Supplementary Material for Chemical Communications

Supplementary Information to:

Excimer Formation by Interstrand Stacked Pyrenes

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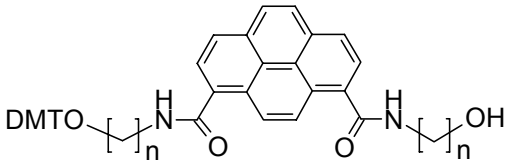
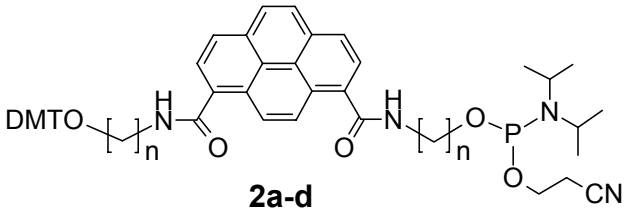
Experimental Section

General. Chemicals, solvents, and reagents for reactions were from *Acros*, *Aldrich*, or *Fluka*, and were of the highest quality available. Solvents for extraction and chromatography were of technical grade and distilled prior to use. Thin layer chromatography (TLC): silica-gel 60 F_{254} glass plates (*Merck*); visualisation by UV and/or A) by dipping in a soln. of anisaldehyde (10 ml), conc. H_2SO_4 (10 ml), and AcOH (2 ml) in EtOH (180 ml) or B) cerium (IV) sulfate (3mM)/ammonium molybdate (250mM in aq. H_2SO_4 (10%) followed by heating. Flash column chromatography (CC): silica gel 60 (40-63 μm, 230-400 mesh, *Fluka*) at low pressure. The chromatography of acid sensitive compounds was carried out with eluent containing 2% NEt_3. ^{1}H- and ^{13}C-NMR: *Bruker AC-300* or *Bruker AMX 400*, δ values in ppm (solvents signals as internal standards), *J* [Hz]; ^{31}P-NMR: *Bruker AC-300*, δ values in ppm (85% H_3PO_4 as external standard). ESI-MS: *VG Platform* single quadrupole ESI mass spectrometer. DMT: 4,4'-dimethoxytrityl; BOP: Benzotriazol-1-yloxy)-tris-(dimthylamino)-phosphonium-hexafluorophosphate; AcOEt: ethyl acetate; r.t: room temperature; HR-ESI-MS: high resolution ESI-MS.

General Procedures



DMT-2a-d



2a-d

a n = 2 **c** n = 4
b n = 3 **d** n = 5

Preparation of the intermediates DMT-2a-d

1 eq. pyrene-1,8-dicarboxylic acid and 5 eq. *Hünig's* base were solved in dry DMF(concentration of the acid 0.2M), then a solution of 1 eq. $HO(CH_2)_nNH_2$ and 1 eq. $DMTO(CH_2)_nNH_2$ in dry pyridine(concentration of the DMT protected linker 0.5M) was added at r.t.. After this 2.2 eq of BOP was added and stirred under N_2 atmosphere for 1h.
The mixture was dilutet with AcOEt washed with 10% *aq.* citric acid and *sat. aq* $NaHCO_3$ *soln.* The org. layer was dried (K_2CO_3) and evaporated under reduced pressure. Purification of the resulting oil bye CC (silica gel; $CH_2Cl_2 \rightarrow CH_2Cl_2$: MeOH (98: 2) (+2% Et_3N)) furnished as yellow foam.

Preparation of phosphoramidites 2a-d

1 eq. of the DMT protected pyrene was solved in dry CH_2Cl_2 and 3 eq. *Hünig's* base. Then 1 eq. of 2-cyanoethyl diisopropylamidochloridophosphite under N_2 atmosphere was added and stirred for 1 h at r.t.. The reaction mixture was directly purified by CC (silica gel; $CH_2Cl_2 \rightarrow CH_2Cl_2$: MeOH (98:2)(+ 2% Et_3N)). Compounds **2a-d** were obtained as yellow foams.

Pyrene-1,8-dicarboxylic acid 1-({2-[(4,4'dimethoxytrityl)-oxy]-ethyl}-amide) 8-[(2-hydroxy-ethyl)-amide] (**DMT-2a**). Yield: 28%, yellow foam. TLC (AcOEt): R_f 0.25

^{1}H-NMR(300MHz,$CDCl_3$): 3.47 (*t*, *J* = 4.7, $ROCH_2$); 3.7-3.85 (*m,* CH_2N, CH_2N'); 3.72 (*s*, 2MeO); 3.97 (m, C*H*$_2$OH); 6.79 (*d*, 4 arom. H); 7.15-8.1 (*m*, 15 arom. H); 8.25 (*m*, 2H). ESI-MS (*pos. mode*): 701[*M*+Na]$^+$

Pyrene-1,8-dicarboxylic acid 1-({3-[(4,4'dimethoxytrityl)-oxy]-propyl}-amide) 8-[(2-hydroxy-propyl)-amide] (**DMT-2b**). Yield: 22%, yellow foam. TLC (AcOEt): R_f 0.25
^{1}H-NMR(300MHz,$CDCl_3$): 1.95 (*m*, 2 $CH_2CH_2CH_2$); 3.33 (*t*, *J* = 5.4, $ROCH_2$); 3.55 (*s*, 2MeO); 3.7-3.9 (*m,* CH_2N, CH_2N', C*H*$_2$OH); 6.59 (*d*, 4 arom. H); 7.0-7.8 (*m*, 15 arom. H); 8.25 (*m*, 2H). ESI-MS (*pos. mode*): 729[*M*+Na]$^+$

Pyrene-1,8-dicarboxylic acid 1-({4-[(4,4'dimethoxytrityl)-oxy]-butyl}-amide) 8-[(2-hydroxy-butyl)-amide] (**DMT-2c**). Yield: 20%, yellow foam. TLC (AcOEt): R_f 0.27
^{1}H-NMR(300MHz,$CDCl_3$): 1.81 (*m*, 2 $CH_2CH_2CH_2CH_2$); 3.14 (*m*, $ROCH_2$); 3.70 (*s*, 2MeO); 3.5-3.8 (*m,* CH_2N, CH_2N', C*H*$_2$OH); 6.70 (*d*, 4 arom. H); 7.1-8.0 (*m*, 15 arom. H); 8.29 (*m*, 2H). ESI-MS (*pos. mode*): 757[*M*+Na]$^+$

Pyrene-1,8-dicarboxylic acid 1-({5-[(4,4'dimethoxytrityl)-oxy]-pentyl}-amide) 8-[(2-hydroxy-pentyl)-amide] (**DMT-2d**). Yield: 17%, yellow foam. TLC (AcOEt): R_f 0.36
^{1}H-NMR(300MHz,$CDCl_3$): 1.69 (*m*, 2 $CH_2CH_2CH_2CH_2CH_2$); 3.11 (*m*, $ROCH_2$); 3.73 (*s*, 2MeO); 3.5-3.8 (*m,* CH_2N, CH_2N', C*H*$_2$OH); 6.79 (*d*, 4 arom. H); 7.1-8.0 (*m*, 15 arom. H); 8.23 (*m*, 2H). ESI-MS (*pos. mode*): 785[*M*+Na]$^+$

Diisopropyl-phosphoramidous acid 2-[(8-{2-[bis-(4,4'dimethoxytrityl)-oxy]-ethylcarbamoyl}-pyrene-1-carbonyl)-amino]-ethyl ester 2-cyano-ethyl ester (**2a**). Yield: 81%, yellow foam. TLC (AcOEt:hexane 6:4 + 2% Et_3N): R_f 0.22
^{1}H-NMR(300MHz,$CDCl_3$): 1.17 (*d*, J = 6.9, 2 *Me*CHN); 2.46 (*t*, J = 6.2, CH_2CN); 3.4-4.0 (*m,* CH_2CH_2N, CH_2CH_2N', OC*H*$_2$$CH_2CN$, 2 Me_2CHN); 3.73 (*s*, 2MeO); 6.79 (*d*, 4 arom. H); 7.1-7.5 (*m*, 9 arom. H); 8.0-8.3(*m*, 6 arom. H); 8.64 (*m*, 2H). ^{31}P-NMR(162MHz, $CDCl_3$): 148.79. HR-ESI-MS (*pos. mode*): 901.3701 ([*M*+Na]$^+$; calc. 901.3706)

Diisopropyl-phosphoramidous acid 2-[(8-{3-[bis-(4,4'dimethoxytrityl)-oxy]-propylcarbamoyl}-pyrene-1-carbonyl)-amino]-propyl ester 2-cyano-ethyl ester (**2b**). Yield: 43%, yellow foam. TLC (AcOEt:hexane 6:4 + 2% Et_3N): R_f 0.24
^{1}H-NMR(300MHz,$CDCl_3$): 1.03, 1.04 (2*d*, J = 6.8, 2 *Me*CHN); 2.03 (*m*, 2 $CH_2CH_2CH_2$); 2.35 (*m*, CH_2CN); 3.3-3.9 (*m,* C*H*$_2$$CH_2CH_2$N, C*H*$_2$$CH_2CH_2$N', OC*H*$_2$$CH_2CN$, 2 Me_2CHN); 3.58 (*s*, 2MeO); 6.59 (*d*, 4 arom. H); 7.0-7.4 (*m*, 9 arom. H); 7.9-8.25(*m*, 6 arom. H); 8.59 (*m*, 2H). ^{31}P-NMR(162MHz, $CDCl_3$): 148.24. HR-ESI-MS (*pos. mode*): 925.4536 ([*M*+Na]$^+$; calc. 925.4543)

Diisopropyl-phosphoramidous acid 2-[(8-{4-[bis-(4,4'dimethoxytrityl)-oxy]-butylcarbamoyl}-pyrene-1-carbonyl)-amino]-butyl ester 2-cyano-ethyl ester (**2c**). Yield: 61%, yellow foam. TLC (AcOEt:hexane 6:4 + 2% Et_3N): R_f 0.42
^{1}H-NMR(300MHz,$CDCl_3$): 1.04, 1.05 (2*d*, J = 6.6, 2 *Me*CHN); 1.82 (*m*, 2 $CH_2CH_2CH_2CH_2$); 2.50 (*t*, J = 6.3, CH_2CN); 3.1-3.8 (*m,* *CH*$_2$$CH_2CH_2CH_2$N, C*H*$_2$$CH_2CH_2CH_2$N', OC*H*$_2$$CH_2CN$,

2 Me_2CHN); 3.70 (*s*, 2MeO); 6.71 (*d*, 4 arom. H); 7.1-7.5 (*m*, 9 arom. H); 7.9-8.25(*m*, 6 arom. H); 8.57 (*m*, 2H). ^{31}P-NMR(162MHz, $CDCl_3$): 147.71. ESI-MS (*pos. mode*): 952 $[M+NH_4]^+$

Diisopropyl-phosphoramidous acid 2-[(8-{5-[bis-(4,4'dimethoxytrityl)-oxy]-pentylcarbamoyl}-pyrene-1-carbonyl)-amino]-pentyl ester 2-cyano-ethyl ester (**2d**). Yield: 68%, yellow foam. TLC (AcOEt:hexane 6:4 + 2% Et_3N): R_f 0.94.
^{1}H-NMR(300MHz,$CDCl_3$): 1.17, 1.18 (2*d*, J = 6.7, 2 *Me*CHN); 1.72 (*m*, 2 $CH_2CH_2CH_2CH_2CH_2$); 2.63 (*m*, CH_2CN); 3.0-3.9 (*m,* $CH_2CH_2CH_2CH_2CH_2N$, $CH_2CH_2CH_2CH_2CH_2N$', OCH_2CH_2CN, 2 Me_2CHN); 3.77 (*s*, 2MeO); 6.81 (*d*, 4 arom. H); 7.1-7.5 (*m*, 9 arom. H); 7.9-8.25(*m*, 6 arom. H); 8.55 (*m*, 2H). ^{31}P-NMR(162MHz, $CDCl_3$): 147.35. HR-ESI-MS (*pos. mode*): 963.4828 ($[M+Na]^+$; calc. 963.4825)

Oligonucleotide Synthesis
Pyrene-derived phosphoramidite building blocks **2a-d** were incorporated into oligonucleotides *via* standard automated oligonucleotide synthesis using I_2/pyridine/water in the oxidation step. Coupling yields with **2a-d** were equal to the ones obtained with standard phosphoramidite building blocks. Oligonucleotides were purified by reverse phase HPLC and characterised by MS (Table 1).

Table 1 suppl.: Molecular weights of oligonucleotides used in this study (*electrospray ionisation time-of-flight*, *ESI-TOF*)

oligonucleotide	calculated for $(M-H)^-$	found
3a	6604.5	6604.4
3b	6632.5	6632.6
3c	6660.5	6660.5
3d	6688.5	6688.6
4a	6506.5	6506.5
4b	6534.5	6534.4
4c	6562.5	6562.4
4d	6590.5	6590.3

Figure 1 suppl.: Circular dichroism curve of hybrid **3d*4d**. Conditions: 1.0 μM oligomers (each strand); 100mM NaCl; 10mM Tris.HCl, pH 7.4, 20°C.

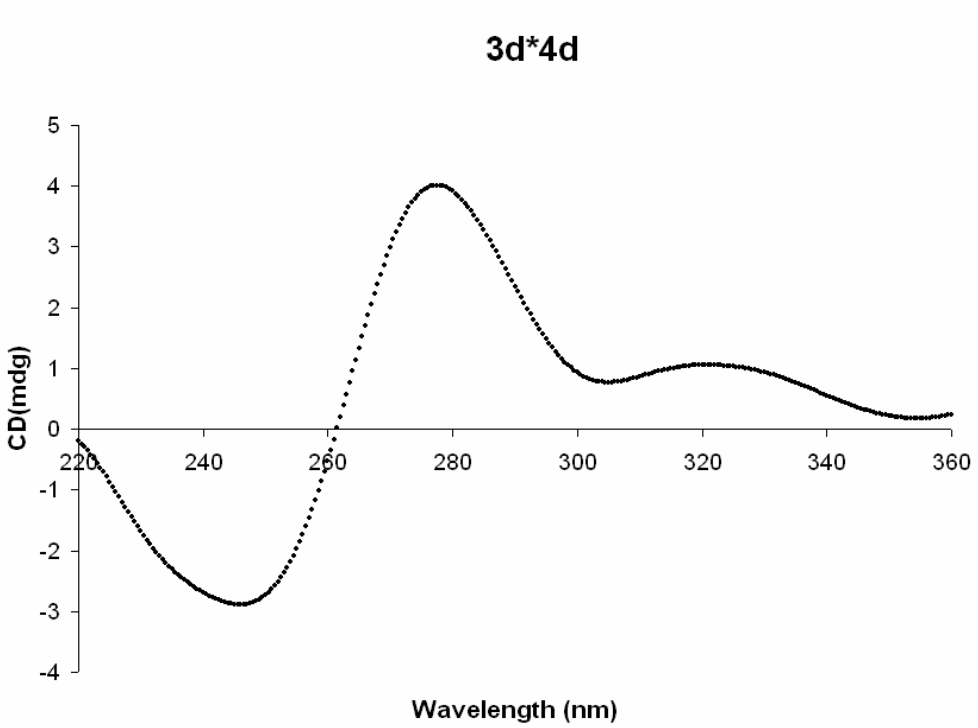
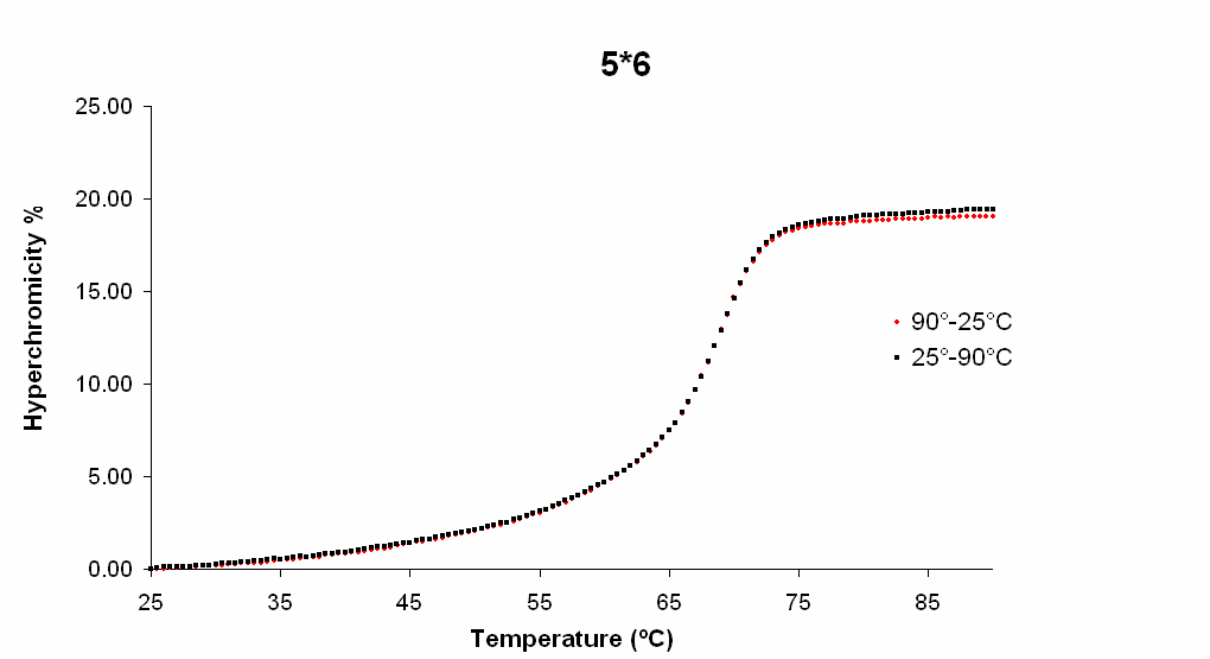


Figure 2 suppl.: Melting curves of different hybrids. Conditions: Oligomer concentration 1.0 µM, 10 mM Tris-HCl, 100 mM NaCl, pH 7.4; temp. gradient: 0.5°C/min. Exptl. error: ± 0.5°C. Absorbance was measured at 260nm. **A**) duplex **5*6**, **B**) duplex **3a*4a**; **C**) duplex **3b*4b**; **D**) duplex **3c*4c**; **E**) duplex **3d*4d**.

A)



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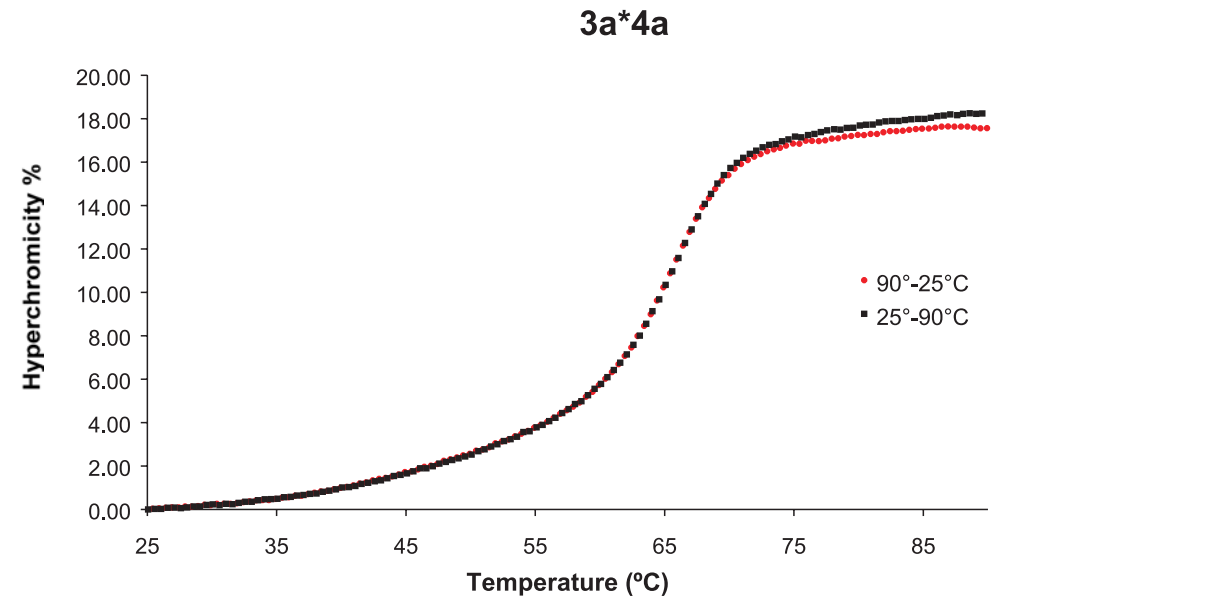

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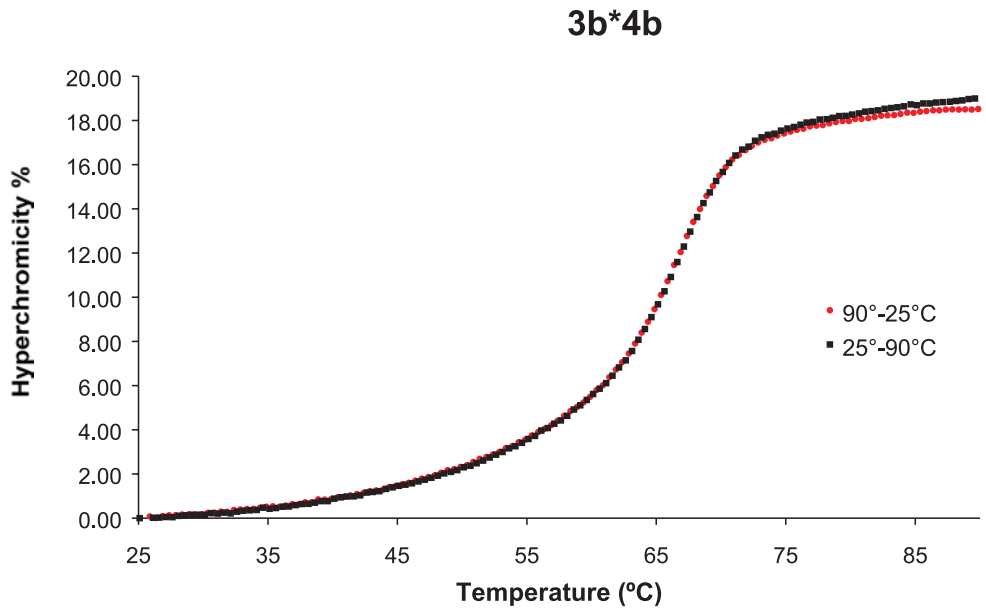
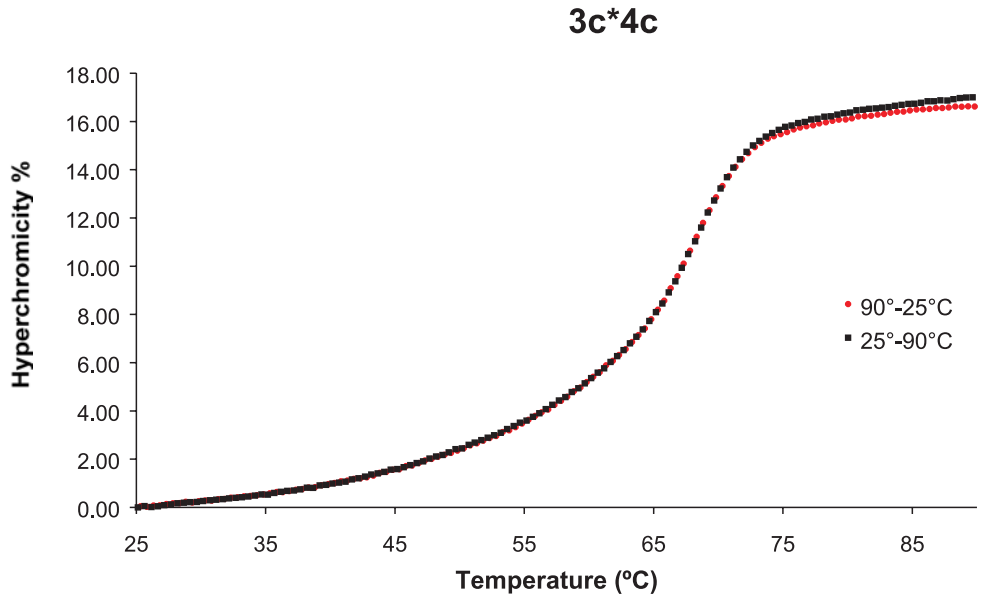


D)



E)