Bioorganic & Medicinal Chemistry Letters 25 (2015) 3999–4004

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and characterization of oligodeoxyribonucleotides modified with 2′-thio-2′-deoxy-2′-*S*-(pyren-1-yl)methyluridine

Brooke A. Anderson, Patrick J. Hrdlicka *

Department of Chemistry, University of Idaho, Moscow, ID 83844-2343, United States

ARTICLE INFO

Article history:
Received 11 June 2015
Revised 30 June 2015
Accepted 2 July 2015
Available online 7 July 2015

Keywords:
Oligonucleotides
DNA recognition
Pyrene
Intercalators

ABSTRACT

Pyrene-functionalized oligonucleotides are intensively explored for applications in materials science and diagnostics. Here, we describe a short synthetic route to 2′-*S*-(pyren-1-yl)methyl-2′-thiouridine monomer **S**, its incorporation into oligodeoxyribonucleotides (ONs), and biophysical characterization thereof. Pseudorotational analysis reveals that the furanose ring of this monomer has a slight preference for *South*-type conformations. ONs modified with monomer **S** display high cDNA affinity but decreased binding specificity. Hybridization is associated with bathochromic shifts of pyrene absorption bands and quenching of pyrene fluorescence consistent with an intercalative binding mode of the pyrene moiety. Monomer **S** was also evaluated as a building block for mixed-sequence recognition of double-stranded DNA via the Invader strategy. However, probes with +1 interstrand arrangements of monomer **S** were found to be less efficient than Invader probes based on 2′-*O*-(pyren-1-yl)methyluridine or 2′-*N*-(pyren-1-yl)methyl-2′-*N*-methyl-2′-aminouridine.

© 2015 Elsevier Ltd. All rights reserved.

Development of pyrene-functionalized oligonucleotides is an area that continues to attract considerable interest due to the prospect of tools for a range of applications in materials science and diagnostics,[1] including generation of self-assembled helical pyrene arrays[2] and the development of probes for detection of complementary DNA/RNA[3] (cDNA/cRNA) and single nucleotide polymorphisms (SNPs).[4] As part of our growing interest in pyrene-functionalized oligonucleotides, we recently introduced an unique approach for recognition of double-stranded DNA (dsDNA),[5–7] which is based on double-stranded oligonucleotide probes that are energetically activated through modification with +1 interstrand zipper[8] arrangements of pyrene-functionalized nucleotides such as 2′-*O*-(pyren-1-yl)methyl RNA or 2′-*N*-(pyren-1-yl)methyl-2′-*N*-methyl-2′-amino DNA monomers (Fig. 1). This particular motif forces the two pyrene moieties to intercalate into the same region of the probe, leading to local perturbation and duplex destabilization as the 'nearest neighbor exclusion principle' is violated.[9] In contrast, each of the two probe strands form very stable duplexes with cDNA as the intercalating pyrene moieties are engaged in efficient π-stacking with neighboring base-pairs. This generates a thermodynamic gradient, which, unlike most other hybridization-based strategies,[10] allows for recognition of mixed-sequence dsDNA target regions at physiologically relevant conditions.[7,11]

Our earlier efforts at optimizing the dsDNA-recognition efficiency of these so-called Invader probes have focused on varying: the number and relative position of the key activating monomers, the nature of the nucleobase and intercalator, and the length of the linker and the orientation between the intercalator and sugar skeleton.[6,7,12–16] In the present work, we set out to study the influence of the 2′-heteroatom of the pyrene-functionalized nucleotide monomer on the dsDNA-recognition characteristics of Invader probes. We hypothesized that the lower electronegativity of the sulfur atom of 2′-*S*-(pyren-1-yl)methyl-2′-thiouridine monomer **S** would weaken the *gauche* effect between O4′ and the 2′-substituent, and thus increase the population of C2′-*endo* (*South*-type) furanose conformations.[17] This, in turn, was expected to result in more favorable conditions for pyrene intercalation, leading to higher cDNA affinity relative to ONs modified with current-generation Invader building blocks 2′-*O*-(pyren-1-yl)methyluridine monomer **O**[18] or 2′-*N*-(pyren-1-yl)methyl-2′-*N*-methyl-2′-aminouridine monomer **N**[18] (Fig. 1).

Here we describe (i) a short synthetic route to 2′-*S*-(pyren-1-yl)methyl-2′-thiouridine phosphoramidite **4** and its incorporation in ONs, and results from (ii) coupling constant analyses, which provide insights into conformational preferences of monomer **S**, (iii) thermal denaturation experiments and thermodynamic parameter analysis, (iv) UV–Vis absorption and fluorescence

* Corresponding author. Tel.: +1 208 885 0108.
E-mail address: Hrdlicka@uidaho.edu (P.J. Hrdlicka).

http://dx.doi.org/10.1016/j.bmcl.2015.07.002
0960-894X/© 2015 Elsevier Ltd. All rights reserved.

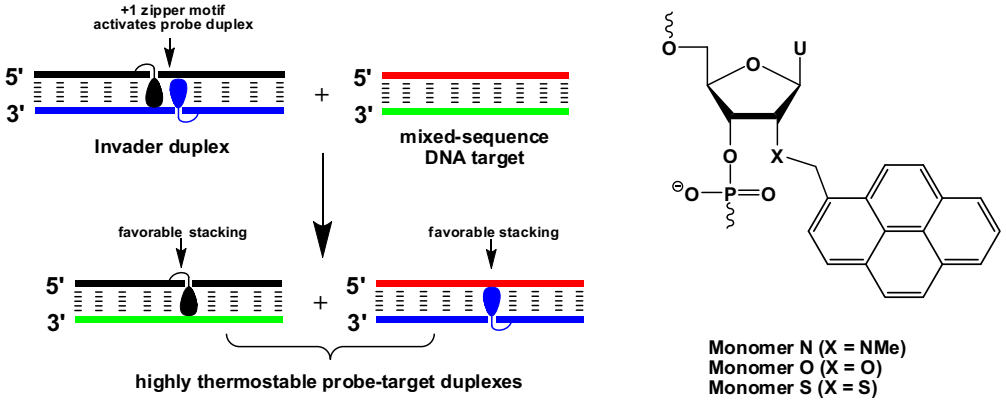


Figure 1. Recognition of dsDNA via the Invader strategy and structures of studied monomers. Droplets denote the intercalating pyrene moiety.

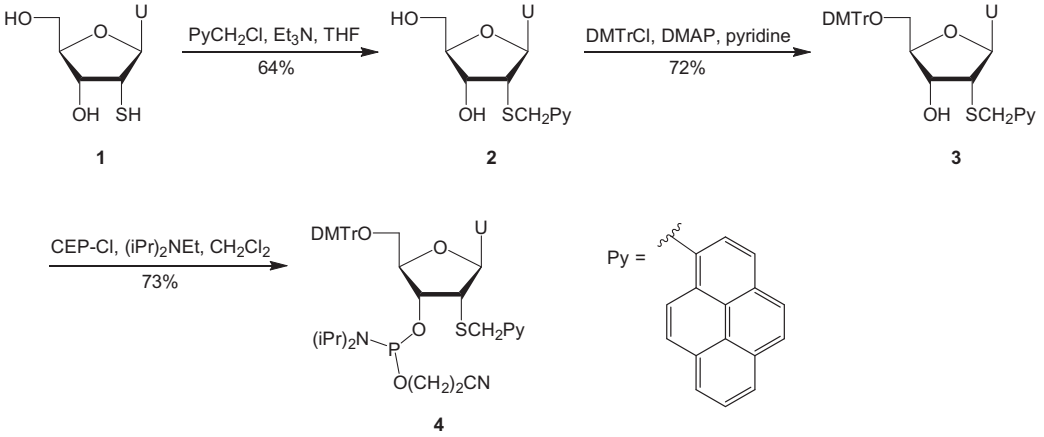
experiments, and (v) dsDNA-recognition experiments, all discussed in relation to ONs and Invader probes based on **O** and **N** monomers.

2′-Deoxy-2′-thiouridine **1**, which was used as the starting material for the synthesis of phosphoramidite **4** (Scheme 1), was prepared from uridine in ~50% yield over three steps as described in the literature.[19] Nucleoside **1** was then alkylated at the S2′-position using 1-pyrenylmethyl chloride under mildly basic conditions,[20] to afford nucleoside **2** in 64% yield. Similar yields were obtained when 1-pyrenylmethyl bromide was used as the alkylating agent (results not shown). Standard O5′-DMT protection afforded nucleoside **3** in 72% yield, which was treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (CEP-Cl) and Hünig's base to give target phosphoramidite **4** in 73% yield.

A coupling constant analysis was performed to determine if the lower electronegativity of the 2′-sulfur of monomer **S** induces a greater proportion of *South* type furanose conformations relative to monomers **N** and **O**. Thus, $^{3}J_{HH}$ scalar coupling constants for the endocyclic sugar protons of nucleoside **3** were used as input in a Matlab-based pseudorotational analysis program,[21] which facilitates determination of pseudorotation phase angles (P) and puckering amplitudes (ϕ_m) for five-membered ring systems, by solving modified Karplus–Diez–Donders equations (Table S1).[21–23] From this analysis, nucleoside **3** is predicted to have a slight preference for *South* conformations ($P = 143°$, $\phi_m = 38°$, $\%S = 61\%$), while the corresponding nucleoside of monomer **O** is predicted to be in two more equally populated conformations, that is, a *North* conformation ($P = 11°$, $\phi_m = 38°$, $\%N = 51\%$) and a *South* conformation ($P = 130°$, $\phi_m = 33°$; $\%S = 49\%$). Interestingly, the corresponding nucleoside of monomer **N** is predicted to exclusively adopt *South* type conformations (main conformer $P = 145°$, $\phi_m = 38°$, 61% frequency; secondary conformer $P = 122°$, $\phi_m = 27°$), presumably due to additional steric interactions in *North* conformations between the 2′-*N*-methyl group and the 3′-oxygen. However, it is important to appreciate that stereoelectronic effects on the nucleoside level may not necessarily fully translate to the oligonucleotide or duplex level. For example, crystal structures of *A*- and *B*-type DNA duplexes modified with 2′-*S*-methyl-uridines show that the modified residues adopt RNA-like C3′-*endo* puckers, demonstrating that replacement of the electronegative 2′-oxygen by a sulfur, does not fundamentally alter the conformational preference of the sugar in the oligonucleotide context,[24] even though these monomers were predicted to adopt DNA-like C2′-*endo* puckers.[25] Ultimately, high-resolution X-ray or solution NMR structures of **N**-, **O**-, and **S**-modified duplexes will be necessary to fully understand the structural underpinnings of the observed trends in thermal denaturation temperatures (T_m) (vide infra).

Phosphoramidite **4** was used in automated solid phase DNA synthesis to incorporate monomer **S** into ONs using extended hand-coupling times (15 min) and 4,5-dicyanoimidazole as an activator, resulting in stepwise coupling yields of >95%. The identity and purity of the modified ONs was established through MALDI-MS (Table S2) and ion-pair reverse phase HPLC (>90% purity).

Thermal denaturation temperatures of duplexes between **S**-modified ONs and cDNA/cRNA were determined from thermal



Scheme 1. Synthesis of target nucleoside **4**. U = uracil-1-yl; DMTr = 4,4′-dimethoxytrityl; DMAP = 4-dimethylaminopyridine; CEP-Cl = 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite.

Download English Version:

https://daneshyari.com/en/article/1358739

Download Persian Version:

https://daneshyari.com/article/1358739

Daneshyari.com