Oligonucleotides containing arylacetylene residues: synthesis and post-synthetic modification *via* [3+2] cycloaddition

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The synthesis of a phosphoramidite reagent for 5′-modification of oligonucleotides by introducing an arylacetylene residue has been described. Using the reaction with 3-(perylen-3-yl)propyl azide as an example, it was shown that the acetylene derivatives of oligonucleotides synthesized using this reagent undergo Cu^{I}-catalyzed [3+2] dipolar cycloaddition. Fluorescent conjugates were obtained in high yields and characterized by mass spectra.

Key words: modified oligonucleotides, [3+2] cycloaddition, conjugation, acetylenes, azides, perylene.

The [3+2] cycloaddition of organic azides to terminal acetylenes, resulting in the formation of 1,2,3-triazoles (first described in the 19th century and studied in detail in the mid-20th),[1] now attracts increasing attention of researchers as a method of combinatorial chemistry,[2,3] in particular, conjugation.[4,5] This process being carried out under mild conditions in aqueous or organic solutions in the absence of any coupling agents can be accelerated by addition of Cu^{I}-species.[6,7] The orthogonality of both the alkyne and azido group with respect to the majority of functional groups allows a pronounced increase in the conjugation multiplicity, *i.e.*, in the number of reactions carried out simultaneously and specifically in one vessel.

However, despite the large potential of this method, only a few examples of catalyzed [3+2] cycloaddition of modified oligonucleotides are known to date.[8—13]

To involve an oligonucleotide into [3+2] cycloaddition, it is necessary to carry out its modification, namely, introduction of a terminal acetylenic or azido group. An azido group can be introduced only post-synthetically,[13,14] because organic azides rapidly undergo the Staudinger reaction[15] with the phosphoramidites used in standard protocols for solid-phase automated oligonucleotide synthesis (ONS). Unlike azides, acetylenes (including terminal ones) are fully compatible with all reagents used in the synthetic cycle and the acetylenic group can be introduced into oligonucleotides using modifying reagents in ONS. Although syntheses of oligonucleotide derivatives containing terminal acetylenes have been reported,[16,17] examples of [3+2] cycloaddition involving them are still unknown. Note that arylethynyl modifications were incorporated into oligonucleotides only once.[18]

Since the method for the synthesis of oligonucleotide conjugates using [3+2] cycloaddition has not been adequately developed, we decided to synthesize a modifying reagent for introducing a terminal alkyne group into oligonucleotides and to optimize the conjugation conditions.

Results and Discussion

Several reactions of azides with modified oligonucleotides containing an acetylenic fragment have been reported; however, all of these cases dealt with alkylethynyl rather than arylethynyl fragment. One could expect that the behavior of alkyl- and arylethynyl derivatives in the [3+2] cycloaddition with azides would differ. Therefore, we have synthesized phosphoramidite **5** containing an arylethynyl fragment (Scheme 1).

The Sonogashira coupling of 4-iodobenzoic acid (**1**) with trimethylsilylacetylene was used to obtain 4-trimethylsilylethynylbenzoic acid (**2**), which reacted with *trans*-4-aminocyclohexanol hydrochloride in the presence of diisopropylethylamine and tris(pyrrolidino)benzotriazol-1-yloxyphosphonium hexafluorophopsphate (PyBOP) (as a coupling reagent) to give amide **3**. Desilylation of the product on treatment with tetrabutylammonium fluoride yielded amide **4** containing a terminal acetylenic fragment. The target reagent **5** was formed upon phosphitylation of amide **4**. Phosphoramidite **5** gives only one signal in the ^{31}P NMR spectrum. Full assign-

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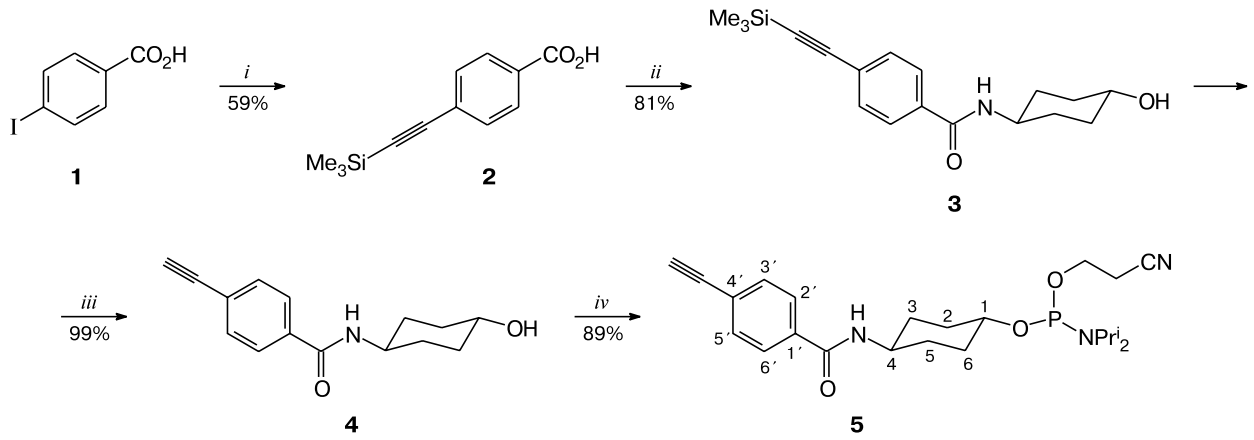
Scheme 1



Reagents and conditions: *i*. $Me_3SiC{\equiv}CH$, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 12 h; HCl; *ii*. tris(pyrrolidino)benzotriazol-1-yloxyphosphonium hexafluorophosphate, $EtNPr^i_2$, 20 min; *trans*-4-hydroxycyclohexylammonium chloride, $EtNPr^i_2$, 4 h; *iii*. NBu_4F, THF, 10 min; *iv*. $(Pr^i_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, CH_2Cl_2, 12 h.

ment of the ^{13}C NMR signals of compound **5** was done using 2D heteronuclear $^1H—^{13}C$ NMR spectra (HMQC and HMBC).

The reagent **5** was used for the synthesis of oligonucleotides **ON1** and **ON2** (Table 1). The structure of oligonucleotides was confirmed by MALDI-TOF mass spectra.

It seemed more challenging to attempt to conjugate acetylene-containing oligonucleotides with nonpolar azides. We chose perylene-derived azide as the model compound having a high fluorescence quantum yield, high chemical stability and photostability. We expected the conjugates to be easily detected during the polyacrylamide gel electrophoresis due to bright fluorescence, while the presence of a nonpolar perylene fragment was expected to substantially increase the HPLC retention time on a reversed-phase column.

Upon the reaction with stabilized ylide, 3-formylperylene **6** (Scheme 2) was converted into unsaturated ester **7**. Its hydrogenation over palladium resulted unexpectedly in unusual product **8** containing a partially hydrogenated perylene nucleus. Unlike the corresponding perylene derivatives, compound **8** is readily soluble in organic solvents, which allows, in particular, its reduction with lithium aluminum hydride to alcohol **9**. The structures of compounds **8** and **9** were confirmed by 2D heteronuclear $^1H—^{13}C$ NMR spectra (HMQC and HMBC). However, treatment of compound **9** with a standard dehydrogenation reagent, *viz.*, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), gave aldehyde **10** in 19% yield together with the target product **11** (yield 9%) and a number of unidentified compounds. Dehydrogenation of **9** with a milder reagent, Ph_3COH and $(CF_3CO)_2O$, in trifluoroacetic acid,[19] afforded the desired product in 16% yield. 3-(Perylen-3-yl)propanol **11** was converted into the target azide **12** *via* the corresponding methanesulfonate.

Table 1. Modified oligonucleotides synthesized using reagent **5** (X is the residue **4**-*p*)

Oligonucleotide	The sequence (5′→3′) (5′→3′)	*M*,[a] found / calculated	τ /min[b]
ON1	X-CATTACATCCAGAC	4495.68 / 4495.84	20.0
ON2	X-TCTGGATGTAATGG	4636.78 / 4636.84	21.0

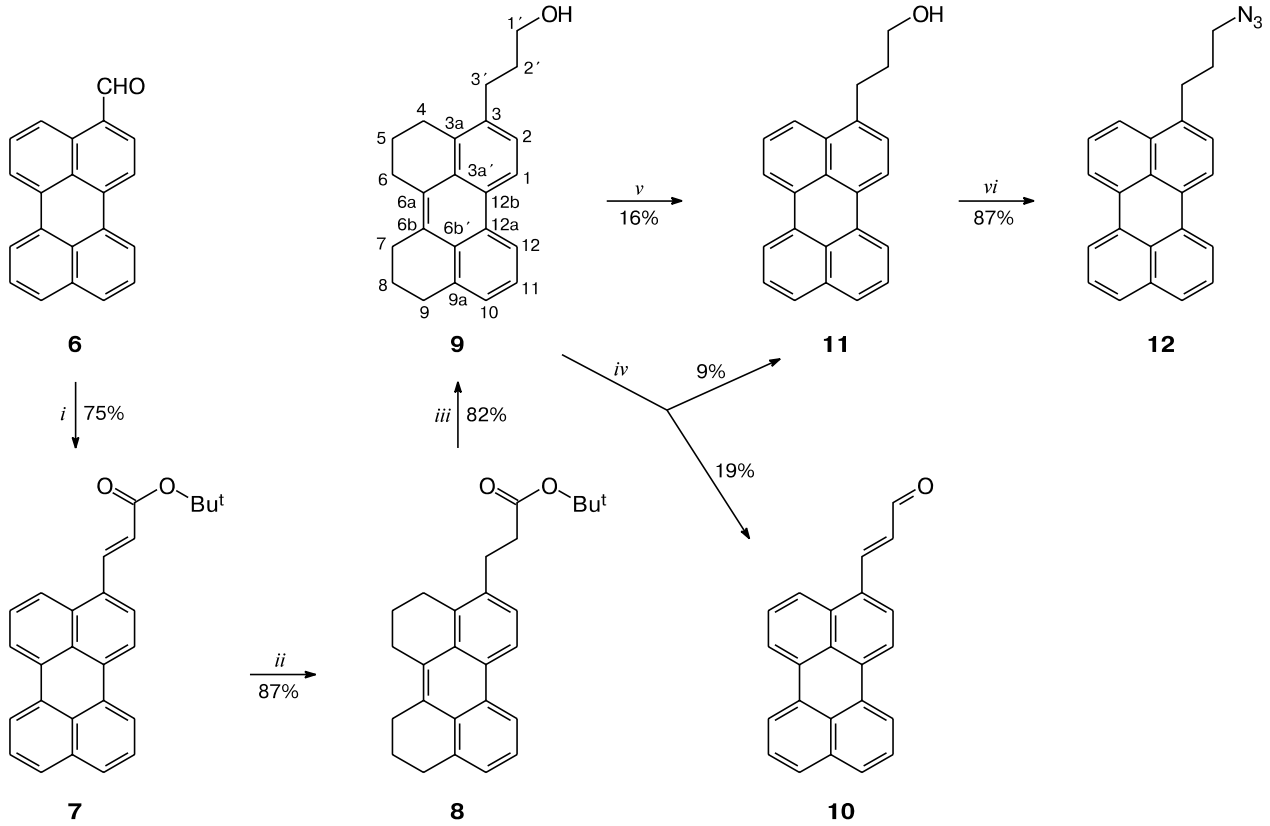
[a] MALDI-TOF mass spectrum; *M* is the mass; the values for the monoisotopic peak are given.

[b] Conditions of chromatography: 5% MeCN in 0.1 *M* aqueous NH_4OAc for 5 min; gradient from 5 to 35% in 30 min and from 35 to 80% in 30 min; τ is the retention time.

Azide **12** was coupled with acetylene-containing oligonucleotides **ON1** and **ON2** (Scheme 3, for **ON1**). The reaction was carried out in a triethylammonium acetate buffer solution in 30% aqueous DMSO (pH 7.0). Copper(I) species in a concentration of 1 mmol L^{-1} generated *in situ* by treatment of copper(II) sulfate with ascorbic acid or tris(2-carboxyethyl)phosphine were used as the catalysts. Note that in the absence of a catalyst, no even traces of the conjugate were formed after 24 h at room temperature or at 60 °C. Meanwhile, in the presence of a catalyst, the reaction proceeds to completion in 24 h at room temperature. According to HPLC, half of the starting oligonucleotide is consumed under the conditions described in approximately 4 h after addition of the catalyst.

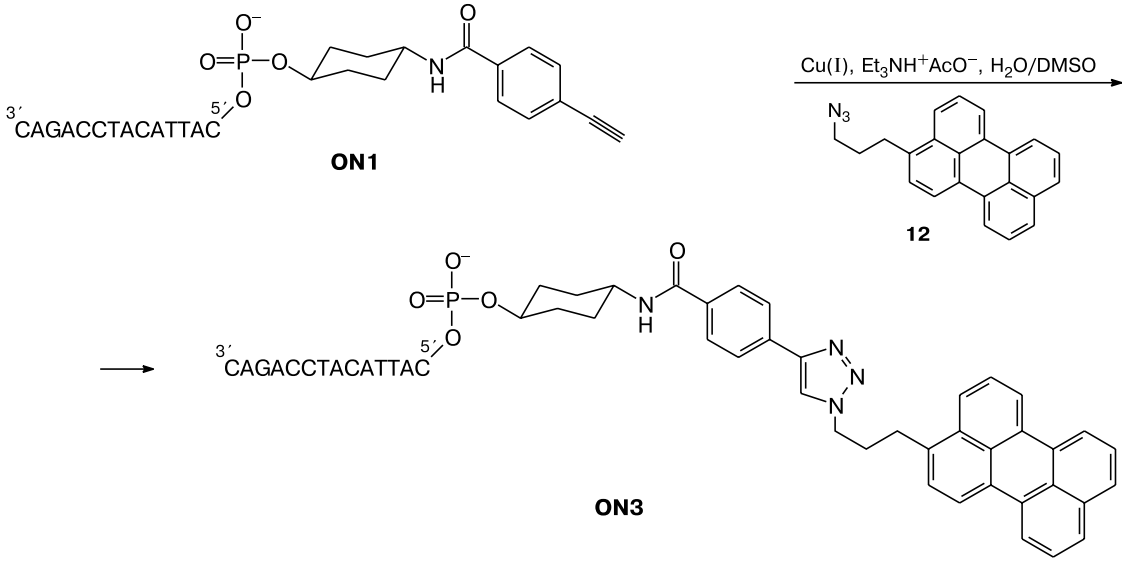
It was found that conjugation should be carried out in an inert atmosphere. When the reaction is carried out in

Scheme 2



Reagents and conditions: *i*. $Bu^tOCOCH{=}PPh_3$, EtOAc, 4 h, Δ; *ii*. H_2, Pd/C, EtOAc, 2 days; *iii*. $LiAlH_4$, Et_2O/THF; *iv*. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, PhMe, 100 °C, 30 min; *v*. Ph_3COH, $(CF_3CO_2)O$, CF_3CO_2H, Δ, 12 h; *vi*. CH_3SO_2Cl, Et_3N, CH_2Cl_2, 1 h; NaN_3, DMF, 12 h.

Scheme 3



air, the product yield substantially decreases according to HPLC, while the starting oligonucleotide is consumed almost completely (Fig. 1). This may be attributable to the known[20] oxidative degradation of oligonucleotides mediated by the Cu^{II}/Cu^{I} redox pair.

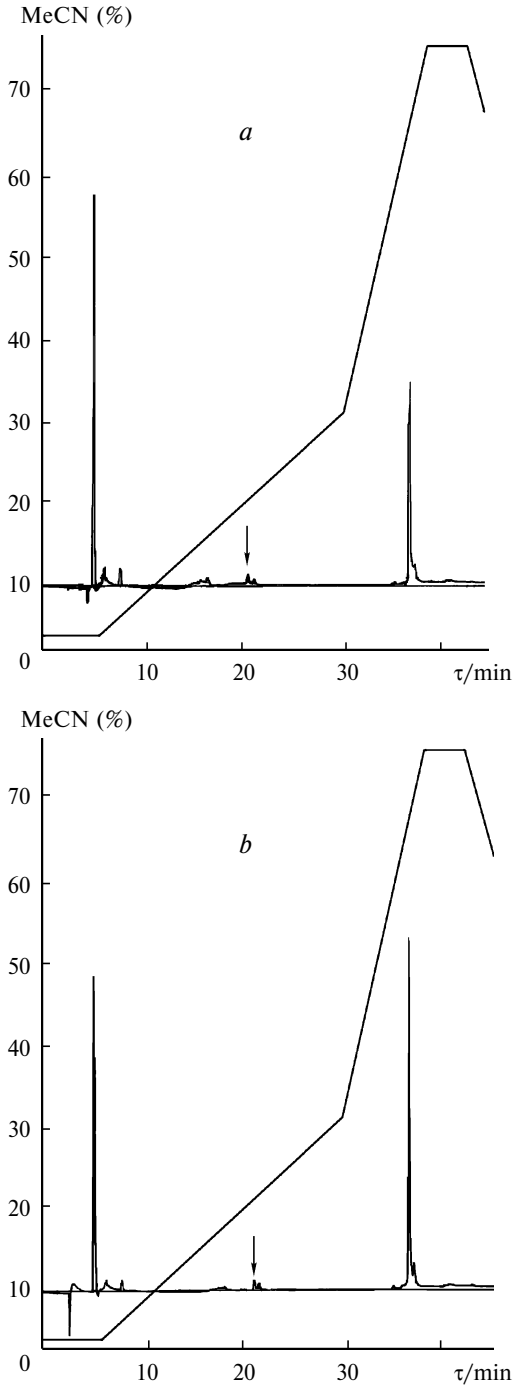


Fig. 1. HPLC of cycloaddition products obtained in air (*a*) and in an inert atmosphere (*b*). The arrow marks the peak of the starting oligonucleotide. The relative sensitivity is the same in both cases.

Table 2. Properties of the perylene conjugates synthesized by [3+2] cycloaddition of azide **12** to oligonucleotides containing an ethynyl group

Conjugate	Starting oligonucleotide	M,[a] found / calculated	τ[b] /min	Y[c] (%)
ON3	**ON1**	4833.07 / 4829.98	41.5	68 (28)
ON4	**ON2**	4973.98 / 4971.98	40.5	71 (30)

[a] MALDI-TOF mass spectrum; M is the mass; the values for the monoisotopic peak are given.
[b] Conditions of chromatography are given in the notes to Table 1.
[c] HPLC-based yields of products. The value in parentheses is the yield of the product isolated by gel electrophoresis; its amount was determined by spectrophotometry. The typical loss during gel electrophoresis isolation of oligonucleotides is about 50% (see Ref. 21) but the loss can be even higher when operating with small amounts of oligonucleotides.

Fluorescent perylene conjugates were isolated by denaturing polyacrylamide gel electrophoresis. The properties and the yields of the products are presented in Table 2. According to HPLC, the conjugate was the only component of the reaction mixture after completion of the process. Therefore, it is obvious that the low isolated yields of the oligonucleotides presented in the Table are mainly due to losses upon gel electrophoresis.

Thus, a modifying reagent for introduction of an ethynyl group into oligonucleotides was synthesized and the possibility of conjugation of acetylene-containing oligonucleotides with hydrophobic aliphatic azides by catalyzed [3+2] cycloaddition was demonstrated.

Experimental

Tetrakis(triphenylphosphine)palladium,[22] 3-formylperylene,[23] diisopropylammonium tetrazolide,[24] and cyanoethoxybis(diisopropylamino)phosphine[25] were synthesized by previously described procedures. Commercially available reagents were used: *trans*-4-aminocyclohexanol hydrochloride, tetrabutylammonium fluoride trihydrate, 4-iodobenzoic acid, trimethylsilylacetylene, triphenylmethanol, trifluoroacetic anhydride, *tert*-butoxycarbonylmethylenetriphenylphosphorane, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tris(2-carboxyethyl)phosphine, and tris(pyrrolidino)benzotriazol-1-yloxyphosphonium hexafluorophosphate (Aldrich, Fluka, Lancaster, and Avocado). Extra pure grade solvents were distilled prior to use. The reactions were monitored by TLC on Kieselgel 60 F_{254} plates (Merck); the spots were visualized in the UV light at 254 nm. Column chromatography was carried out on silica gel Kieselgel 60 (Merck), particle size 40—63 μm. The solutions were concentrated on a rotary evaporator in a water jet pump vacuum at a bath temperature of 30—50 °C. NMR spectra were recorded on a Bruker AC-500 spectrometer at 500 MHz (^{1}H),

125.7 MHz (^{13}C), and 202.4 MHz (^{31}P). The spectra were calibrated using the residual signals of the DMSO-d_6 protons (δ_H 2.50 and δ_C 39.7) or $CDCl_3$ (δ_H 7.25 for ^1H and δ_C 77.0); the chemical shifts were referred to $SiMe_4$ (^1H and ^{13}C) or 85% H_3PO_4 (^{31}P). 2D heteronuclear $^1H—^{13}C$ gradient selected NMR spectra, HMQC and HMBC, were obtained by using $2048(t_2)\times256(t_1)$ complex point data sets, zero filled to $2048(F_2)\times1024(F_1)$. The spectral window widths were 13 and 200 ppm for ^1H and ^{13}C, respectively. The HMBC spectra were recorded with a 50 ms delay for evolution of long-range couplings. The melting points were determined on a Boetius hot stage (not corrected). The oligonucleotide synthesis was carried out on a BiosSet ASM-700 instrument on a 200 nmol scale by standard procedures. MALDI-TOF mass spectra were measured on a Bruker Ultraflex spectrometer. A Beckman 153 chromatograph and a Supelco Discovery C18 column (250×4 mm) were used for HPLC.

(4-Trimethylsilylethynyl)benzoic acid (2). A solution of 4-iodobenzoic acid (2.480 g, 10 mmol) in DMF (15 mL) was degassed three times by evacuating the flask and then filling it with argon. In an argon flow, trimethylsilylacetylene (1.83 mL, 13 mmol), triethylamine (4.17 mL, 30 mmol), tetrakis(triphenylphosphine)palladium (577 mg, 0.5 mmol), and copper(I) iodide (190 mg, 1 mmol) were added successively. The mixture was kept under argon at room temperature for 16 h and partitioned between ethyl acetate (100 mL) and 10% HCl (100 mL). The organic layer was separated, washed with water (3×100 mL) and brine (100 mL), dried with Na_2SO_4, and concentrated. The residue was chromatographed on silica gel in a 20% Me_2CO—PhMe system, and the reaction product was recrystallized from hexane. Yield 1.293 g (59%). Colorless crystals, m.p. 153—156 °C (hexane), R_f 0.46 (20% Me_2CO—PhMe). ^1H NMR ($CDCl_3$), δ: 0.26 (s, 9 H, C<u>H</u>$_3$); 7.54, 8.03 (both d, 2 H each, CH_{Ar}, J = 8.2 Hz).

***trans*-4-(4-Trimethylsilylethynylbenzoylamino)cyclohexanol (3).** Tris(pyrrolidino)benzotriazol-1-yloxyphosphonium hexafluorophosphate (1.147 g, 2.205 mmol) and diisopropylethylamine (0.78 mL, 4.5 mmol) was added to a solution of 4-(trimethylsilylethynyl)benzoic acid (491 mg, 2.25 mmol) in DMF (5 mL). The mixture was stirred for 20 min at ~20 °C, then a solution of *trans*-4-aminocyclohexanol hydrochloride (319 mg, 2.25 mmol) and diisopropylethylamine (0.78 mL, 4.5 mmol) in DMF (5 mL) was added. After 4 h, the reaction mixture was poured in a mixture of water (50 mL) and EtOAc (120 mL). The organic layer was separated and washed with water (2×50 mL), a saturated solution of $NaHCO_3$ (50 mL), again water (50 mL), and brine (50 mL), dried with Na_2SO_4, and concentrated. The residue was recrystallized from PhMe—MeOH. Yield 576 mg (81%). Colorless crystals, m.p. 228—229 °C (PhMe—MeOH). R_f 0.28 (10% Me_2CO—$CHCl_3$). ^1H ($CDCl_3$), δ: 0.25 (s, 9 H, C<u>H</u>$_3$); 1.24—1.34 (m, 2 H); 1.40—1.51 (m, 3 H) ($H_{ax}(2)$, $H_{ax}(3)$, $H_{ax}(5)$, $H_{ax}(6)$, O<u>H</u>); 2.02, 2.11 (both m, 2 H each) ($H_{eq}(2)$, $H_{eq}(3)$, $H_{eq}(5)$, $H_{eq}(6)$); 3.64 (m, 1 H, H(4)); 3.94 (m, 1 H, H(1)); 5.86 (d, 1 H, N<u>H</u>, J = 7.4 Hz); 7.49, 7.66 (both d, 2 H each, H(2′), H(3′), H(5′), H(6′), J = 8.2 Hz).

***trans*-4-(4-Ethynylbenzoylamino)cyclohexanol (4).** A solution of tetrabutylammonium fluoride (555 mg, 2.121 mmol) in THF (5 mL) was added to a solution of compound **3** (539 mg, 1.767 mmol) in THF (15 mL). The mixture was stirred for 10 min at ~20 °C, diluted with PhMe (50 mL), and concentrated, and the residue was chromatographed on silica gel in a 20% MeOH—$CHCl_3$ system. Yield 427 mg (99%). Colorless crystals, m.p. 220—221 °C (hexane—$CHCl_3$). R_f 0.29 (10% MeOH—$CHCl_3$). ^1H NMR (DMSO-d_6), δ: 1.18—1.28 (m, 2 H); 1.31—1.41 (m, 2 H), ($H_{ax}(2)$, $H_{ax}(3)$, $H_{ax}(5)$, $H_{ax}(6)$); 1.76—1.88 (m, 4 H, $H_{eq}(2)$, $H_{eq}(3)$, $H_{eq}(5)$, $H_{eq}(6)$); 3.39 (m, 1 H, H(4)); 3.70 (m, 1 H, H(1)); 4.33 (s, 1 H, ≡C<u>H</u>); 4.53 (d, 1 H, O<u>H</u>, J = 4.2 Hz); 7.54 (d, 2 H, H(3′), H(5′), J = 8.3 Hz); 7.83 (d, 2 H, H(2′), H(6′), J = 8.3 Hz); 8.23 (d, 1 H, N<u>H</u>, J = 7.9 Hz).

***trans*-1-[(*N*,*N*-Diisopropylamino)-(2-cyanoethoxy)phosphinoxy]-4-(4-ethynylbenzoylamino)cyclohexane (5).** Diisopropylammonium tetrazolide (240 mg, 1.400 mmol) and bis(diisopropylamino)cyanoethoxyphosphine (422 mg, 1.400 mmol) were added under argon to a stirred suspension of *trans*-4-(4-ethynylbenzoylamino)cyclohexanol (**4**) (227 mg, 0.933 mmol) in dry CH_2Cl_2 (30 mL). The mixture was kept at room temperature under argon for 16 h, diluted with CH_2Cl_2 (50 mL), and poured in a water (50 mL)—CH_2Cl_2 (50 mL) mixture. The organic layer was separated, washed with water (50 mL), a saturated aqueous solution of $NaHCO_3$ (2×50 mL), and again with water (50 mL), dried with Na_2SO_4, and concentrated. The residue was chromatographed on silica gel in 10% Me_2CO and 1.5% Et_3N in PhMe. Yield 369 mg (89%). Colorless crystals, t.dec. >110 °C. R_f 0.47 (10% Me_2CO in PhMe). ^1H NMR (DMSO-d_6), δ: 1.14 (d, 12 H, C<u>H</u>$_3$, J = 6.7 Hz); 1.42 (m, 4 H, $H_{ax}(2)$, $H_{ax}(3)$, $H_{ax}(5)$, $H_{ax}(6)$); 1.80—2.05 (m, 4 H, $H_{eq}(2)$, $H_{eq}(3)$, $H_{eq}(5)$, $H_{eq}(6)$); 2.80 (t, 2 H, C<u>H</u>$_2$CN, J = 6.0 Hz); 3.53—3.62 (m, 2 H, Me_2C<u>H</u>N); 3.64—3.80 (m, 4 H, H(1), H(4), C<u>H</u>$_2$CH$_2$CN); 4.33 (s, 1 H, ≡CH); 7.58 (d, 2 H, H(3′), H(5′), J = 8.3 Hz); 7.87 (d, 2 H, H(2′), H(6′), J = 8.3 Hz); 8.25 (d, 1 H, N<u>H</u>, J = 7.7 Hz). ^{13}C NMR (DMSO-d_6), δ: 19.89 (d, <u>C</u>H$_2$CN, $^3J_{P,C}$ = 6.9 Hz); 24.31 (d, 2 C, Me, $^3J_{P,C}$ = 6.9 Hz); 24.43 (d, 2 C, Me, $^3J_{P,C}$ = 6.9 Hz); 29.91, 29.97 (C(3), C(5)); 32.71 (d, $^3J_{P,C}$ = 3.4 Hz); 32.80 (d, $^3J_{P,C}$ = 3.4 Hz) (C(2), C(6)); 42.54 (d, PN<u>C</u>H, $^2J_{P,C}$ = 12.6 Hz); 47.55 (C(4)); 58.01 (d, PO<u>C</u>H$_2$, $^2J_{P,C}$ = 17.8 Hz); 71.90 (d, C(1), $^2J_{P,C}$ = 17.7 Hz); 82.68 (≡<u>C</u>H); 82.96 (<u>C</u>≡CH); 119.05 (<u>C</u>N); 124.25 (C(4′)); 127.59 (C(2′), C(6′)); 131.54 (C(3′), C(5′)); 134.84 (C(1′)); 164.78 (<u>C</u>O). ^{31}P NMR (DMSO-d_6), δ: 146.21.

***tert*-Butyl *trans*-3-(perylen-3-yl)acrylate (7).** Solid (*tert*-butoxycarbonylmethylene)triphenylphosphorane (361 mg, 0.96 mmol) was added to a suspension of 3-formylperylene (192 mg, 0.8 mmol) in EtOAc (75 mL), the mixture was stirred for 12 h, an additional portion of (*tert*-butoxycarbonylmethylene)triphenylphosphorane (90 mg, 0.24 mmol) was added, and the mixture was refluxed for 4 h. The solution was cooled, concentrated, and then toluene (100 mL) was added to, and distilled from the residue, and the residue was chromatographed on silica gel in $CHCl_3$ and recrystallized from a $CHCl_3$—MeOH system. Yield 227 mg (75%). Bright red crystals, m.p. 223—225 °C (MeOH—$CHCl_3$). R_f 0.56 ($CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.58 (s, 9 H, C<u>H</u>$_3$); 6.47 (d, 1 H, H(2′), $J_{2',3'}$ = 15.7 Hz); 7.49 (m, 2 H, H(8), H(11)); 7.56 (m, 1 H, H(5)); 7.70 (m, 2 H, H(9), H(10)); 7.74 (d, 1 H, H(2), J = 8.0 Hz); 8.05 (d, 1 H, H(4), J = 8.5 Hz); 8.14—8.26 (m, 4 H, H(1), H(6), H(7), H(12)); 8.36 (d, 1 H, H(3′), $J_{2',3'}$ = 15.7 Hz).

***tert*-Butyl 3-(4,5,6,7,8,9-hexahydroperylen-3-yl)propionate (8).** Compound **7** (1.09 g, 3.00 mmol) and 5% Pd/C (300 mg) were suspended in EtOAc (150 mL). The suspension was evacuated, flushed with hydrogen, stirred for 48 h in a hydrogen atmosphere, and filtered through a glass fiber filter. The filtrate

was concentrated and the residue was chromatographed on silica gel in PhMe. Yield 1.01 g (87%). Yellowish crystals, m.p. 156—160 °C (MeOH—$CHCl_3$), R_f 0.44 (PhMe). ^{1}H NMR ($CDCl_3$), δ: 1.45 (s, 9 H, C<u>H</u>$_3$); 2.09 (m, 4 H, H(5), H(8)); 2.57 (t, 2 H, H(2´), $J_{2´,3´}$ = 8.0 Hz); 3.02—3.14 (m, 10 H, H(4), H(6), H(7), H(9), H(3´)); 7.33 (d, 1 H, H(10), $J_{10,11}$ = 7.0 Hz); 7.39 (d, 1 H, H(2), $J_{1,2}$ = 8.5 Hz); 7.44 (dd, 1 H, H(11), $J_{10,11}$ = 7.0 Hz, $J_{11,12}$ = 8.5 Hz); 8.47 (d, 1 H, H(1), $J_{1,2}$ = 8.5 Hz); 8.49 (d, 1 H, H(12), $J_{11,12}$ = 8.5 Hz).

3-(4,5,6,7,8,9-Hexahydroperylen-3-yl)propanol (9). A solution of *tert*-butyl ester **8** (920 mg, 2.38 mmol) in an ether (10 mL) and THF (10 mL) mixture was added dropwise to a stirred suspension of $LiAlH_4$ (190 mg, 5 mmol) in anhydrous ether (5 mL) at such a rate that the mixture was maintained at boiling. Then the mixture was kept for 10 min, propan-2-ol (0.5 mL) and water (5 mL) were added dropwise, and the reaction mixture was poured into Et_2O (100 mL) and 10% HCl (50 mL). The organic layer was washed with water (2×50 mL) and brine (50 mL), dried with Na_2SO_4, and concentrated. The residue was recrystallized from a PhMe—light petroleum mixture. Yield 620 mg (82%). Colorless crystals, m.p. 94—96 °C (PhMe—petroleum ether), R_f 0.30 (10% Me_2CO in PhMe). ^{1}H NMR ($CDCl_3$), δ: 1.93 (m, 2 H, H(2´)); 2.05—2.13 (m, 4 H, H(5), H(8)); 2.91 (t, 2 H, H(3´), $J_{2´,3´}$ = 7.8 Hz); 3.03—3.13 (m, 8 H, H(4), H(6), H(7), H(9)); 3.74 (t, 1 H, H(1´), $J_{1´,2´}$ = 6.3 Hz); 7.31 (d, 1 H, H(10), $J_{10,11}$ = 7.0 Hz); 7.39 (d, 1 H, H(2), $J_{1,2}$ = 8.5 Hz); 7.48 (dd, 1 H, H(11), $J_{10,11}$ = 7.0 Hz, $J_{11,12}$ = 8.5 Hz); 8.49 (m, 2 H, H(1), H(12)). ^{13}C NMR ($CDCl_3$), δ: 22.83, 23.13 (C(5), C(8)); 27.34, 27.95, 28.33 (C(4), C(6), C(7)); 29.78 (C(3´)); 31.50 (C(9)); 33.40 (C(2´)); 62.63 (C(1´)); 120.63, 120.67 (C(1), C(12)); 124.79 (C(11)); 125.29 (C(10)); 126.90 (C(2)); 128.10 (C(12b)); 128.32 (C(6b´)); 128.86 (C(3a´)); 129.09 (2 C, C(6a), C(6b)); 129.61 (C(12a)); 133.39 (C(3a)); 136.35 (2 C, C(3), C(9a)).

***trans*-3-(Perylen-3-yl)acrolein (10) and 3-(perylen-3-yl)propanol (11).** A solution of 3-(4,5,6,7,8,9-hexahydro-3-perylenyl)propanol **9** (474 mg, 1.50 mmol) and 1,2-dichloro-5,6-dicyanobenzo-1,4-quinone (1.362 g, 6 mmol) in PhMe (15 mL) was kept for 30 min at 100 °C. The solution was cooled, 6 *M* NaOH (20 mL) was added, and the mixture was filtered through celite. The organic layer was separated, washed with water (2×50 mL), dried with Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (4→8% Me_2CO in PhMe). The yield of 3-(perylen-3-yl)propanol (**11**) was 40 mg (9%). Yellow crystals, m.p. 143—145 °C, R_f 0.20 (20% Me_2CO in PhMe). ^{1}H NMR (DMSO-d_6), δ: 1.80—1.87 (m, 2 H, H(2´)); 3.04 (t, 2 H, H(3´), $J_{2´,3´}$ = 7.6 Hz); 3.53 (t, 2 H, H(1´), $J_{1´,2´}$ = 6.4 Hz); 4.56 (br.s, 1 H, O<u>H</u>); 7.41 (d, 1 H, H(2), $J_{1,2}$ = 7.9 Hz); 7.49—7.55 (m, 2 H, H(8), H(11)); 7.58 (m, 1 H, H(5)); 7.76 (m, 2 H, H(9), H(10)); 7.96 (d, 1 H, H(4), $J_{4,5}$ = 8.5 Hz); 8.27 (d, 1 H, H(1), *J* = 7.9 Hz); 8.30 (d, 1 H, *J* = 7.3 Hz), 8.34 (d, 1 H, *J* = 7.6 Hz) (H(7), H(12)); 8.37 (d, 1 H, H(6), $J_{5,6}$ = 7.3 Hz). The yield of *trans*-3-(perylen-3-yl)acrolein (**10**) was 88 mg (19%). Red crystals, m.p. 234—236 °C, R_f 0.47 (10% Me_2CO in PhMe). ^{1}H NMR ($CDCl_3$), δ: 6.87 (dd, 1 H, H(2´), $J_{2´,3´}$ = 15.6 Hz, $J_{1´,2´}$ = 7.6 Hz); 7.51 (m, 2 H, H(8), H(11)); 7.59 (m, 1 H, H(5)); 7.71 (d, 1 H, *J* = 8.2 Hz), 7.74 (d, 1 H, *J* = 8.2 Hz) (H(9), H(10)); 7.79 (d, 1 H, H(2), $J_{1,2}$ = 7.9 Hz); 8.01 (d, 1 H, H(4), $J_{4,5}$ = 8.5 Hz); 8.16 (d, 1 H, H(1), $J_{1,2}$ = 7.9 Hz); 8.19—8.27 (m, 4 H, H(6), H(7), H(12), H(3´)); 9.84 (d, 1 H, C<u>H</u>O, $J_{1´,2´}$ = 7.6 Hz).

3-(Perylen-3-yl)propanol (11). A solution of 3-(4,5,6,7,8,9-hexahydroperylen-3-yl)propanol (130 mg, 0.41 mmol), triphenylmethanol (321 mg, 1.23 mmol), and trifluoroacetic anhydride (0.23 mL, 1.64 mmol) in CF_3CO_2H (10 mL) was refluxed for 12 h and concentrated. The residue was dissolved in MeOH (10 mL) and added to a solution of NaOH (100 mg). After 1 h, the solution was acidified with AcOH (200 μL) and concentrated. The residue was treated with PhMe (50 mL), concentrated, and chromatographed on silica gel (10% Me_2CO in PhMe). Yield 21 mg (16%).

3-(Perylen-3-yl)propyl azide (12). Methanesulfonyl chloride (26 μL, 0.34 mmol) and triethylamine (35 μL, 0.25 mmol) were added successively to a suspension of 3-(perylen-3-yl)propanol (52 mg, 0.17 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h at ~20 °C, the solvent was evaporated, the residue was dissolved in DMF (5 mL), and sodium azide was added (82 mg, 1.3 mmol). The mixture was kept for ~16 h and poured into an H_2O (50 mL)—EtOAc (100 mL) mixture. The organic layer was washed with H_2O (2×50 mL), saturated aqueous $NaHCO_3$ (2×50 mL), again H_2O (50 mL), and brine (50 mL), dried with Na_2SO_4, and concentrated. The residue was chromatographed on silica gel in PhMe. Yield 49 mg (87%). Yellow crystals, m.p. 128—130 °C, R_f 0.39 (30% PhMe in hexane). ^{1}H NMR ($CDCl_3$), δ: 2.05 (m, 2 H, H(2´)); 3.11 (t, 2 H, H(3´), *J* = 7.6 Hz); 3.39 (t, 2 H, H(1´), *J* = 6.5 Hz); 7.33 (d, 1 H, H(2), $J_{1,2}$ = 7.8 Hz); 7.46 (m, 2 H, H(8), H(11)); 7.52 (m, 1 H, H(5)); 7.66 (m, 2 H, H(9), H(10)); 7.84 (d, 1 H, H(4), $J_{4,5}$ = 8.5 Hz); 8.11 (d, 1 H, H(1), $J_{1,2}$ = 7.8 Hz); 8.15 (d, 1 H, *J* = 7.3 Hz); 8.18 (d, 1 H, *J* = 7.6 Hz) (H(7), H(12)); 8.21 (d, 1 H, H(6), $J_{5,6}$ = 7.3 Hz).

Oligonucleotide synthesis was carried out in an automated mode using standard phosphoramidites (dA^{Bz}, dC^{Bz}, dG^{iBu}, dT) according to manufacturer´s protocols. The condensation of the terminal phosphoramidite **5** was carried out for 1 min (capping and removal of the dimethoxytrityl group were omitted). Oligonucleotides were cleaved from the support and deprotected by treatment with concentrated (28%) ammonia (0.75 mL). After deprotection, the resulting solution was freeze-dried, the residue was dissolved in water (150 μL), and the oligonucleotide was precipitated with acetone (1.5 mL), centrifuged, washed with acetone (1 mL), dissolved in 50% aqueous formamide (200 μL), and subjected to electrophoresis in 20% denaturing (7 *M* urea) polyacrylamide gel (20 cm, 400 V, 20 mA, Bromophenol Blue and Xylene Cyanol as leading dyes). Oligonucleotides were visualized in the gel by absorption, eluted with 0.5 *M* $LiClO_4$ for 12 h, filtered to remove the gel, and desalted on Sephadex G-50 columns. The oligonucleotide concentration was determined by spectrophotometry by measuring absorption at 260 nm. The mass spectra of the oligonucleotides were recorded using a 1 : 1 (v/v) freshly prepared mixture of solutions of 2,6-dihydroxyacetophenone (40 mg in 1 mL of methanol) and diammonium citrate (80 mg in 1 mL of water) as the ionization matrix for MALDI-TOF.

Conjugation (general procedure). Dimethyl sulfoxide (15 μL), an aqueous solution of triethylammonium acetate (0.2 *M*, pH 7, 32 μL), a solution of azide **12** in DMSO (1.19 mmol L^{-1}, 16.8 μL), and an aqueous solution of copper(II) sulfate pentahydrate (7.94 *mM*, 12.6 μL) were mixed in a 1.5 mL disposable plastic tube. The solution was degassed by purging with argon for 30 s, and then an aqueous solution of ascorbic acid (12.5 *mM*, 8.0 μL) and a solution of the oligonucleotide (0.1 *mM*,

20 μL) were added. The tube was purged with argon once again, closed, and kept at room temperature for 24 h. The oligonucleotide was precipitated with acetone (1.4 mL) and centrifuged. The precipitate was dissolved in 50% aqueous formamide (15 μL) and subjected to electrophoresis in 20% denaturing (7 *M* urea) polyacrylamide gel (20 cm, 550 V, 15 mA, Bromophenol Blue and Xylene Cyanol as leading dyes). The oligonucleotides were visualized in the gel based on fluorescence, eluted with 0.5 *M* $LiClO_4$ for 12 h, filtered to remove the gel, and desalted on Sephadex G-50 columns.

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