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RNA as scaffold for pyrene excited complexes

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Abstract—Synthesis and spectral properties of 1-ethynylpyrene base modified RNA are reported. The fluorophore attached to the 2-position of adenosine is directed into the easily accessible minor groove in RNA. Through an intermolecular interaction of the pyrene residues in twofold labelled RNA, single and double strands can be distinguished by their fluorescence maxima around 450 and 480 nm, respectively. This behaviour allows the kinetic investigation of RNA hybridisation and folding by fluorescence spectroscopy.

1. Introduction

DNA as a building block for nanoscale materials[1] has attracted considerable interest due to its possible application in molecular devices. RNA, on the other hand, has much less been utilized.[2] Both nucleic acids form predictable three dimensional structures based on Watson-Crick complementarity. These duplexes are either of the B-type with a wide major and a narrow minor groove mostly for DNA or with a deep and narrow major groove and a shallow minor groove for the A-type mostly found in double-stranded RNA. Here, the concept of site specifically mono-functionalized oligonucleotides led us to utilize the easily accessible minor groove site in RNA. In order to achieve this, the group of interest has to be attached to the purine 2-position, which is possible with A and G.[3] We started to derivatize adenosine in RNA with pyrene using an acetylenic linker.

1-Ethynylpyrene as a highly fluorescent chromophore has initially been introduced into the 5-position of deoxy-uridine.[4] This rigid label is expected to minimally disturb the duplex geometry and to align the fluorophore in a predictable fashion due to its rigid acetylenic linker. Pyrene and 1-ethynylpyrene are commonly introduced into the base at the 5-position of pyrimidines and at the 8-position of the purines.[5] Pyrene was also introduced at the 2′-sugar-position in DNA, RNA and LNA[6] using flexible linkers. Alternatively pyrene can also substitute the whole nucleic base or nucleotide.[7]

A first simple molecular model constructed with Insight-II (Fig. 1) using an ideal, unperturbed RNA structure as scaffold showed that two 1-ethynylpyrene residues attached to the 2-position in different strands can be placed in the minor groove of double-stranded RNA without sterical hindrance. In addition we predicted good stacking between two pyrenes only when fixed on opposite strands. Otherwise, only a partial overlap is expected. The distance between the two pyrene residues in the model is about 3 Å, a distance suitable for complex formation.

Herein we report the introduction of 1-ethynylpyrene into the 2-position of adenosine in RNA oligonucleotides, their characterization and their fluorescence properties. The site specific functionalization is achieved by Sonogashira cross-coupling on solid support in the course of oligonucleotide synthesis,[8] an innovative method rarely used by now.[9]

Keywords: Fluorescence; Pyrene; Excited complex; Interstrand stacking.
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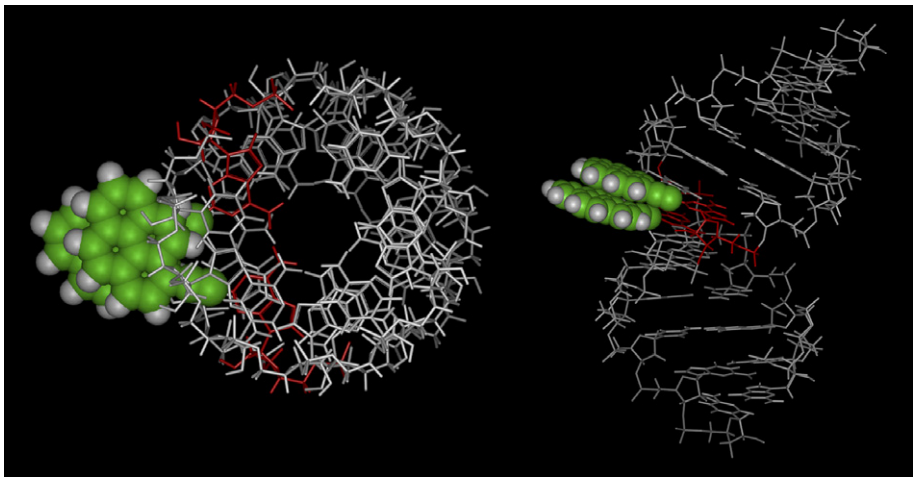


Figure 1. Molecular model of a 12-membered RNA oligomer containing an AU-core with an interstrand pyrene dimer in the minor groove, red: adenosine nucleoside, green: 1-ethynylpyrene.

2. Results and discussion

2.1. Synthesis

Whereas 5-halo-pyrimidines and 8-halo-purines used by other research groups are commercially available, 2-iodoadenosine has to be synthesized according to literature procedures. Compound **9** was synthesized in four steps from guanosine in an overall yield of 45%. For the preparation of **6**, the use of acetonitrile as solvent and DMAP/triethylamine as catalyst/co-catalyst[10] was preferred to DMF/pyridine as solvent as better yields could be achieved and the interference of residual DMF with $POCl_3$ (formation of Vilsmeier-Haack-reagent) in the synthesis of **7** could be avoided. 2′,3′,5′-Tri-*O*-acetylguanosine **6** was treated with $POCl_3$ to afford the chloro-compound **7**.[11] Conversion to the 2-iodo-6-chloro-purine derivative **8** was performed with a variant of the Sandmeyer reaction of then in situ generated diazonium salt of **7**.[12] Nucleophilic aromatic substitution at the 6-position of the purine with methanolic ammonia and subsequent treatment with NaOMe in MeOH to deprotect the remaining acetyl groups yielded 2-iodoadenosine **9** (Scheme 1).

As we have had experienced excellent results with the recently established ACE® chemistry used for our spin-labelling experiments, this method was also applied to the solid-phase synthesis of the pyrene-modified RNA. The ACE® chemistry[13] developed by Dharmacon uses an inverted protection group strategy with a fluoride-labile 5′-silyl-group and an acid labile 2′-orthoester group in comparison to the established TBDMS-strategy. The synthesis of the 2′-bis-acetoxyethyloxy-5′-silyl protected amidite[14] **15** for solid-phase RNA synthesis starts with the protection of the exocyclic amine with *N*,*N*-dimethylacetamide-dimethylacetal in DMF as the corresponding amidine **10**.[15] For discrimination of 2′- and 3′-hydroxy function the Markiewicz strategy[16] was used. Reaction of the N-protected 2-iodoadenosine with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane affords **11**. Reaction of **11** with tris-(acetoxyethyloxy)methylester (ACE) and subsequent treatment with TEMED-HF in acetonitrile yielded 2′-ACE protected **13**. Protection of the 5′-hydroxyl group with benzhydryloxy-bis(trimethylsilyloxy)chlorsilane (BzH-Cl) and reaction with methyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite afforded the 2′-ACE-5′-BzH-protected phosphoramidite **15** suitable for solid-phase synthesis in an overall yield of 49% starting with 2-iodoadenosine (Scheme 2).

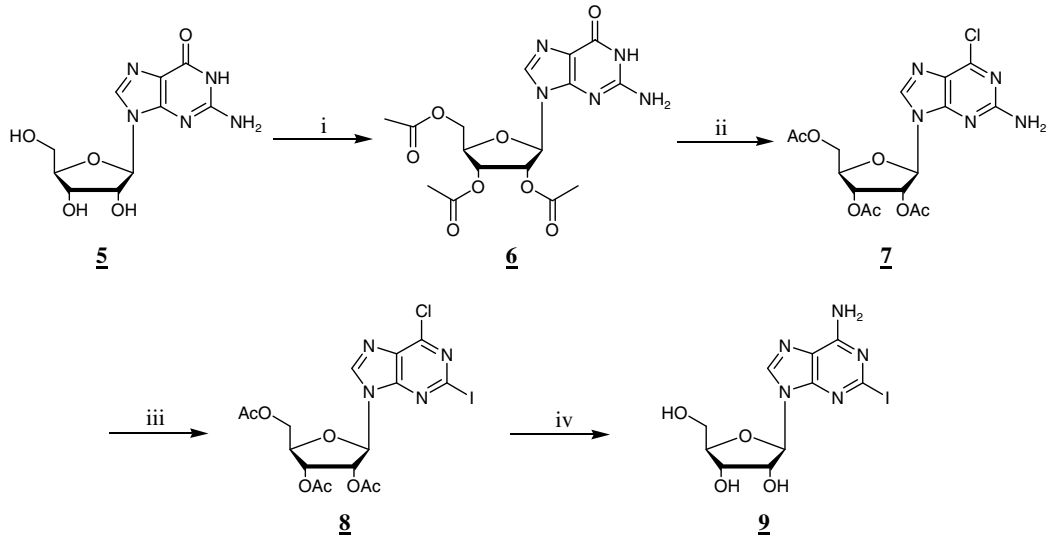
Solid-phase RNA synthesis was performed on a 0.2 μmol scale using standard Dharmacon protocols. The introduction of 1-ethynylpyrene was achieved by Sonogashira cross-coupling on solid support: the oligonucleotide solid-phase synthesis was interrupted after the incorporation of 2-iodoadenosine without deprotection of the 5′-hydroxyl group. The column was then removed from the synthesizer and the reagent mixture for Sonogashira cross-coupling of 1-ethynylpyrene with the iodinated base was loaded to the column using syringes.[9] The coupling was performed twice for 2.5 h each to ensure quantitative reaction (Fig. 2).

After each Sonogashira cross-coupling the solid support was washed thoroughly with CH_2Cl_2, dried in vacuo and the solid-phase synthesis was resumed. After removing the protecting groups, the oligonucleotides were purified by anion exchange chromatography. Following desalting and deprotection of the residual 2′-protecting group, the oligonucleotides were analysed by MALDI-TOF mass spectroscopy.

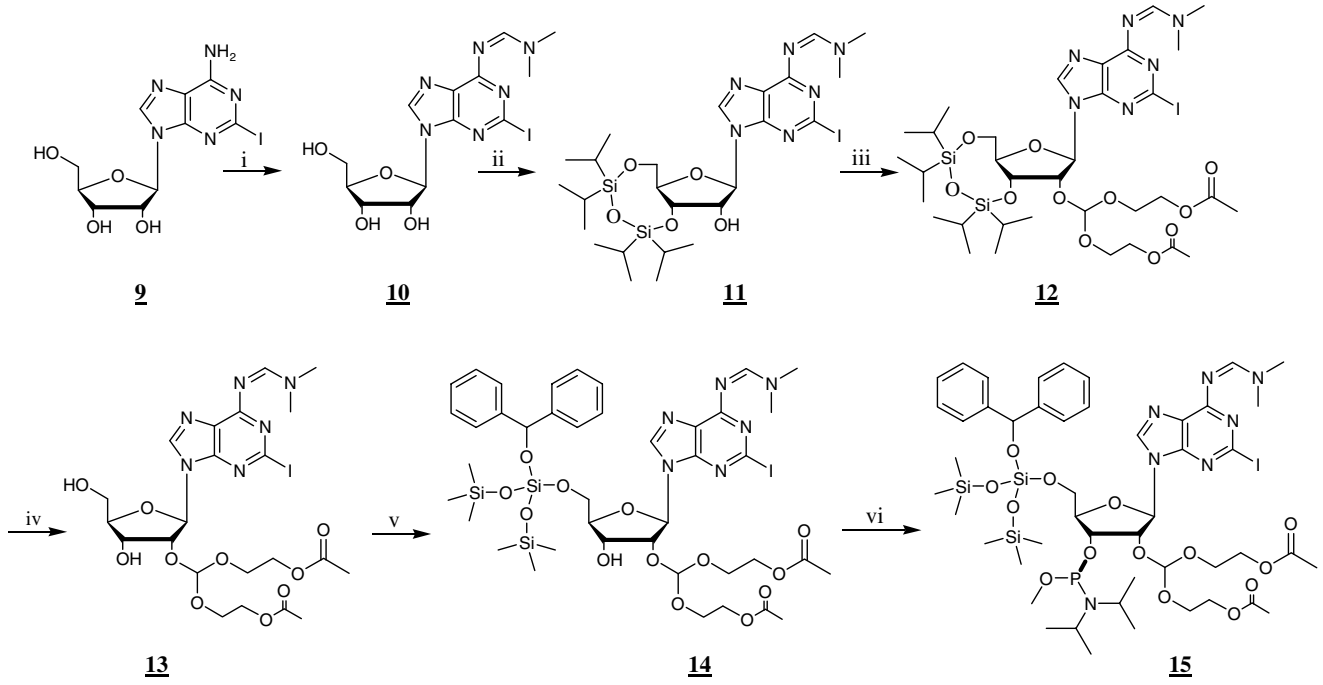
We synthesized self-complementary oligonucleotides with an AU core and 5′- and 3′-GC pairs as well as non self-complementary sequences to be able to investigate singly 1-ethynylpyrene-modified single and double strands in addition to the twofold modified duplexes. The sequences and analytic data of the synthesized oligonucleotides are listed in Table 1.

2.2. Thermal stability and CD spectra

To determine the influence of 1-ethynylpyrene in the 2-position of adenosine on the stability of the synthesized oligonucleotides, melting curves and CD spectra were



Scheme 1. Synthesis of 2-iodoadenosine **9**. Reagents and conditions: (i) Ac_2O, MeCN, Et_3N, *N*,*N*-dimethylaminopyridine, 30 min, rt, 91%; (ii) $POCl_3$, MeCN, 15 min, 110 °C, 80%; (iii) iso-pentylnitrite, THF, I_2, CH_2I_2, CuI, 45 min reflux, 89%; (iv) NH_3/MeOH, 24 h, rt, 70%.



Scheme 2. Synthesis of ACE-protected phosphoramidite **15** starting with 2-iodoadenosine **9**. Reagents and conditions: (i) DMF-dimethylacetal, DMF, 1 h, 50 °C, 86%; (ii) tetraisopropyldisiloxandichloride (TIPS-Cl_2), pyridine, 2 h, 0 °C → rt, 83%; (iii) tri-(2-acetoxyethyl)-ortho formate, CH_2Cl_2, 48 h, rt, 89%; (iv) TEMED-HF, MeCN, 3 h, rt, 96%; (v) benzhydryloxy-bis-(trimethylsilyloxy)chlorsilane, CH_2Cl_2, diisopropylamine, 1 h, rt, 92%; (vi) bis-(*N*,*N*-diisopropylamino)-methoxyphosphine, CH_2Cl_2, 5 min, 0 °C, 13 h, rt, 86%.

measured. Melting temperatures (T_m) of synthesized double-stranded oligonucleotides are given in Table 2. Modified self-complementary oligonucleotides are significantly more stable (+11.1, +11.0 and +8.5 °C for **IB**, **IIB** and **IIIB**, respectively) than the corresponding unmodified duplexes. In case of the non self-complementary sequence the double-stranded singly modified oligonucleotide duplexes **IVB**–**VA** and **IVA**–**VB** are drastically less stable (−6.3 °C and −12.2 °C, respectively) than the unmodified sequence in contrast to the twofold modified duplex **IVB**–**VB** that is thermally more stable (+3.2 °C) than the unmodified sequence. The stabilisation of inherently twofold modified RNA could be attributed to an attractive π–π-interaction between the aromatic fluorophores as it is also the case for the twofold modified non self-complementary duplex **IVB**–**VB**. In contrast, a single pyrene residue, lacking attractive π–π-interaction, seems to disturb the stack and therefore lowers the melting temperature with respect to the unmodified sequence.

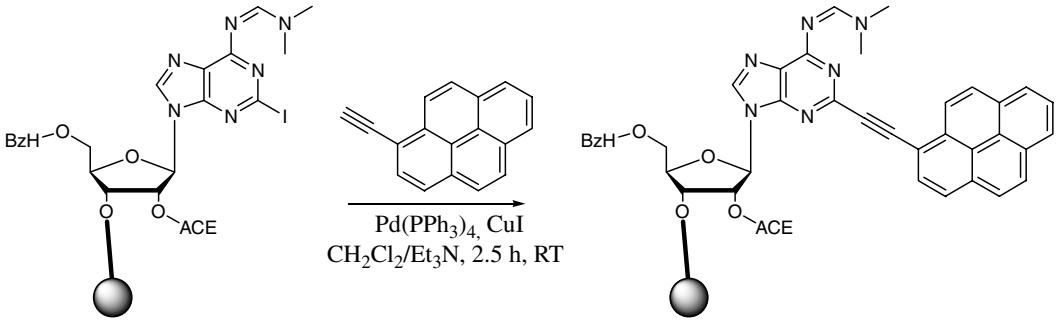


Figure 2. Conditions for the solid-phase Sonogashira cross-coupling between the polymer bound oligonucleotide and 1-ethynylpyrene.

Table 1. Sequences and calculated masses of synthesized modified and unmodified oligonucleotides together with the corresponding analytic data found by MALDI-TOF mass spectroscopy

ON	Sequence	Mass calcd	Mass found
IA	5′-GCGCA UGCGC-3′	3174.99	3174.02
IB	5′-GCGCA^{Py}UGCGC-3′	3399.62	3398.95
IIA	5′-GCGAA UUCGC-3′	3159.98	3157.66
IIB	5′-GCGAA^{Py}UUCGC-3′	3384.23	3382.04
IIIA	5′-GCAAA UUUGC-3′	3144.97	3142.63
IIIB	5′-GCAAA^{Py}UUUGC-3′	3369.22	3367.68
IVA	5′-CUUUUCA UUCUU-3′	3632.15	3631.40
IVB	5′-CUUUUCA^{Py}UUCUU-3′	3856.42	3855.05
VA	3′-GAAAAGUA AGAA-5′	3913.51	3913.16
VB	3′-GAAAAGUA^{Py}AGAA-5′	4137.78	4136.69

Table 2. Thermal denaturation studies of modified oligonucleotides

Duplex	Sequence	T_m (°C)	ΔT_M
IA–IA	5′-GCGCA UGCGC-3′	68.3 ± 0.9	—
IB–IB	5′-GCGCA^{Py}UGCGC-3′	79.4 ± 0.6	+11.1
IIA–IIA	5′-GCGAA UUCGC-3′	42.0 ± 0.2	—
IIB–IIB	5′-GCGAA^{Py}UUCGC-3′	53.0 ± 0.6	+11.0
IIIA–IIIA	5′-GCAAA UUUGC-3′	26.5 ± 0.2	—
IIIB–IIIB	5′-GCAAA^{Py}UUUGC-3′	35.0 ± 0.2	+8.5
IVA–VA	5′-CUUUUCAUUCUU-3′ 3′-GAAAAGUAAGAA-5′	42.2 ± 0.4	—
IVB–VA	5′-CUUUUCA^{Py}UUCUU-3′ 3′-GAAAAGU AAGAA-5′	35.9 ± 0.1	−6.3
IVA–VB	5′-CUUUUCAU UCUU-3′ 3′-GAAAAGUA^{Py}AGAA-5′	30.0 ± 0.1	−12.2
IVB–VB	5′-CUUUUCA^{Py}UUCUU-3′ 3′-GAAAAGUA^{Py}AGAA-5′	45.4 ± 0.1	+3.2

CD spectra of both modified and unmodified self-complementary as well as non self-complementary sequences show maxima at 265 nm (global), 222 nm (local) and minima at 245 nm (global), 211 nm (local) corresponding to an A-form RNA. CD spectra indicate that the overall structure of the modified oligonucleotides compared to the corresponding unmodified ones is conserved. This is even the case for the destabilised singly modified non self-complementary duplexes **IVA–VB** and **IVB–VA** (Fig. 3). Furthermore, the twofold modified oligonucleotides show a CD signal between 350 and 450 nm for the pyrene absorption with a negative Cotton effect with a minimum around 430 nm and a maximum at 380 nm, which implies an ordered arrangement of the pyrene groups in the chiral environment.

In the case of the modified non self-complementary sequences no CD signal can be observed in this range if just one strand is modified (as in **IVB–VA** and **IVA–VB**) in contrast to the twofold modified duplex **IVB–VB** which also exhibits a signal similar to the self-complementary duplexes (Fig. 3).

These findings imply that two pyrene residues are needed for an ordered arrangement presumably as a π–π-stack in the minor groove inhibiting rotation around the acetylenic bond for both 1-ethynylpyrene residues. This is in contrast to results presented for 5-(1-ethynylpyrene)-2′-deoxyuridine modified DNA where it is stated that at least three pyrene residues are needed for a detectable CD signal.[5c] In the duplexes with only one pyrene modification an extended rotational freedom can be assumed, which explains the absence of a CD signal due to the lack of ordered structure.

2.3. Fluorescence spectra

In order to investigate the changes of pyrene fluorescence in the twofold modified RNA upon hybridisation, temperature dependent fluorescence spectra were recorded. The self-complementary sequence **IIIB** (5′-GCAAA^{Py}UUUGC-3′) shows a broad unstructured fluorescence spectrum with a maximum at 471 nm. Upon thermal denaturation of the double strand, this maximum is blue-shifted by 27 nm to 444 nm (Fig. 4). These findings are comparable to the results of Wagenknecht and co-worker where, in contrast to the presented work, 2′-deoxyuridine in DNA is modified with 1-ethynylpyrene.[5c]

The shift of the emission band is biggest in the region of the oligonucleotide melting point (35 °C for **IIIB**). Above 40 °C and under 28 °C only a slight change in fluorescence maximum can be observed. This behaviour is visualized in more clarity by plotting the fluorescence maxima against temperature (Fig. 5). The estimated melting temperature computed from a fitted curve is 34.9 °C ± 3.9 °C, which is in good agreement with the melting temperature obtained by UV measurements.

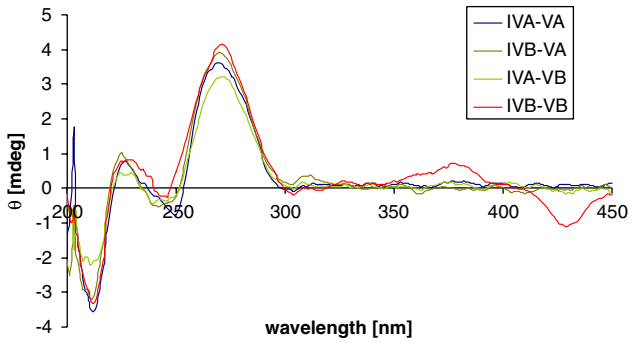
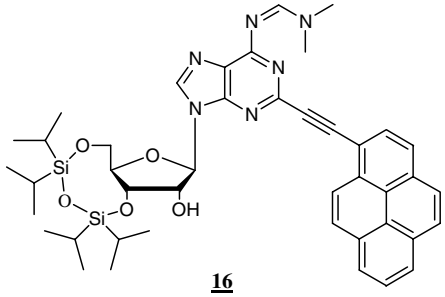


Figure 3. CD spectra of unmodified (**IVA**–**VA**), singly modified (**IVB**–**VA**, **IVA**–**VB**) and twofold pyrene labelled (**IVB**–**VB**) double strands in phosphate buffer, oligonucleotide concentration 5 μM.

The fluorescence spectrum of the single strand also shows no structure as it would be expected for pyrene with its three maxima around 385, 405 and 425 nm. 1-Ethynylpyrene itself shows the typical structured pyrene emission spectrum and formation of an excimer band at 490 nm at concentrations above 10^{-4} M (Fig. 6). The protected 2-(1-ethynylpyrene)-adenosine derivative **16** exhibits a different behaviour.



16

Compound **16** only exhibits a broad unstructured fluorescence spectrum with a red-shifted maximum around 437 nm at a concentration of 10^{-5} M in methanol compared to 1-ethynylpyrene with its three maxima between 380 and 430 nm (Fig. 6).

A similar behaviour for conjugated pyrene-modified nucleosides was also found for 8-(1-ethynylpyrene)-adenosine modified oligonucleotides.[17]

Quantum yield for **16** estimated on the basis of quinine sulfate in sulfuric acid is 0.72. Quantum yields of the oligonucleotides are generally lower in the range of 0.49 (for **IVB** in duplex with **VA**) to 0.68 for self-complementary **IIIB**.

Considering the fluorescence spectrum of 1-ethynylpyrene-modified nucleoside **16** and the concentration of 5 μM of oligonucleotides in thermal denaturation studies what excludes formation of an intermolecular excimer of the pyrene residues, the broad fluorescence can be assigned to the fluorescence of the singly pyrene-modified single strand without an interstrand interaction of the two pyrene residues. The loss of vibronic structure leads to a broad emission band for 2-(1-ethynylpyrene)-adenosine derivative presumably due to a strong electronic coupling in the conjugated system.

In the case of the non self-complementary oligonucleotides it is possible to investigate the fluorescence spectra of singly modified double strands together with the twofold modified strands. Both 1-ethynylpyrene-modified single strands **IVB** (5′-CUUUUCA^{Py}UUCUU-3′) and **VB** (5′-AAGAA^{Py}UGAAAAG-3′) showed broad emission spectra with maxima at 451 and 452 nm, respectively. Upon hybridisation with the complementary unmodified strand **VA** and **IVA**, respectively, the wavelengths of emission maxima did not change within the experimental error. There is, however, a significant drop of approximately 50% in fluorescence intensity. This can most likely be attributed to quenching mechanisms, for example by means of an energy transfer into the base stack and a subsequent radiationless deexcitation. Like

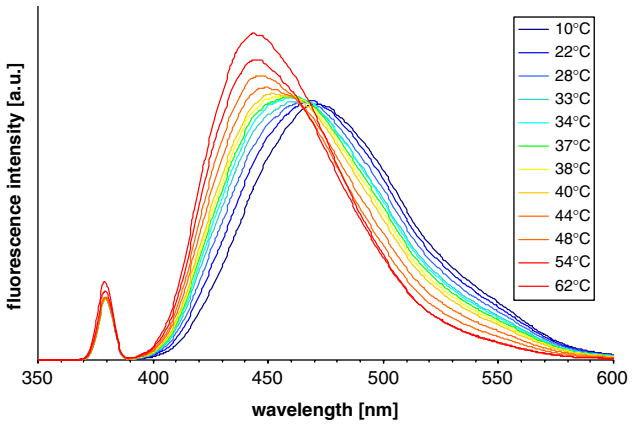


Figure 4. Temperature dependent emission spectra of pyrene-modified self-complementary strand **IIIB** (5′-GCAAA^{Py}UUUGC-3′) in phosphate buffer, oligonucleotide concentration 5 μM, λ_{exc} = 380 nm.

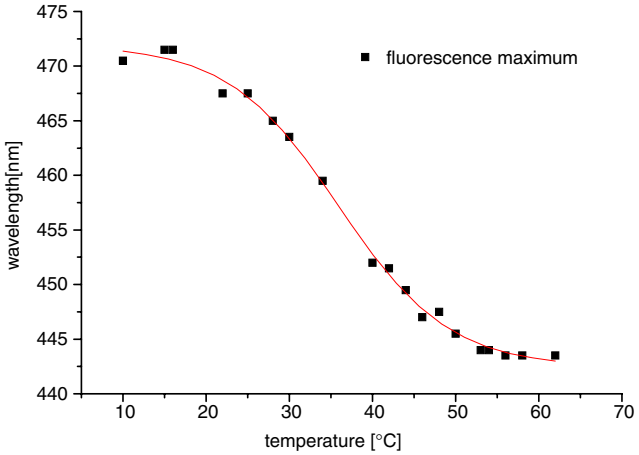


Figure 5. Temperature dependency of the fluorescence maxima, same conditions as in Figure 4.

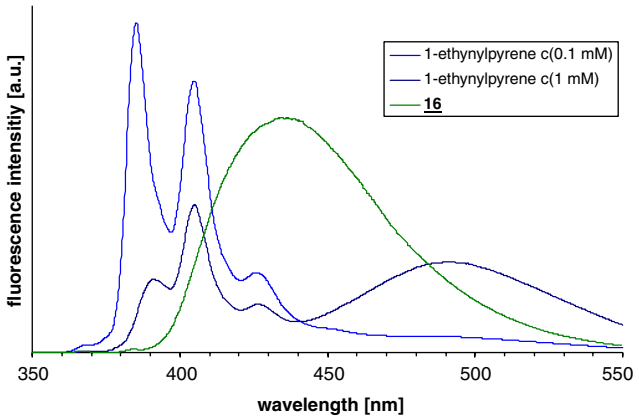


Figure 6. Fluorescence spectra of 1-ethynylpyrene (blue) at concentrations of 10^{-3} M and 10^{-4} M in MeCN and **16** (green) at a concentration of 10^{-5} M in MeOH, λ_{exc} = 365 nm.

self-complementary sequences, but in contrast to the singly modified double strands, the twofold modified duplex **IVB–VB** emits with a maximum at 481 nm corresponding to a bathochromic shift of 30 nm. This wavelength shift can be followed by the naked eye (Fig. 7).

These findings indicate that the bathochromic shift of 27 nm for self-complementary strands and 30 nm in case of twofold labelled non self-complementary duplexes is not just an effect of hybridisation with the complementary strand but gives rise to the assumption of an interaction of the two pyrene residues located on the different strands. Twofold ethynylpyrene-modified uridine in DNA has already been investigated by femtosecond time resolved spectroscopy and an electronic interaction of the two chromophores was reported for adjacent chromophores as well as across an intermediate base pair, leading to a wavelength shift half as large as the one we observed for our system.[18] In contrast to this work, where chromophores are located on the same strand, in our system energy transfer through the base stack is not expected since the two pyrene residues are located on different strands. In agreement with this interpretation, the temperature dependent fluorescence measurements showed no shift for the fluorescence maxima wavelength in case of the singly modified duplexes **IVA–VB** or **IVB–VA** but a hypsochromic shift from 481 to 451 nm of the twofold modified duplex **IVB–VB** upon thermal denaturation.

Comparison of the absorption spectra shows small differences in the region of the $S_0 \rightarrow S_1$ transition. For the thermally denatured case, a small blue shift can be seen and the two absorption maxima are more pronounced. Further measurements to clarify the significance of these differences in the absorption spectrum are currently being carried out.

3. Conclusion

In summary, we described a convenient way to introduce fluorophores like 1-ethynylpyrene into RNA oligonucleotides by site specific functionalization on solid support during oligonucleotide synthesis using Sonogashira cross-coupling. Upon bonding 1-ethynylpyrene to adenosine in the 2-position the structured pyrene fluorescence spectrum changed to a broad unstructured band. This is attributed to an electronic coupling between pyrene and the nucleic base[18] in the extended conjugated system. The nature of this electronic coupling has still to be investigated in detail.

The increase in T_m values and the CD spectra presented together with the fluorescence spectra of singly and twofold 1-ethynylpyrene-modified oligonucleotides allows us to assume that the two pyrene residues can actually interact with each other and show a broad red-shifted fluorescence on the RNA as a scaffold. The fluorescence wavelength shift is quite pronounced and can be followed by the naked eye. By this fluorescence shift, one can distinguish between single-stranded and double-stranded RNA during thermal denaturation. This

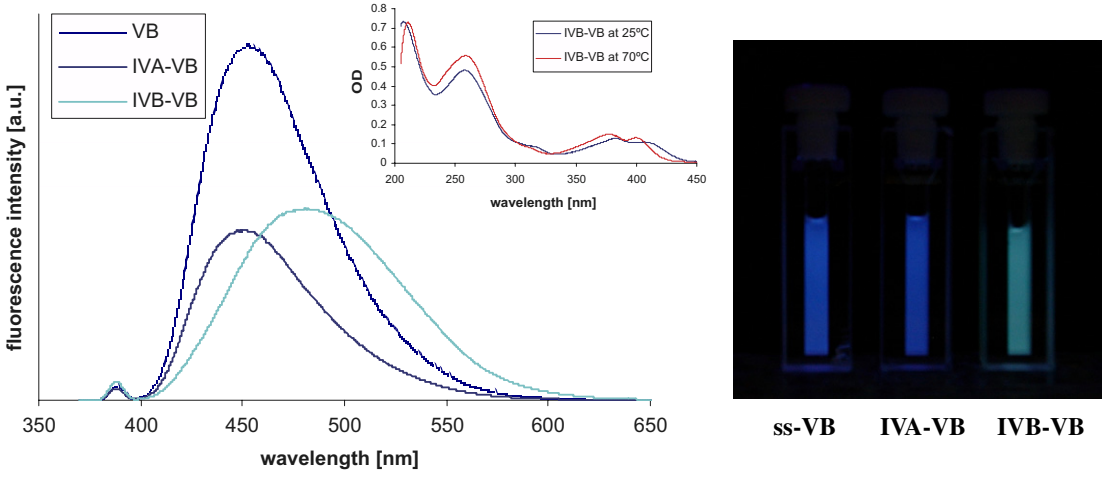


Figure 7. (Left) Fluorescence spectra of single-stranded **VB**, double-stranded singly pyrene-modified **IVA–VB** and double-stranded twofold modified **IVB–VB**, phosphate buffer, oligonucleotide concentration 5 μM, λ_{exc} = 385 nm; inset: UV spectrum of duplex **IVB–VB**, same conditions. (Right) Photographs of cuvettes containing the corresponding solutions of oligonucleotides, excitation with a standard UV lamp at λ_{exc} = 365 nm.

behaviour could be used for the time resolved investigation of RNA hybridisation and folding. The exact nature of the pyrene–pyrene-interaction remains yet to be examined. Femtosecond fluorescence measurements are currently performed to investigate this point.

4. Experimental

The synthesis of compounds **7**–**15** will be reported elsewhere.[19] The reactions were monitored by thin-layer chromatography (TLC) analysis on silica gel aluminium plates (silica gel 60 F_{254}, 0.2 mm, Merck). Column chromatography was performed on silica gel (40–63 μm, Merck). Technical solvents were used after distillation for chromatography; absolute solvents, dried over molecular sieve, were purchased from FLUKA. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX250 at 250 MHz. Electron Spray Ionisation (ESI) masses were collected on a VG Platform II (Fisons Instruments).

4.1. 2′,3′,5′-Tri-*O*-acetylguanosine (6)

Guanosine (5 g, 17.7 mmol) and *N*,*N*-dimethylaminopyridine (162 mg, 1.3 mmol, 7 mol%) were dissolved in 220 mL acetonitrile. After addition of TEA (9.7 mL, 69.9 mmol, 1.1 equiv) and acetic acid anhydride (6 mL, 63.5 mmol, 3.6 equiv) the mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of 3 mL MeOH and the solvent evaporated in vacuo. The oily residue was recrystallized from 2-propanol to afford **2** as a white powder (6.6 g, 91%). $R_f = 0.60$ (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (250 MHz, DMSO-d_6) δ [ppm] 10.73 (s, 1H, NH), 7.92 (s, 1H, H8), 6.53 (s, 2H, NH_2), 5.97 (d, 1H, H1′), 5.78 (t, 1H, H2′), 5.48 (dd, 1H, H3′), 4.39–4.21 (m, 3H, H4′, H5′), 2.10 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc). ^{13}C NMR (63 MHz, DMSO-d_6) δ [ppm] 170.05, 169.41, 169.24, 156.58, 153.58, 151.08, 135.50, 116.80, 84.37, 79.51, 72.01, 70.28, 63.04, 20.50, 20.35, 20.16; ESI-MS(+) *m*/*z* (%) = 410.0 (100) $[M+H]^+$, 258.7 (50) [tri-*O*-acetylribose+H]$^+$, 152.5 (60) [guanine+H]$^+$.

4.2. 2-(1-Ethynylpyrenyl)-3′,5′-*O*-(tetraisopropyldisiloxano)-N^6-(*N*′,*N*′-dimethylamino-methyleno)-adenosine (16)

Protected 2-iodoadenosine **11** (90 mg, 0.13 mmol) and 1-ethynylpyrene (40 mg, 0.18 mmol, 1.36 equiv), CuI (5 mg, 26 μmol, 15 mol%) and $(PPh_3)_4Pd$ (20 mg, 17 μmol, 10 mol%) were dissolved in a degassed solvent mixture of 5 mL DMF and 300 μL TEA. After 24 h of stirring at room temperature, the reaction mixture was diluted with 30 mL CH_2Cl_2 and extracted twice with 10 mL 0.3 M EDTA (aqueous solution) and the aqueous phase extracted with CH_2Cl_2. After evaporation of the solvent and column chromatography (CH_2Cl_2/MeOH = 90:10; $R_f = 0.58$) the product was obtained as a yellow solid (80 mg, 78%) ^{1}H NMR (250 MHz, DMSO-d_6) δ [ppm] 9.08 (s, 1H, CH), 8.80 (m, 2H, ArH, H8), 8.35–8.04 (m, 8H, ArH), 6.17 (d, 1H, H1′), 5.04 (m, 1H, H2′), 4.74 (m, 1H, H3′), 4.22-4.07 (m, 3H, H4′, H5′), 3.33 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), MALDI-TOF *m*/*z* (%) = 790.73 (100) $[M+H]^+$.

4.3. Oligonucleotide synthesis and purification

The oligonucleotides were synthesized on a 0.2 μmol scale on a rebuilt ABI 392 synthesizer (Applied Biosystems) with phosphoramidites purchased from Dharmacon. Every RNA synthesis was stopped after incorporation of the 2-iodoadenosine phosphoramidite without deprotecting the 5′-hydroxyl group. The column was removed from the synthesizer and maintained under argon atmosphere. In the meantime CuI (6.0 mg) was dissolved in dried and deoxygenated CH_2Cl_2/Et_3N (17.5:7.5 mL). 150 μL of this solution was added under argon to a mixture of $(PPh_3)_4Pd$ (3.5 mg, 15 equiv) and 1-ethynylpyrene (2.1 mg, 45 equiv) with syringe (equivalents related to the 0.2 μmol scale). The orange solution was given onto the column and moved in it back and forth using two syringes. After a reaction time of 2.5 h the column was washed with 10 mL of dried CH_2Cl_2, dried for 10 min in vacuo and flushed with argon. The Sonogashira cross-coupling was performed a second time as described above. After the second coupling the column was reinstalled on the synthesizer to end the oligonucleotide synthesis. Afterwards the phosphate protecting groups were cleaved on solid support by treatment with 2 mL of a 0,4 M solution of disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate-trihydrate (S_2Na_2) in DMF/H_2O = 98:2 within 60 min. Deprotection of the exocyclic protection groups and cleavage of acetate of the 2′-ACE group as well as cleavage of the oligonucleotides from solid support are effected by treatment with a 40% aqueous solution of methylamine for 10 min at 55 °C. The crude oligonucleotide was purified by anion exchange chromatography (A: demineralised water, B: 1 M LiCl, gradient: 0–80% B within 40 min) on JASCO HPLC-system 900 with a semi-preparative Dionex DNAPac® PA-100 column (9 × 250 mm). After desalting with PD-10 Sephadex columns from Amersham Biosciences, the final deprotection of the 2′-ACE protecting group was performed under sterile conditions with a TEMED/acetic acid buffer adjusted to pH 3.8 for 30 min at 60 °C. The obtained oligonucleotides were characterized by MALDI-TOF mass spectrometry (VOYAGER DE-PRO mass spectrometer from Applied Biosystems).

4.4. Spectroscopic measurements

All spectroscopic measurements were performed in a phosphate buffer containing 10 mM NaH_2PO_4, 10 mM Na_2HPO_4 and 140 mM NaCl adjusted to pH 7.0. Oligonucleotide concentration was 5 μM. Melting curves were recorded on a Cary UV–vis spectrophotometer from Varian by detecting the optical density at 260 nm. The solution was heated to 80 °C for 1 min and then cooled to 10 °C within 10 min to form the duplex. The melting curves were recorded using a heating rate of 0.5 °C/min. CD spectra were recorded on a JASCO J-710 spectropolarimeter at a temperature of 10 °C between 200 and 450 nm. The fluorescence spectra were recorded on a

Hitachi F-4500 fluorescence spectrophotometer with excitation at 385 or 365 nm.

Quantum yields were estimated on the basis of quinine sulfate quantum yield of 0.55 in 0.5 M sulfuric acid. For organic solvents a correction based on diffractive indices was done, for aqueous solutions no such correction was carried out.

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