A click chemistry approach towards nucleic acid major groove functionalization

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ABSTRACT

A synthetic strategy towards new aromatic nucleoside derivatives introducing additional aromatic functionality placed in the major groove of a modified DNA duplex is presented. The functionalities are introduced using Click Chemistry conditions and found to increase the overall duplex stability.

INTRODUCTION

Hydrogen-bonding and \(\pi-\pi\)-stacking are important for a large part of the overall duplex stability.\textsuperscript{1} The use of biphenyl as nucleobase replacements with polar and electron withdrawing substituents as well as donors have been proven by Leumann and co-workers to have interesting properties with respect to stability and fluorescence.\textsuperscript{2,3} We have been focusing on increasing the overall duplex stability by introducing triazole linked aromatic functionalities on the natural pyrimidines with inherent ability to increase \(\pi-\pi\)-interaction into the major groove\textsuperscript{4} and by using Click Chemistry as a convenient methodology.\textsuperscript{5,7} The aim of this investigation is to obtain a method for introducing various functional moieties that may find application within DNA-based nanotechnology.

RESULTS AND DISCUSSION

In continuation of previous results obtained in our group\textsuperscript{4} we decided to further study the effects of introducing polar aromatic substituents in the major groove of DNA by using the synthetic monomers 2, 3 and 4. Furthermore, we aim at extending this uridine series with the corresponding cytidine analogs and have initiated this work by synthesizing the corresponding cytidine analog of 2. Monomer 2 was obtained according to the previously published procedure (Scheme 1),\textsuperscript{4} and monomer 3 was obtained using a similar protocol. Monomer 4 was synthesized using the novel TBTA Cu\textsuperscript{I} ligand\textsuperscript{8} from the DMT\textsubscript{R} protected nucleoside 5 (Scheme 1), and then converted to the corresponding phosphoramidite.

The use of TBTA and CuI without an additional reductive additive such as sodium ascorbate proved to give comparable yields and to shorten the reaction time considerably (from 30 min, 100 °C using MW irradiation or rt over night to 2 h at rt). When incorporated into ON’s the TBS group of 3 was removed in the global deprotection by NH\textsubscript{3}(aq).

\textbf{Scheme 1} a) 1) azide generated \textit{in situ},\textsuperscript{9} 2) nucleoside 1, CuI, Na-ascorbate, EtOH, H\textsubscript{2}O, rt.; b) 1) azide generated \textit{in situ},\textsuperscript{9} 2) TBTA, CuI, Nucleoside 5; c) DMT\textsubscript{Cl}, pyridine, rt.; d) (i-Pr\textsubscript{2}N)(O(CH\textsubscript{3})\textsubscript{2})CN, 20% DIPEA in DCE (v/v), rt.

The cytidine analog was obtained in five consecutive steps from commercially available 5-iodo-2'-deoxycytidine (scheme 2). We initially decided based upon previously published work\textsuperscript{10} to use the N\textsubscript{2},N\textsubscript{3}-dimethylaminomethyliden as protecting group for the exocyclic amine. However, this proved to be incompatible with our strategy most probably due to the conjugated \(\pi-\pi\)-system (nucleobase and triazole moiety). This discovery led to a change of strategy, and it was decided to use benzoyl at a late stage to protect the exocyclic amine resulting in a working strategy towards phosphoramidite 11 (Scheme 2).
Monomer 2\textsuperscript{4} and 3 have been successfully incorporated into ON’s and studied in DNA:DNA and DNA:RNA duplexes. Almost similar results for the two modifications were found (data not shown). Single incorporation led to large decreases in duplex stability, whereas four consecutive incorporations led to large increases in DNA:RNA duplex stability due to aromatic stacking in the major groove.\textsuperscript{5}

To estimate the overall duplex conformation, simple force field molecular modeling was performed with all monomers 2, 3 and 4. The torsion angle of the bi-aromatic bond was determined using \textit{ab initio} calculation\textsuperscript{6} and the simulation indicates that 2, 3 and 4 are similar in respect to placement in the major groove (Figure 1). Monomer 8 appears from \textit{ab initio} calculations to have a different placement of its aromatic moiety in the major groove.

CONCLUSION

We have successfully synthesized four pyrimidine nucleotide building blocks, which by incorporation into nucleic acid duplexes increase the π-π-stacking into the major groove contributing significantly to the overall duplex stability.

REFERENCES

9. All azides are generated \textit{in situ} using the appropriate bromo derivative, sodium azide, N,N-dimethyl-ethylenediamine in EtOH:H\textsubscript{2}O (7:3, v/v) heated at 150 °C, 900 s followed by cooling to 60 °C, 10 s then reheated at 150 °C, 900 s using MW irradiation.

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