Chemical Science

EDGE ARTICLE

Observation of the Rare Chrysene Excimer

Oleg Khorev, Caroline Bösch, Markus Probst and Robert Häner*

Formation of the so far elusive chrysene excimer in solution is achieved by using DNA as a supramolecular scaffold. Oligonucleotides possessing one or two chrysene building blocks have been synthesized. Chrysene excimer fluorescence has been unambiguously observed in DNA double strands, as well as in single strands containing two neighbouring chrysenes.

Introduction

Chrysene (Figure 1) is an alternant polycyclic aromatic hydrocarbon (PAH).[1] Compared to other PAHs like pyrene, benzo[a]pyrene, or linear acenes, it has met rather limited interest as a substrate for biological or electronic applications or for use in the materials sciences.[2] While extensive reviews on organic electronics are available for, e.g., pyrene[3] and linear acenes[2] none exist for chrysene even though it has recently been explored for organic light-emitting diode (OLED) applications.[4]

One of the main photophysical characteristics of PAHs is their ability to form excimers in solution,[5] in the solid state (e.g. polymers, crystals) and in organized assemblies (e.g. membranes, micelles, LB films). Excimers (*exci*ted di*mers*) can be easily observed in the fluorescence emission spectrum[6] and were first described by Förster in 1954 for pyrene.[7] Since then, excimers have been observed with many PAHs, the limiting factor being primarily the low solubility of larger PAHs at relatively high concentrations ($\sim 10^{-2}$ M) that are needed for excimer formation.[8] The excimer band exhibits a broad, Gaussian shape that lacks vibrational structure. It is strongly red-shifted with respect to the monomer emission band.[5,8]

Although the photophysical properties of chrysene have been extensively studied,[9-11] one question remains unanswered: can chrysene form an excimer in solution under standard conditions? The history of the chrysene excimer is noteworthy. Early attempts to detect it in solution failed, leading J. B. Birks himself to postulate that perhaps chrysene is the only PAH that cannot form an excimer.[12] Chrysene excimer emission could not be detected in LB films,[13] nor was it observed in pure single crystals.[14,15] So far the only experimental evidence for its formation has been obtained from a high pressure study of chrysene microcrystals.[16] Furthermore, a possible excimer component was observed in chrysene containing vinyl copolymers.[17]

DNA has been successfully applied as a supramolecular scaffold for organizing and studying organic chromophores.[18-45] We have previously reported on the introduction of PAHs into DNA, which leads to the appearance of remarkable spectroscopic and electronic effects, such as the formation of excimers,[46,47] exciplexes,[48] J- and H-aggregates,[49] energy transfer[50] or aggregation-induced fluorescence.[51] Non-nucleosidic dialkynyl PAH building blocks,[52,53] as well as nucleoside-derived, alkynyl-substituted PAHs have been introduced into DNA by several groups.[54-59] Here, we report on the formation and characterization of the chrysene excimer in single and double stranded DNA (Figure 1).
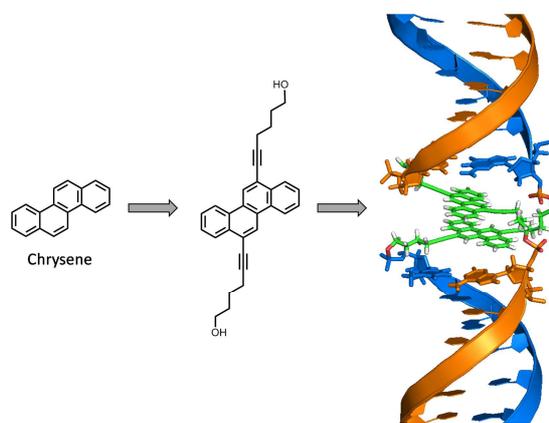

Figure 1 Illustration of structural organization of chrysene molecules in a DNA supramolecular scaffold (HyperChem, minimized with *amber* force field).[60]

Results and discussion

Synthesis

Chrysene (**1**, Scheme 1) was brominated using published procedures[61,62] giving 6,12-dibromochrysene (**2**) in an 84% yield. Compound **2** was then coupled with 5-hexyn-1-ol under

Sonogashira conditions giving the dialkynyl diol **3** in a 42% yield. Finally mono-DMT protection (→ **4**) and phosphitylation gave the phosphoramidite **5**. The latter was incorporated into DNA by standard solid-phase synthesis procedures to furnish oligonucleotide strands with one (**6** and **7**) or two (**8** and **9**) chrysene incorporations per strand (Table 1).
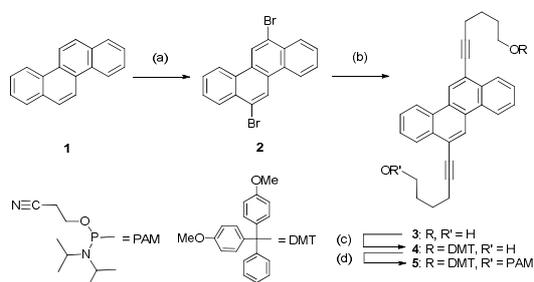

Scheme 1 Synthesis of chrysene phosphoramidite **5**. Conditions: (a) Br_2, 1,2-dichloroethane, 85 °C, 18 h, 84%; (b) 5-hexyn-1-ol, Pd[PPh$_3$]$_2$Cl$_2$, CuI, THF/Et$_3$N (1:1), 80 °C, 46 h, 42%; (c) DMTCl, Et$_3$N, THF, 24 h, rt, 53%; (d) PAMCl, Et$_3$N, DCM, 1 h, rt, 71%.

Duplex stability

Thermal denaturation experiments (ESI) observed at 260 nm gave melting temperature (T_m) values of 77 °C for **6*****7** and 74 °C for **8*****9** (Table 2). The T_m of the unmodified reference duplex (**refA*****refB**) was 71 °C.

Table 1 Oligonucleotide sequences

refA	5'-AGC TCG GTC ATC GAG AGT GCA-3'
refB	3'-TCG AGC CAG TAG CTC TCA CGT-5'
6	5'-AGC TCG GTC A**X**C GAG AGT GCA-3'
7	3'-TCG AGC CAG T**X**G CTC TCA CGT-5'
8	5'-AGC TCG GTC A**XX** GAG AGT GCA-3'
9	3'-TCG AGC CAG T**XX** CTC TCA CGT-5'
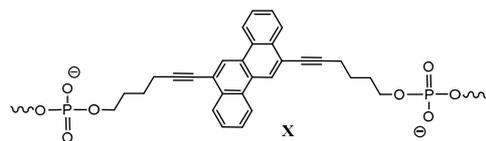
UV-vis absorption and fluorescence spectra

In general, ethynyl substitution on PAHs causes a red shift in the absorption and emission bands as well as an increase in the fluorescence quantum yield.[63,64] The UV-vis absorption spectra of the chrysene diol **3** (Figure 2) reveal pronounced conjugation effects on the absorption and emission properties upon triple bond addition. As a result of a reduction in symmetry, the forbidden $S_0 \rightarrow S_1$ transition of chrysene becomes allowed which is reflected by an increase in the absorption coefficient (ε).[65] The emission maximum of **3** is red-shifted by 23 nm compared to chrysene (**1**). Furthermore, the fluorescence quantum yield (Φ_F) increases from 0.14 for chrysene to 0.41 for the dialkynyl derivative **3** (Table 2), also as a result of the

aforementioned symmetry decrease. Similar effects have been reported for pyrene, where the symmetry-forbidden S_0-S_1 transition also becomes allowed upon ethynyl disubstitution.[63]

The UV-vis spectra of the different oligomers and hybrids are shown in Figure 3. The absorption by chrysene is seen between 320 and 390 nm. As can be seen, (Figure 3, inset), the relative heights of the 0→0 and the 0→1 vibronic bands (370 and 350 nm, respectively) in oligomers **6** and **7** are the same as those of compound **3** (Figure 2). However, in oligomers **8** and **9**, with two consecutive chrysene incorporations, a pronounced inversion of the relative peak heights can be seen, which serves as an indication for π-π stacking.[66-68]
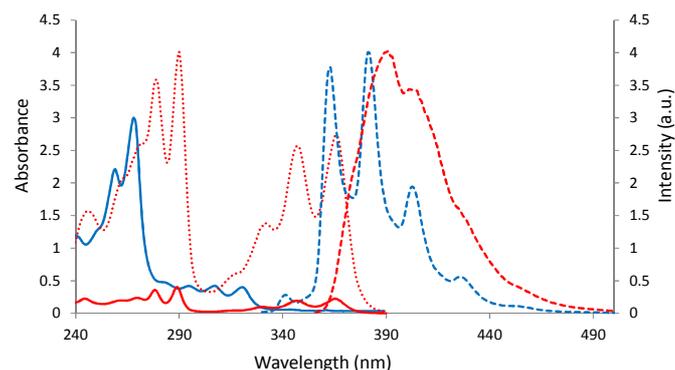

Figure 2 Absorption (solid line), fluorescence (scaled, dashed line) and excitation (scaled, dotted line) spectra of chrysene (**1**; blue; 2.45×10-5 M) and compound **3** (red; absorption: 5.35×10-6 M; emission: 1.33×10-6 M) in THF.

Table 2 Spectroscopic data of chrysene, compound 3, oligomers **6-9** and hybrids **6*****7** and **8*****9**

	abs λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	emission λ_{max} (nm)	Φ_F	T_m (°C)
1	361	700[a]	362	0.14[a]	-
3	367	41000[b]	391	0.41[b]	-
6	371	-	387	0.08	-
7	371	-	387	0.02	-
8	371	-	446	0.21	-
9	371	-	455	0.07	-
6***7**	372	-	471	0.30	77
8***9**	372	-	471	0.16	74

a) In cyclohexane;[69] b) in THF

The UV-vis spectra of duplexes **6*****7** and **8*****9** are also shown in Figure 3. An inversion of the relative heights of the vibronic bands is observed after hybridisation of single strands **6** and **7** to form duplex **6*****7**, whereas little change occurs upon hybridisation of **8** and **9**. The latter indicates that adjacent chrysenes are already π-stacked in single strands **8** and **9**.
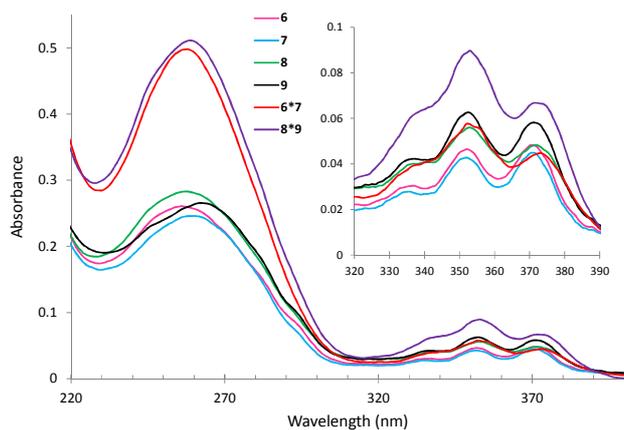
Figure 3 Absorption spectra of single strands **6-9** and corresponding duplexes **6*7** and **8*9**. Inset: 320-400 nm range. Conditions: 10 mM sodium phosphate buffer pH 7.0, 0.1 M NaCl, oligomer conc. 1.0 μM single and double strands.
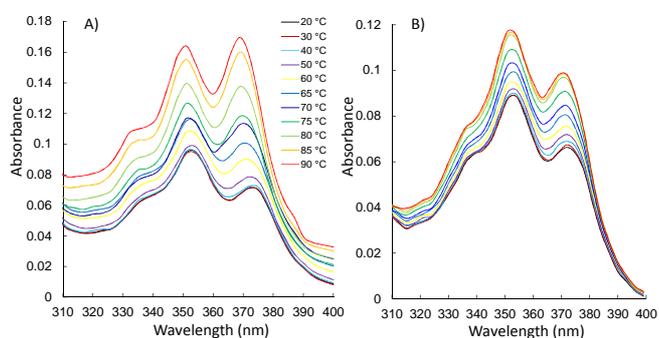

Figure 4 Temperature-dependent UV-vis spectra of duplex **6*7** (A) and **8*9** (B). Conditions: see Fig. 3; oligo conc. 1.75 μM each strand.

The temperature-dependent UV-vis spectra of the duplexes are shown in Figure 4. As duplex **6*7** is heated from 20 to 90 °C, there is a slight blue shift (4 nm) accompanied by inversion of vibronic band heights indicating breakdown of intermolecular π-stacking interactions. A minor blue shift (2 nm) is observed for duplex **8*9** upon heating and there is almost no change in the vibronic band structure further indicating that, although the interstrand interaction is gone, considerable intramolecular π-stacking between neighbouring chrysenes still exists within each DNA single strand.

Fluorescence spectra (Figure 5) provide clear evidence for chrysene excimer formation. Single strands **6** and **7** exhibit only monomer fluorescence. However, hybridization of the two strands results in a complete change of the spectrum showing exclusively an excimer emission centered around 471 nm, which originates from the formation of an excimer via interstrand stacking of the two chrysene molecules. Intrastrand excimer formation can already be seen in single strands **8** and **9** which possess two neighbouring chrysene units. A shoulder on the left side of the excimer emission band in oligomer **8** is attributable to residual monomer fluorescence. The maxima of the emission bands are 446 and 455 nm, respectively.

Hybridization of these two strands leads to a stack of four chrysene units and a red-shift of the excimer band with a maximum at the same wavelength as the one in hybrid **6*7**.
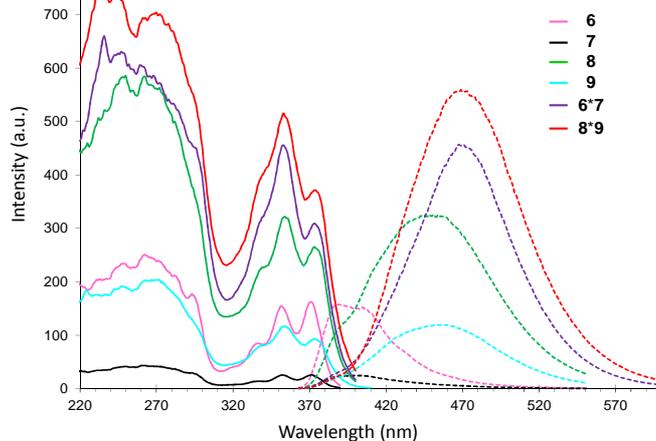

Figure 5 Fluorescence (dashed line) and excitation (solid line) spectra of single strands **6-9** and hybrids **6*7** and **8*9**. Conditions: see Fig. 3; oligo conc. 1.0 μM each strand. Excitation: 353 nm; emission: 400 nm (for **6** and **7**); 450 nm (for **8** and **9**); 470 nm (for **6*7** and **8*9**).

A similar emission band with a maximum of 450 nm was observed by Offen in chrysene crystals at 17 kbar pressure, but not at atmospheric pressure (1 atm).[16] Since chrysene forms a type A crystal in which the molecules are not packed face-to-face,[70] the author attributed the excimer emission band to defects formed within the lattice at extremely high compressions. Chiellini et al. have studied optically active chrysene-based vinyl copolymers,[17] and have described an excimer-like emission band. However, the reported band is complex, consisting of several superimposed components, two distinct maxima and a non-Gaussian shape, therefore its exact identity remains uncertain.

Φ_F increases significantly upon hybridization of **6** and **7** (Table 2). The nucleobase type next to the chrysene has a significant effect in the fluorescence intensity[71] (Figure 5) and quantum yield (Table 2). The lower Φ_F value of duplex and **8*9** (0.16) relative to duplex **6*7** (0.30) remains unclear, but was also observed in the dialkynyl pyrene case.[52]

Upon heating and thermal denaturation of hybrid **6*7** (Figure 6A), the intensity of the excimer band decreases with a slight blue shift of its maximum (12 nm, 20→75 °C) while the emission of the chrysene monomer (λ_{max} 391 nm) increases with increasing temperature. A distinct isoemissive point is present at 415 nm, which indicates that there are only two emitting species present, i.e., chrysene monomer and excimer.[72] The temperature-dependent fluorescence spectra of duplex **8*9** (Figure 6B) show a behaviour that differs in one major way from hybrid **6*7**: as the temperature is increased from 20 °C and the strands begin to dissociate, the excimer fluorescence band also gradually becomes less intense and blue shifted until ~70 °C (the approximate T_m, see Table 2) is reached. On further rise of the temperature, the intensity of the excimer band

increases again with progressive blue shifting to reach a maximum around 450 nm at 90 °C, which coincides with the value obtained also for the single strands (Figure 5). Such behaviour is consistent with the initial dissociation of the intermolecular excimer (λ_{max} 470 nm) in the duplex, followed by the formation of an intrastrand (λ_{max} 450 nm) excimer between neighbouring chrysenes in a DNA single strand. We have previously observed a similar blue shift upon transition from interstrand to intrastrand excimer in DNA sequences with a pyrene fluorophore.[73] In general, a more red-shifted excimer band is correlated with greater stability and transannular π-π interactions.[74] Thus, the present finding indicates that the intrastrand chrysene excimer is less stable than the interstrand excimer.
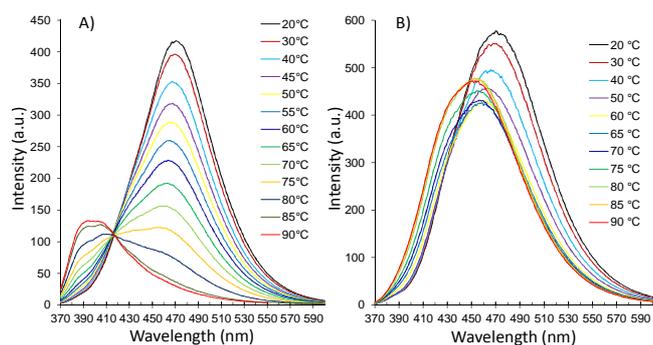

Figure 6 Temperature-dependent fluorescence spectra of duplex **6*7** (A) and **8*9** (B). Conditions: see Fig. 3; oligo conc. 1.0 µM each strand.
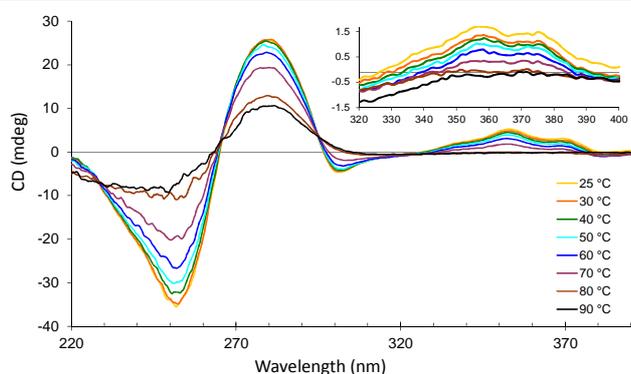

Figure 7 Temperature-dependent CD spectrum of duplex **6*7**. Conditions: see Fig. 3; oligo conc. 5.0 µM each.

Circular dichroism

Compared to other PAHs, relatively few CD studies on chrysene derivatives have been published.[17] Although studies on DNA alkylated by chrysene metabolites have been reported, the compounds no longer possess a chrysene aromatic core due one ring being saturated and thus behaving like a phenanthrene chromophore.[75] Figure 7 shows the CD spectrum of duplex **6*7**. At 25 °C, the 220-300 nm region shows the standard bisignate signal due to double stranded B-DNA. The strong S_0-S_2 transition of **3** between 250-300 nm (cf. Figure 2), overlaps with the DNA base dichroic absorptions. An induced CD (ICD)

of the dialkynylchrysene chromophore S_0-S_1 transition, with 3 vibronic bands (336, 352 and 362 nm), is clearly visible, which coincides well with the absorption band of duplex **6*7** (Figure 3). Upon heating from 25 °C to 90 °C, all signal intensities of the dichroic effects decrease, and the ICD of the dialkynylchrysene completely disappears after the 70 to 80 °C heating step (Figure 7, inset), which is in the region of the melting temperature of duplex **6*7**. The CD behaviour of duplex **8*9** (ESI) is similar to that of **6*7**.

Conclusion

We have described the first unambiguous observation of a chrysene excimer formed in solution under standard conditions. Association of individual chrysene units is enabled by a DNA supramolecular scaffold. Alkyne-substituted chrysene building blocks were incorporated into oligodeoxynucleotides. Excimer formation is observed upon duplex formation. Excimer emission is also observed from neighbouring chrysenes in single strands. The fluorescence spectrum exhibits the classic red-shifted, Gaussian-shaped excimer band between 450 and 470 nm with quantum yields as high as 40%. The high sensitivity of this excimer to spatial proximity opens possibilities for using chrysene as a fluorescent label for bioconjugation.[76]

Experimental section

1. General procedures

All reagents and solvents were purchased from commercial suppliers and used without further purification. Water was taken from a MilliQ system. NMR spectra were obtained on a Bruker AV 300 (300 MHz) spectrometer at 298 K. Mass-spectrometric data were obtained on Thermo Fisher LTQ Orbitrap XL using Nano Electrospray Ionization (NSI). UV-vis spectra were measured on a Cary 100 Bio spectrophotometer. Fluorescence and excitation spectra were measured on a Cary Eclipse spectrofluorimeter. CD spectra were measured on a Jasco J-715 spectropolarimeter. Unless otherwise indicated, all experiments were performed in 10 mM sodium phosphate buffer pH 7.0, 0.1 M NaCl. Reference oligonucleotides (**refA** and **refB**) were purchased from Microsynth (Switzerland).

2. Synthesis of compound 5

6,12-Bis(6-hydroxyhex-1-yn-1-yl)chrysene (3): Compound **2**[61,62] (0.595 g, 1.54 mmol) was dissolved in THF (10 ml) and Et$_3$N (10 ml). 5-Hexyn-1-ol (1.54 ml, 9 eq.), CuI (20 mg) and Pd[Ph$_3$P]$_2$Cl$_2$ (50 mg) were added. The reaction mixture was stirred under reflux at 80 °C under argon in a Schlenk flask. After 22 h 5-hexyn-1-ol (0.51 ml, 3 eq.) and CuI (20 mg) and Pd[Ph$_3$P]$_2$Cl$_2$ (50 mg) were added, and the reaction mixture was stirred under reflux for another 24 h. After cooling, H$_2$O (50 ml) was added, and the reaction mixture was extracted with DCM. The organic layer was dried over Na$_2$SO$_4$, filtered, the

solvent was removed *in vacuo*, and the product was purified by silica gel chromatography (DCM/MeOH 1:0→98:4, stepwise gradient) to give **3** contaminated with a little 5-hexyn-1-ol. The yellowish solid was further purified by dissolving it in a minimal amount of acetone, followed by precipitation with hexane. The white precipitate was filtered off and dried under high vacuum to yield pure **3** (0.17 g, 42%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.85 (s, 2H, chrysene H-5, H-11), 8.75 (m, 2H, chrysene), 8.50 (m, 2H, chrysene), 7.72 (m, 4H, chrysene), 3.80 (m, 4H, 2×C*H*$_2$OH), 2.71 (m, 4H, 2×CCC*H*$_2$), 1.89 (m, 8H, 2×C*H*$_2$C*H*$_2$CH$_2$OH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 131.09 (2C), 129.28 (2C), 127.72 (2C), 127.58 (2C), 126.85 (2C), 126.16 (2C), 125.15 (2C), 123.97 (2C), 120.90 (2C), 96.94 (2C), 78.86 (2C), 60.22 (2C), 31.84 (2C), 25.04 (2C), 18.97 (2C). HRMS-NSI (*m/z*): [M+H]$^+$ calcd for C$_{30}$H$_{29}$O$_2$, 421.2168; found, 421.2156.

6-[6-(4,4'-Dimethoxytriphenylmethyloxy)hex-1-yn-1-yl]-12-(6-hydroxyhex-1-yn-1-yl)chrysene (4): Compound **3** (1.1 g, 2.62 mmol) was dissolved in THF (100 ml) and Et$_3$N (15 ml), and DMTCl (0.89 g, 1 eq.) was added in one portion. The reaction mixture was stirred at rt for 24 h under argon. Monitoring by TLC (DCM/MeOH/Et$_3$N 94:5:1) showed remaining starting material. Therefore, DMTCl (0.444 g, 0.5 eq.) was added and the reaction mixture was stirred for another 24 h at rt. The solvent was removed in vacuo and the product was purified by silica gel chromatography with DCM/Et$_3$N (99:1) as eluent. Compound **4** was isolated as a solid (1.01 g, 53%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.85-8.84 (d, 2H, chrysene H-5, H-11), 8.76-8.72 (m, 2H, chrysene), 8.52-8.48 (m, 2H, chrysene), 7.74-7.65 (m, 4H, chrysene), 7.50-7.47 (m, 2H, DMT), 7.39-7.34 (m, 4H, C$_6$H$_4$, DMT), 7.31-7.26 (m, 2H, DMT, CHCl$_3$), 7.22-7.18 (m, 1H, DMT), 6.83-6.80 (m, 4H, C$_6$H$_4$, DMT), 3.80 (m, 2H, C*H*$_2$OH), 3.76 (s, 6H, 2×OCH$_3$), 3.19 (t, *J* = 5.8 Hz, 2H, C*H*$_2$ODMT), 2.71 (m, 2H, CCC*H*$_2$), 2.64 (t, *J* = 6.5 Hz, 2H, CCC*H*$_2$) 1.93-1.86 (m, 8H, 2×C*H*$_2$C*H*$_2$CH$_2$O). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 157.94, 145.19, 136.00, 131.09, 129.53, 129.28, 129.27, 128.87, 127.73, 127.67, 127.61, 127.54, 127.50, 126.85, 126.83, 126.52, 126.16, 126.11, 125.16, 125.13, 123.91, 120.92, 120.84, 113.08, 96.92 (alkyne), 96.66 (alkyne), 85.24 (C$_q$, *C*(Aryl)$_3$, DMT), 79.05 (alkyne), 78.86 (alkyne), 62.36 (2C, 2×CH$_2$CH$_2$CH$_2$O), 60.23 (2C, 2×CH$_2$CH$_2$*C*H$_2$O), 54.92 (2C, 2×OCH$_3$), 31.85, 28.83, 25.37, 25.05, 18.98. HRMS-NSI (*m/z*): [M]$^+$ calcd for C$_{51}$H$_{46}$O$_4$, 722.3391; found, 722.3399.

Phosphoramidite 5: Compound **4** (0.30 g, 0.41 mmol) was dissolved in dry DCM (30 ml) and Et$_3$N (0.22 ml, 1.58 mmol) followed by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.303 g, 1.28 mmol) were added. After 1 h of stirring at rt under argon, the solvent was removed in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate/Et$_3$N 7:3:0.1) to give a colorless oil, which was dissolved in a minimal amount DCM, followed by addition of an excess of hexane. After decanting,

the solid precipitate was dried under high vacuum to give compound **5** as a colorless solid (0.27g, 71%). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.00-8.98 (m, 4H, chrysene H-5, H-11), 8.43 (m, 2H, chrysene), 7.77 (m, 4H, chrysene), 7.40 (d, *J* = 7.5 Hz, 2H, DMT), 7.33 – 7.16 (m, 7H, DMT), 6.85 (d, *J* = 8.8 Hz, 4H, DMT), 3.80 – 3.70 (m, 10H, 2×CH$_2$OP, 2×OCH$_3$), 3.58 (m, 2H, 2×C*H*(CH$_3$)$_2$), 3.09 (t, *J* = 5.4 Hz, 2H, C*H*$_2$ODMT), 2.78 – 2.64 (m, 6H, 2×CCCH$_2$, CH$_2$CN), 1.83 (m, 8H, 2×C*H*$_2$C*H*$_2$CH$_2$O), 1.13 (dd, *J* = 6.8, 3.0 Hz, 12H, 2×CH(C*H*$_3$)$_2$). ^{13}C NMR (75 MHz, DMSO) δ 157.93, 145.19, 136.00, 131.09, 131.07, 129.53, 129.27, 127.73, 127.68, 127.61, 127.52, 126.85, 126.83, 126.52, 126.12, 125.17, 123.94, 120.86, 118.92, 113.09, 96.71 (alkyne), 96.65 (alkyne), 85.23(C$_q$, *C*(Aryl)$_3$, DMT), 79.04 (alkyne), 78.99 (alkyne), 62.77, 62.55, 62.36, 58.21, 57.97, 54.92, 42.51, 42.35, 30.14, 30.05, 28.82, 25.35, 24.96, 24.38, 24.36, 24.28, 24.26, 19.87, 19.77, 18.95, 18.80. ^{31}P NMR (121.5 MHz, DMSO-d$_6$): δ 146.22 (phosphoramidite). HRMS-NSI (*m/z*): [M+H]$^+$ calcd for C$_{60}$H$_{64}$O$_5$N$_2$P, 923.4547; found, 923.4554.

4. Oligonucleotides and duplexes

The oligonucleotides **6**-**8** were prepared on an Applied Biosystems 394 DNA/RNA synthesizer. A standard cyanoethyl phosphoramidite coupling protocol was used beginning with nucleoside-loaded controlled pore glass (CPG) supports. Commercially available natural nucleoside phosphoramidites were dissolved in CH$_3$CN to yield 0.1 M solutions. Compound **5** was dissolved in 1,2-dichloroethane to yield a 0.1 M solution. The CPG-bound oligonucleotides were cleaved and deprotected by treatment with NH$_4$OH at 55 °C for 16 h. The supernatant was collected and the debris was washed three times with 1 ml EtOH/H$_2$O 1:1. After lyophilisation the crude oligonucleotides were purified by reversed phase HPLC (Merck LiChroCART 250-4; LiChrospher 100, RP-18, 5 μm). A gradient starting with 0% up to 100% CH$_3$CN in 0.1 M aqueous triethylammonium acetate was set at a flow rate of 1.0 ml/min. The purified oligonucleotides were dissolved in 1 ml H$_2$O. Samples of the stock solutions were diluted 50 times and the absorbance at 260 nm was measured. The molar extinction coefficients of the oligonucleotides were calculated using the ε$_{260}$ values of 15300, 11700, 7400 and 9000 for A, G, C and T bases, respectively, and 36,653 for the chrysene building block. Formation of duplexes **6*****7** and **8*****9**: equimolar amounts of single strand solutions were combined in buffer and were kept at 90 °C for 10 min, before being allowed to cool down overnight.

Acknowledgements

Notes and references

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland. E-mail: robert.haener@ioc.unibe.ch

Chemical Science Accepted Manuscript

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