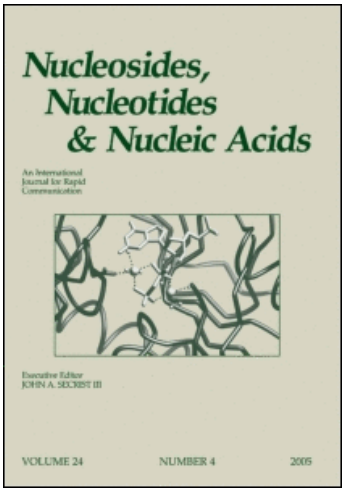

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SYNTHESIS AND INCORPORATION OF A SIMPLE ACYCLIC FURAN CONTAINING PHOSPHORAMIDITE

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□ *A novel furan containing phosphoramidite was synthesized and incorporated into model oligonucleotides. This glycol nucleic acid based building block contains a furan unit substituting the natural base, and can be used for post synthetic oligonucleotide modifications by orthogonal chemistries such as Schiff base formation after in situ oxidation or Diels-Alder cycloadditions.*

Keywords Phosphoramidite; furan; cross-linking; modified oligonucleotide

INTRODUCTION

Interstrand cross-links have been extensively studied in the past since they are among the most toxic DNA damage.[1] They are lesions that can be formed by various anti tumor drugs, such as cis-diaminedichloroplatinum (II) and mitomycin C.[2] For the study of DNA-repair, sufficient amounts of uniformly cross-linked DNA are needed. Since isolation of cross-linked DNA from natural sources is difficult, various interstrand cross-linked duplexes were chemically synthesized.[3–6]

Recently our group developed a new method for DNA interstrand cross-linking based on the use of a furan moiety as masked reactive functionality.[7] Through selective oxidation the furan can be transformed into a 4-oxo-enal. This unsaturated aldehyde can react with various nucleophiles, resulting in interstrand cross-links when positioned inside a DNA-duplex (Figure 1). Furan containing oligonucleotides further find applications for the conjugation of fluorescent labels[8,9] and surface enhanced resonance Raman scattering-probes[10] via Diels-Alder strategies and in the development of new orthogonal base pairs.[11]

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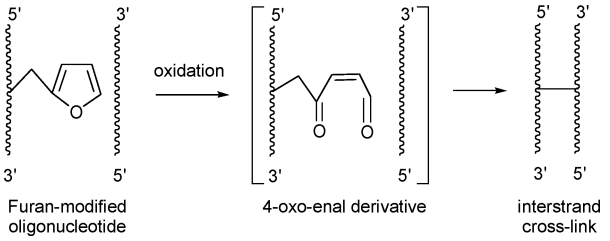


FIGURE 1 Furan based cross-link strategy.

Previously, we concentrated on a nucleoside building block containing a furan moiety linked via an amide bond to the 2′ position of the ribose. New furan containing building blocks should be easily synthetically accessible and display a high atom economy. Inspired by the glycol nucleic acid[12] (GNA) building blocks, developed by the Meggers group, we here present a simplified furan phosphoramidite accessible via a very short and convenient synthesis route (Figure 2). In order to ensure accessibility of the oxidized furan moiety by the complementary bases a two carbon linker between the formal C3′ and the furan was introduced.

RESULTS AND DISCUSSION

The synthesis is outlined in Scheme 1. The first step involves tosylation of the commercially available S-(+)-3,4-O-isopropylidenedioxybutan-1-ol. Direct substitution of the tosylate with furyllithium failed to give conversion to the desired product. Therefore, upon conversion to a bromide smooth substitution with furyllithium yields compound **3** in satisfactory yields. Deprotection of the acetonide was performed at 0°C since cleavage of the furan ring can occur at higher temperatures under these acidic conditions. In order to avoid formation of the double dimethoxytritylated compound, also this protection reaction was performed at low temperature. In this way dimethoxytritylation of the secondary alcohol can be minimised. Although

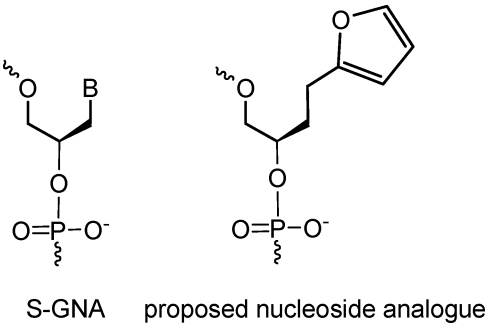
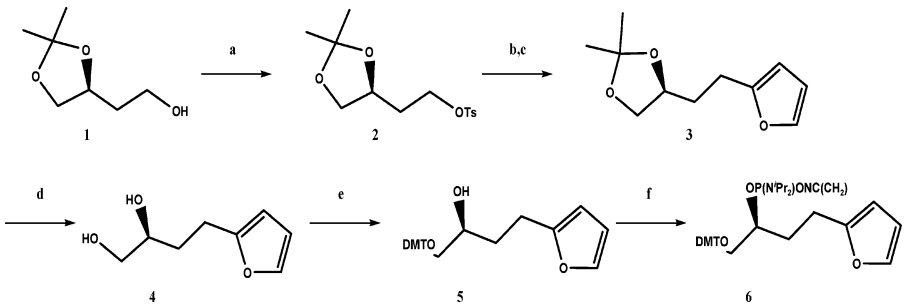


FIGURE 2 Structure of glycol nucleic acid building block S-GNA and the proposed furan derivative.



SCHEME 1 Synthesis a) pTsCl (1.15 eq.), pyridine, 0°C, 2.25 hours, 91%; b) LiBr (5 eq.), DMF, 60°C, 1 hour; c) Furan, BuLi, THF, −78–0°C, 16 hours, 62% over two steps; d) 4M HCl, THF, 3 hours, 0°C, 89%; e) DMTCl (1 eq.), pyridine, 0°C, 1.25 hours, 66%; f) (iPr$_2$N)(NCCH$_2$CH$_2$O)PCl (2.5 eq.), DIPEA, CH_2Cl_2, 1.5 hours, rt, 79%.

no completely selective reaction takes place, separation of mono- and ditritylated compounds was achieved by flash chromatography yielding the desired monoprotected **5** in good yield. The correct positioning of the DMT-group was confirmed by NMR. The phosphoramidite could be easily generated under standard conditions which yielded **6** as a colorless oil.

The building block was then incorporated into oligonucleotide strands on an Applied Biosystems 394 DNA synthesizer. For a 1 μmol synthesis, 0.4 ml of a 50 mM solution of **6** in acetonitrile was dried over molecular sieves (3 Å) for 1 hour. A 1 M 4,5-dicyanoimidazole solution in acetonitrile was used as activator.[13] The coupling of the modification was performed manually with a prolonged coupling time of 10 minutes. The coupling yields under these conditions were estimated to be more than 99%. The modified phosphoramidite was incorporated into four different sequences (see Table 1) varying each time the nearest neighboring bases. It was thus confirmed that coupling to different bases did not result in observed differences in coupling yield. These oligonucleotide sequences will be further used to explore the specificity and sequence context dependence of cross-linking. All masses of the synthesized oligonucleotides were confirmed by MALDI-TOF.

In conclusion we have synthesized a novel furan containing phosphoramidite which is easily accessible via a short and convenient route and

TABLE 1 Synthesized oligonucleotide sequences where X represents the modified residue. Comparison of the calculated masses with the masses obtained with MALDI-TOF

Sequence	Calculated mass (Da)	Measured [M+1] (m/z)
5′-CTGACGG**X**GTGC-3′	3589.63	3589.44
5′-CTGACGT**X**TTGC-3′	3539.62	3540.51
5′-CTGACGC**X**CTGC-3′	3509.54	3509.86
5′-CTGACGA**X**ATGC-3′	3557.56	3557.53

which can be efficiently incorporated into oligonucleotide strands. The current building block allows for internal introduction of a furan moiety into an oligonucleotide. Selective reactions with furan, such as Diels-Alder cycloaddition or oxidative transformation into a 4-oxo-enal, allow for versatile and orthogonal postsynthetic chemistries. Studies on the influence of the newly incorporated building block on the duplex stability and cross-link potential are currently in progress.

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