Triplex glue by synthesizing conjugated flexible intercalators

Erik B. Pedersen\textsuperscript{1,}*, Amany M. A. Osman\textsuperscript{1}, Daniel Globisch\textsuperscript{1}, Manikanand Paramasivam\textsuperscript{2}, Susanna Cogo\textsuperscript{2}, Niels Bomholt\textsuperscript{1}, Per T. Jørgensen\textsuperscript{1}, Luigi E. Xodo\textsuperscript{2} and Vyacheslav V. Filichev\textsuperscript{1,3}

\textsuperscript{1}Nucleic Acid Center, Institute of Physics and Chemistry, University of Southern Denmark, DK-5230 Odense M, Denmark, \textsuperscript{2}Department of Biochemical Science and Technology, P.le Kolbe, 33100 Udine, Italy and \textsuperscript{3}Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

\textbf{ABSTRACT}

Bulge insertions of conjugated intercalators into the DNA triplex structure are found to give a dramatic contribution to the triplex stability. On the other hand insertions of conjugated intercalators are found to diminish quadruplex structures and in this way breaking down the self association of G-rich oligonucleotides under physiologically potassium ion conditions. A large number of intercalators are described here and they all result in dramatic increases of thermal stability of the corresponding triplets. Another interesting aspect of conjugated intercalators is their use for assembling alternate strand triplex.

Targeting of neighboring purine sequences on each their strand in the duplex DNA is a challenge for the 5'–5' connectivity of the TFOs because of a large distance between the 5'-ends. The intercalator approach offers a linkage with the proper combination of flexibility and rigidity to produce alternate strand triplex with higher stability than a similar wild type triplex of the same total length.

\textbf{RESULTS AND DISCUSSION}

We now found that the type of linker and its length to the conjugator are important factors. Enhancement of aromatic stacking interactions with nucleobases of the TFO was achieved by increasing the aromatic surface using the corresponding naphthalene derivative (R)-1-O-[4-(1-pyrenylethyl)naphthylmethyl]glycerol.

![](https://academic.oup.com/nass/article-abstract/52/1/37/1106690/fig1)

Also we synthesized a TFO with an amino side chain attached to the aromatic system that under physiological conditions is positively charged and forms an ionic interaction to a negatively charged phosphate in the purine backbone.

Another promising type of flexible intercalators was easily achieved via a condensation reaction of 9,10-phenanthrenequinone with (S)-4-(2-(2,2-dimethyl-1,3-
Alternate strand TFOs that target homopurine tracts located on each strand of the dsDNA have been designed to increase number of targets in dsDNA. Alternate strand TFO consists of minimum two independent oligonucleotides covalently linked at their 3'- or the 5'-ends. Since each oligonucleotide of the probe should bind simultaneously to the target, in combination with specific point of strand alternation, a better specificity to dsDNA can be foreseen when compared to targeting a homopurine sequence.

Herein we report the synthesis of novel 5'-5' linkers obtained via on-column postsynthetic derivatization of DNA. The postsynthetically palladium(0)-catalyzed reaction between 2-(3-iodobenzylxyloxy)ethyl phosphate linked to the 5'-end of DNA and 1,3-diethynylbenzene resulted in different types of on-column ON cross-linking.

The linkage, X, was formed by Sonogashira reactions between two separate terminal 2-(3-iodobenzylxyloxy)ethyl phosphates and 1,3-diethynylbenzene. In addition, the linkage Z, were presumably formed by Sonogashira reactions between two separate terminal 2-(3-iodobenzylxyloxy)ethyl phosphates and two 1,3-diethynylbenzene followed by oxidative arylacetylenic homocoupling(s).

To our knowledge this is the first observation of one pot postsynthetic Sonogashira coupling and acetylenic homocoupling between neighboring on-column ONs, representing a new technique in postsynthetically modifications of on-column. These structures were investigated by molecular modeling and this suggested that minor structural modifications could result in even more effective alternate strand linkages. These were then synthesized by traditional synthesis of the required amidites.

REFERENCES

*Corresponding Author. E-mail: ebp@ifk.sdu.dk