, *J* 8.0 Hz, H-1, H-2), 4.50 (4H, t, *J* 6.0 Hz, H-1′), 3.89 (4H, t, *J* 6.0 Hz, H-2′), 3.73 (4H t, *J* 5.0 Hz, H-3′), 3.34 (4H, t, *J* 5.0 Hz, H-4′). Anal. Calcd for $C_{32}H_{24}N_8O_6$: C, 62.33; H, 3.92; N, 18.17. Found: C, 61.12; H, 4.00; N, 18.24.

4.8. General conjugation procedure

Alkyne-modified oligonucleotide (2 nmol) was dissolved in deionized water (25 μL) and placed into a pressure-tight vial. A solution of *N*,*N*′-bis(2-[2-azidoethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (either 1 or 2 nmol, in 7 or 14 μL DMSO) was then added followed by additional amount of DMSO (36 or 29 μL, respectively), triethylammonium acetate buffer stock solution (10 μL, 2 M in water, pH 7.0), and ascorbic acid (10 μL, 5 mM in water). The solution was degassed by argon bubbling through solution for 30 s. Then, a mixture of TBTA (7.0 μL, 10 mM in DMSO) and $CuSO_4$ (5.0 μL, 10 mM in water) was added. The vial was purged with argon, closed, sealed with parafilm and placed into hot (95 °C) water. The water was allowed to cool to rt for 15 h, the oligonucleotide material was precipitated with Me_2CO, and the conjugates were purified by PAGE.

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