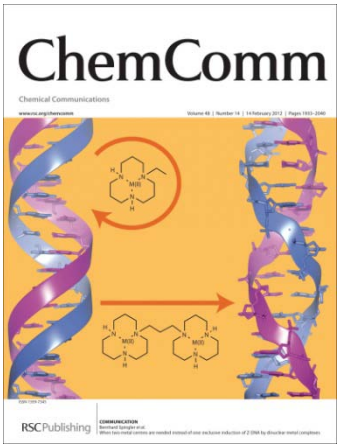

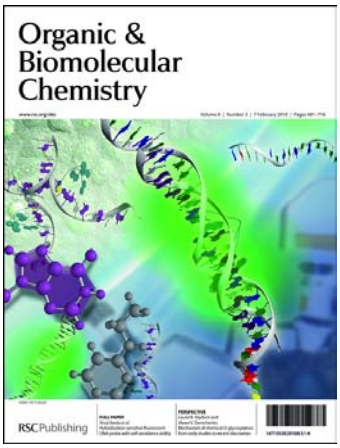

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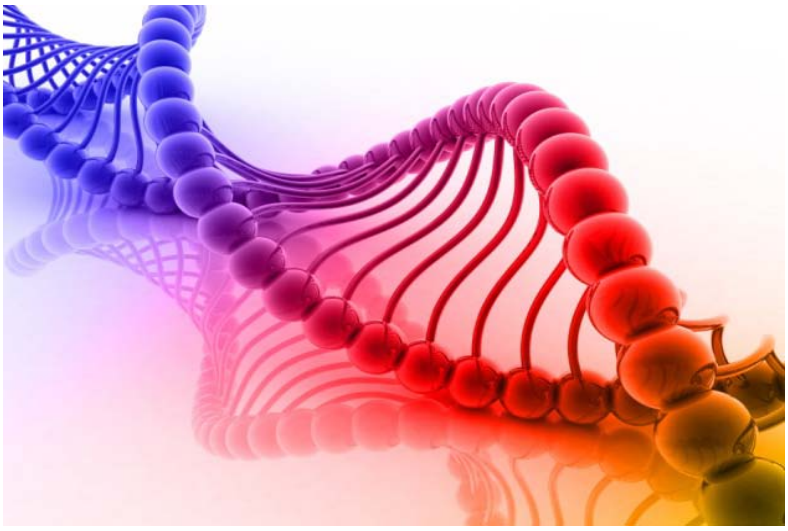

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COMMUNICATION

Synthesis and hybridization properties of oligonucleotides modified with 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridines†‡

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Oligonucleotides modified with consecutive incorporations of 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers X–Z display strong thermal affinity and binding specificity toward RNA targets, due to formation of chromophore arrays in the major groove.

The use of nucleic acids as scaffolds for organization of chromophore arrays is an area of considerable focus, which is fuelled by the promise for materials with interesting photophysical and electronic properties.[1–6] A frequently employed approach toward this end entails self-assembly of duplexes involving oligonucleotides (ONs), which are densely modified with chromophore-functionalized nucleotide monomers. Examples of building blocks include monomers where chromophores replace nucleobase moieties[7–9] or are attached to non-nucleosidic linkers,[10,11] sugar skeletons[12–14] or nucleobase moieties. Among the latter class, C5-functionalized pyrimidine monomers in which chromophores are either directly attached to the nucleobase moiety or attached *via* an alkynyl linker have been studied in particular detail and demonstrated to facilitate array formation in the major groove.[15–19] While array formation often partially counteracts the prominent duplex destabilization induced by these monomers, the resulting duplexes still only display moderate thermostability. Development of nucleotide building blocks, which enable formation of chromophore arrays in the major groove without compromising duplex thermostability, therefore remains a desirable goal.

Nielsen and coworkers have recently demonstrated that ONs, which are consecutively modified with 5-(1-phenyl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers, display strong and highly specific affinity toward RNA targets due to the formation of stabilizing chromophore arrays in the major groove.[20–22] While the influence of phenyl substitution on array formation has been studied in detail,[21,23] the question how the size of the aromatic moiety influences stacking efficiency and thermostability has not been systematically addressed. Following a hypothesis that chromophores with larger aromatic surfaces are likely to result in stronger stacking interactions in the spacious major groove, we set out to study the hybridization properties of ONs, which are modified with 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers **X–Z** featuring three differentially sized aromatic moieties at the 1-position of the triazole ring (Fig. 1).

Phosphoramidites **3X** and **3Y** were obtained *via* the same general strategy, which we recently used for the synthesis of **3Z** (Scheme 1).[24] Thus, O5′-protected 5-ethynyl-2′-deoxyuridine **1**[25] was reacted with azidobenzene[26] or 1-azidonaphthalene[27] in a Cu^{I} catalyzed [3 + 2] azide–alkyne cycloaddition[28] to afford nucleosides **2X** and **2Y** in 74% and 78% yield. Subsequent O3′-phosphitylation using 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (*i.e.*, PCl-reagent) and *N*,*N*′-diisopropylethylamine (DIPEA) provided 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine phosphoramidites **3X**[20] and **3Y** in 60% and 73% yield, respectively.

The phosphoramidites were incorporated into ONs *via* machine-assisted solid-phase DNA synthesis (hand-coupling 20 min, 4,5-dicyanoimidazole as an activator; coupling yields >95%, >95% and ~92% for monomers **X**, **Y** and **Z**, respectively). The composition and purity of the modified ONs was verified by MALDI-ToF MS analysis (Table S1‡) and ion-pair reverse-phase HPLC, respectively.

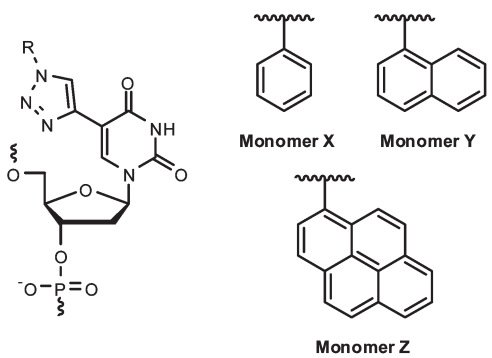

Fig. 1 Structures of 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridines studied herein.

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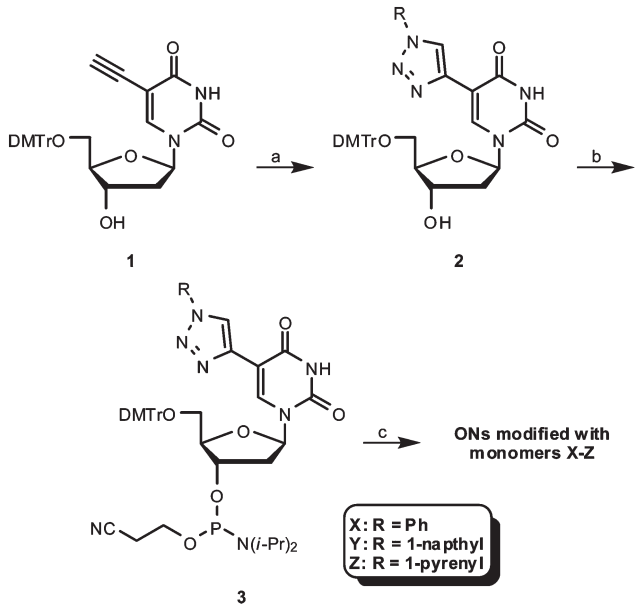
† Dedicated to the memory of Professor H. Gobind Khorana.

‡ Electronic supplementary information (ESI) available: General experimental section; synthetic protocols and NMR spectra for nucleosides **2X–3Y**; protocols for ON synthesis and purification; MS-data for modified ONs; description of thermal denaturation experiments; T_m data recorded in a medium salt buffer; additional fluorescence emission spectra. See DOI: 10.1039/c2ob26717a

Monomers **X–Z** were incorporated once, twice or four times into a 9-mer T-rich sequence that has been used to study and prepare self-assembling chromophore arrays.[20] Thermal denaturation temperatures (T_m's) of duplexes between modified ONs and complementary DNA/RNA targets were determined in buffers of high or medium ionic strength (Tables 1 and S2‡, respectively).

Singly modified ONs display substantially lower thermal affinity toward DNA and RNA complements than corresponding unmodified ONs (see ΔT_m's for the **B1**-series, Table 1), which is commonly observed for ONs modified with C5-chromophore-functionalized pyrimidine monomers.[15,16,29–31] Duplex thermostability decreases progressively as the size of the aryl substituent increases, most likely due to increased perturbation of the hydration spine in the major groove. Incorporation of two 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers as next-nearest neighbors results in further duplex destabilization, although the energetic penalty associated with monomer **Y** is partially reversed (see ΔT_m/mod for the **B2**-series, Table 1). In contrast, incorporation of two or four consecutive **X** or **Y** monomers strongly reverses duplex destabilization, especially in **X**-modified duplexes with RNA targets (compare ΔT_m/mod trends for **X1→X3→X4** and **Y1→Y3→Y4**, Table 1). Interestingly, **Z4**, which features four consecutive incorporations of monomer **Z**, displays very high affinity toward RNA as well as DNA targets (see ΔT_m/mod for **Z4**, Table 1) although it should be noted that broad transitions are observed (Fig. S4‡).



Scheme 1 (a) RN_3, aq. sodium ascorbate, aq. $CuSO_4$, THF : H_2O : *t*BuOH, rt (**2X**: 74%; **2Y**: 78%; **2Z**:[24] 52%); (b) PCl-reagent, DIPEA, CH_2Cl_2, rt (**3X**: 60%; **3Y**: 73%; **3Z**:[24] 73%); (c) machine-assisted DNA synthesis. R = phenyl, 1-naphthyl and 1-pyrenyl for the **X**-, **Y**- and **Z**-series, respectively.

Computational studies have previously linked the increased thermostability of **X4** : RNA to formation of chromophore arrays in the major groove.[21] This, along with the observed T_m trends for **Y**/**Z**-modified duplexes and results from additional structural studies (*vide infra*), suggests that incorporation of consecutive 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers is a general strategy toward formation of stable chromophore arrays in the major groove. The inherently destabilizing effect of these building blocks is, most likely, counteracted by favorable hydrophobic interactions between chromophores upon array formation. However, the correlation between duplex/array stability and size/hydrophobicity of the aryl moiety is complex (trend in ΔT_m/mod values: **Z4** ≥ **X4** > **Y4**).

Next, the thermostability of duplexes between **B1**- or **B4**-series ONs and RNA targets featuring a centrally mismatched nucleotide was determined to study the binding specificity of these probes (Table 2). Singly modified ONs display mismatch discrimination profiles that differ from the corresponding unmodified ONs in the following manner: (i) **X1** and **Y1** display less efficient discrimination of U-mismatches, (ii) **Z1** displays markedly poorer discrimination of rC-mismatches, and (iii)

Table 1 Thermal denaturation temperatures (T_m values) for duplexes between **B1B4** and complementary DNA/RNA in a high salt buffer[a]

			T_m (ΔT_m/mod) [°C]					
			DNA			RNA		
			3′-CACAAAACG			3′-CACAAAACG		
ON	Sequence	**B** =	**X**	**Y**	**Z**	**X**	**Y**	**Z**
B1	5′-GTGT **B** TTGC		35.5 [−4.5]	32.0 [−8.0]	25.0[b] [−15.0]	35.0 [−3.0]	29.0 [−9.0]	25.0[b] [−13.0]
B2	5′-GTG **B**T**B** TGC		28.5 [−5.8]	27.5 [−6.3]	—	29.5 [−4.3]	29.5 [−4.3]	—
B3	5′-GTGT **BB** TGC		32.5 [−3.8]	29.5 [−5.8]	—	40.0 [+1.0]	32.0 [−3.0]	—
B4	5′-GTG **BBBB** GC		38.0 [−0.5]	26.0[b] [−3.5]	52.5[b] [+3.0]	55.0 [+4.3]	39.0 [+0.3]	55.5[b] [+4.4]

[a] T_m's determined as the first derivative maximum of thermal denaturation curves (A_{260} *vs.* *T*) recorded in a high salt buffer ([Na^+] = 710 mM, [Cl^-] = 700 mM, pH 7.0 (adjusted with NaH_2PO_4/Na_2HPO_4)), using 1.0 μM of each strand. T_m's are averages of at least two measurements within 1.0 °C. ΔT_m/mod = change in T_m's per modification relative to unmodified reference duplexes (+DNA complement: T_m = 40.0 °C; +RNA complement: T_m = 38.0 °C). "—" denotes no transition. [b] Weak/broad transition.

Table 2 T_m values for duplexes between the **B1**/**B4**-series and centrally mismatched RNA targets[a]

			RNA: 3′-CAC A**M**A ACG			
			T_m/°C	ΔT_m/°C		
ON	Sequence	**M** =	**A**	**C**	**G**	**U**
D1	5′-GTGTTTTGC		38.0	−14.0	−6.0	−20.0
X1	5′-GTGT **X** TTGC		35.0	−14.5	−11.0	−13.0
X4	5′-GTG **XXXX** GC		55.0	−20.0	−13.5	−20.0
Y1	5′-GTGT **Y** TTGC		29.0	−11.0	−7.0	−12.5
Y4	5′-GTG **YYYY** GC		39.0	−13.5	−7.0	−22.0
Z1	5′-GTGT **Z** TTGC		25.0	−3.0[b]	<−10.0[c]	<−10.0[c]
Z4	5′-GTG **ZZZZ** GC		55.5	−12.5[b]	−12.5[b]	−12.5[b]

[a] For conditions of thermal denaturation experiments, see Table 1. ΔT_m = change in T_m relative to the matched DNA : RNA duplex (**M** = A). [b] Weak transition. [c] No transition above 15 °C.

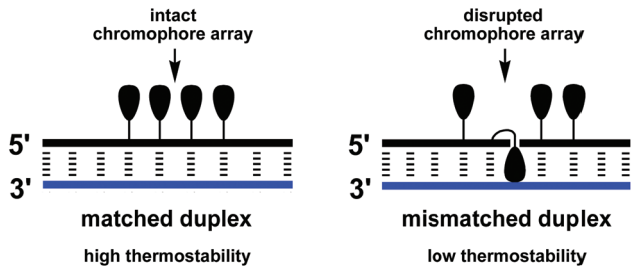


Fig. 2 Proposed structural model that rationalizes increased affinity and specificity of ONs modified with four consecutive 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers.

X1 and **Z1** display improved discrimination of rG-mismatches (Table 2). Interestingly, the target specificity of **X4**, **Y4** and, possibly, **Z4** is markedly improved relative to their singly modified counterparts (compare ΔT_m for the **B4**- *vs.* **B1**-series, Table 2). In fact, **X4** and **Y4** display base pairing fidelity that compares favorably with the unmodified reference strand, suggesting that chromophore arrays may have beneficial impacts on target affinity as well as target specificity. Interestingly, while ONs with stretches of 5-ethynyl-2′-deoxyuridine monomers are known to display improved target specificity due to long-range cooperativity,[32] ONs with stretches of C5-chromophore-functionalized 2′-deoxyuridine monomers typically display poor mismatch discrimination.[15,17]

The following structural model accounts for the observed T_m-trends (Fig. 2): (i) hybridization of **X4**/**Y4**/**Z4** with complementary RNA targets results in the formation of a stabilizing chromophore array in the major groove, whereas (ii) hybridization with mismatched targets results in array disruption, reduced duplex stability and improved mismatch specificity.

The model is substantiated by steady-state fluorescence emission experiments with **Z1**–**Z4**.§ Thus, duplexes involving the singly modified **Z1** display typical pyrene monomer peaks at ~380 nm and ~400 nm (Fig. S5‡). In contrast, duplexes involving probes with two or four incorporations of monomer **Z** generally display a broad and prominent peak at ~475 nm, which is indicative of pyrene–pyrene excimer formation and π–π-stacking

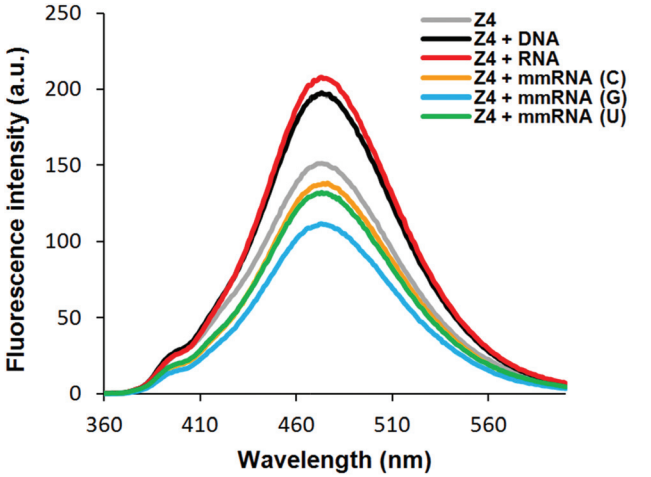


Fig. 3 Steady-state fluorescence emission spectra of **Z4** in the presence or absence of complementary DNA/RNA or mismatched RNA targets (λ_{em} = 344 nm; T = 10 °C).

(Fig. 3 and S5‡). Interestingly, duplexes between **Z4** and centrally mismatched RNA targets display lower excimer intensity (Fig. 3), which suggests perturbation of the pyrene array as proposed in the structural model (Fig. 2).

In summary, we demonstrate that ONs with stretches of 5-(1-aryl-1,2,3-triazol-4-yl)-2′-deoxyuridine monomers display improved RNA affinity and specificity relative to reference strands, due to formation of stabilizing chromophore arrays in the major groove. This design principle is expected to have important implications in the design of supramolecular nucleic acid based π-functional materials and antisense ONs.

Acknowledgements

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Notes and references

§ Broad peaks in the UV-Vis spectra of **Z1**–**Z4** with/without DNA/RNA targets precluded determination of hybridization-induced changes in absorption maxima, which could have provided additional insight into pyrene binding modes (results not shown).

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