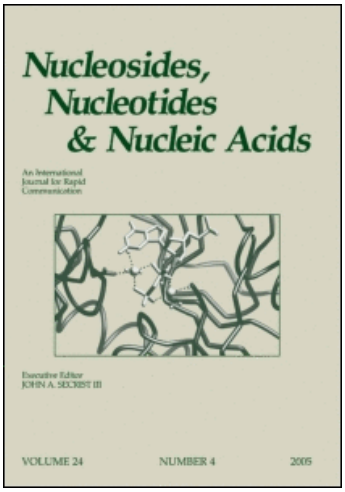

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THE SYNTHESIS OF DIENE-CONTAINING NUCLEOSIDE PHOSPHORAMIDITES AND THEIR USE IN THE LABELING OF OLIGONUCLEOTIDES

Emma Anderson and Douglas Picken ▫ *Link Technologies Ltd., Scotland, UK*

▫ *A variety of furan-modified nucleoside phosphoramidite monomers has been prepared and efficiently incorporated into oligonucleotides. These take part in Diels-Alder reactions with fluorescent maleimides to give fluorescent-labeled oligonucleotides. This represents a strategy for oligonucleotide labeling that is orthogonal to amine-based methods.*

INTRODUCTION

The need to detect DNA sequences with high sensitivity[1] has seen the development of a plethora of techniques in molecular biology such as fluorogenic 5′ nuclease assays, molecular beacons, and scorpion probes. Central to the use of all these techniques is the ability to synthesize oligonucleotides modified with suitable reporter groups at specific positions. While the chemistry for the synthesis of oligonucleotides is now very well established, the incorporation of some desirable labels is problematic because of their incompatibility with the phosphoramidite method, most especially the deprotection conditions. While techniques have been developed to overcome these difficulties by using amino modifications for post-synthetic labeling, some significant drawbacks remain.

In this work we have set out to expand the repertoire of available techniques for the labelling of oligonucleotides by the synthesis of a variety of phosphoramidite monomers containing electron-rich dienes that can participate in a Diels-Alder reaction with electron-deficient alkenes. Many fluorophores are available as maleimide derivatives, which are suitable dienophiles.

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MONOMER SYNTHESIS

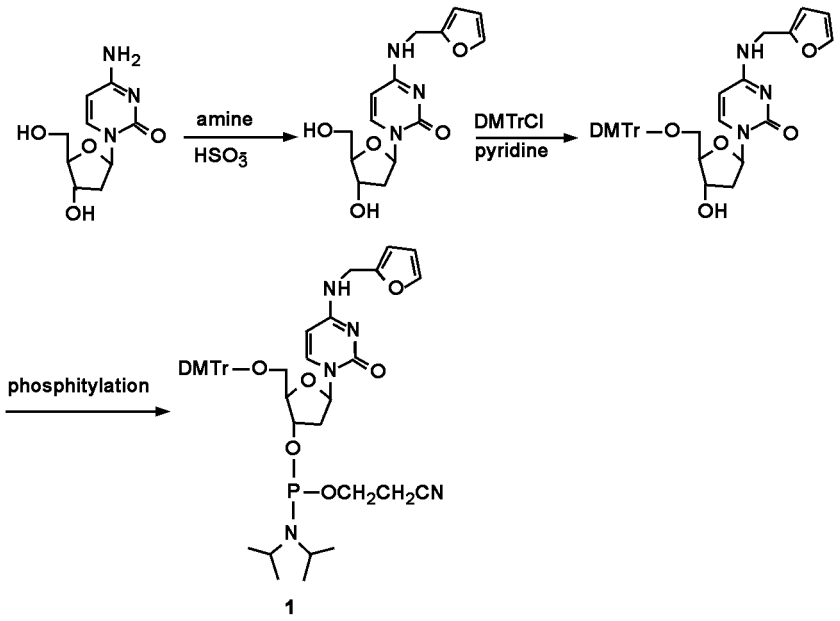
The furan ring has been widely used as an electron-rich diene in the Diels-Alder reaction. Due to the convenience of its introduction and relative stability we have chosen to concentrate on this modification.

As a first approach to the modification of oligonucleotides with furan residues, we chose to prepare phosphoramidite **1**, where the modification is at the N4-position of a deoxycytidine residue. The synthetic route to this compound is shown in Scheme 1.

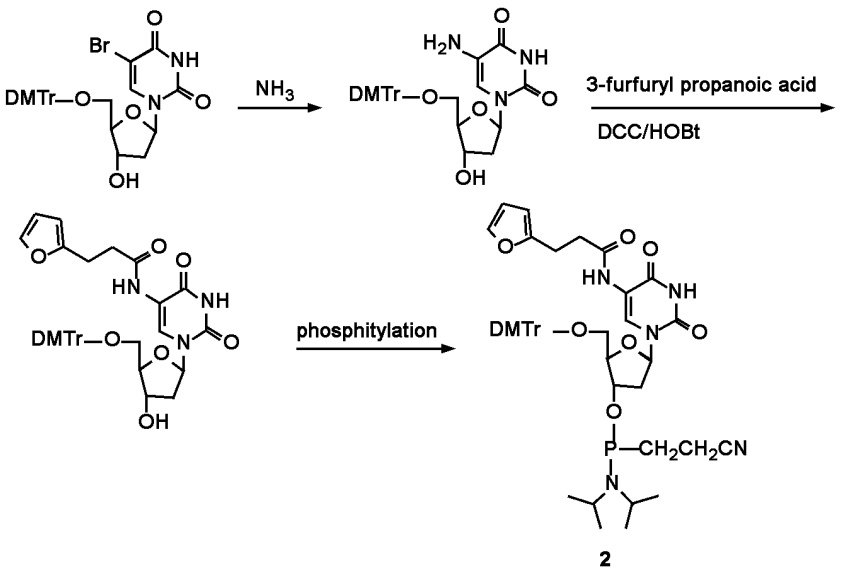
While it was felt that this particular monomer was useful in the preparation of terminally modified oligonucleotides, it would be desirable to have a method of modification that would not interfere with Watson-Crick hydrogen bonding.

One of the most widely used positions for base modification is the 5-position of pyrimidine residues and we have prepared two different compounds bearing furan groups at this position.

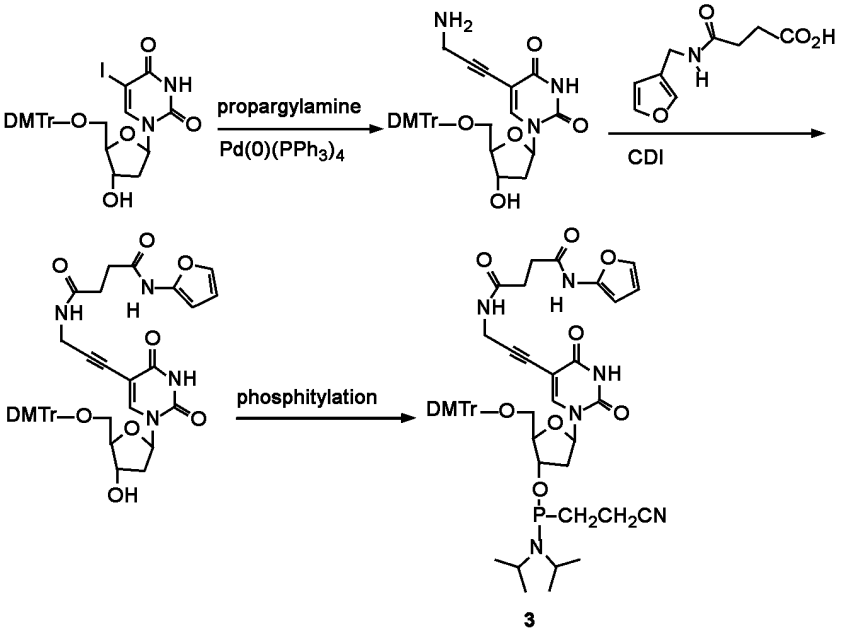
The synthesis of monomer **2** starts from 5′-DMTr-5-bromo-deoxyuridine (see Scheme 2), which is transformed by reaction with liquid ammonia[3] into the 5-amino compound. Coupling of this with 3-furfuryl propanoic acid and subsequent phosphitylation leads to the desired phosphoramidite **2** in an overall yield of 53%.



SCHEME 1 Starting from deoxycytidine, the amino group was transaminated with furfuryl amine in a bisulphite-catalysed reaction,[2] followed by standard dimethoxytrityl protection of the 5′ hydroxyl group and subsequent phosphitylation. This series of reactions led to compound **1** in an overall yield of 40%. The coupling of this phosphoramidite using standard cycles occurs as efficiently as standard monomers.



SCHEME 2



SCHEME 3

This monomer was used for oligonucleotide synthesis and behaves in all respects as a standard nucleoside phosphoramidite.

A further member of this series was prepared in order to provide a monomer with a spacer portion where the reactive furan is further away from the oligonucleotide, since it was felt that this would be a desirable feature for many experiments. Starting with 5′-DMTr-5-iodo-deoxyuridine, propargylamine was coupled using a Sonogoshira procedure[4] (see Scheme 3). Subsequent carbonyl diimidazole-mediated coupling with furfuryl succinamidic acid and phosphitylation yielded the required phosphoramidite **3** in 38% overall yield. This compound is not soluble in acetonitrile at the concentrations used for synthesis, but can be successfully incorporated into oligonucleotides by using THF as co-solvent.

CONJUGATIONS

Each of the monomers described above has been used in the synthesis of oligonucleotides modified both at the 5′ end and at internal positions. Conjugation experiments with commercially available fluorescent maleimides were then carried out. In general, the conditions used have been to incubate a five-fold molar excess of the maleimide with the oligonucleotide in 100 mM citrate/phosphate buffer pH 5.0 for several hours at 37°C. A typical example of a crude conjugation mixture is shown in Figure 1, where Alexa 488 C5 maleimide is conjugated to the sequence indicated, where X represents monomer **2**. Experiments to optimize the conjugation reactions are ongoing.

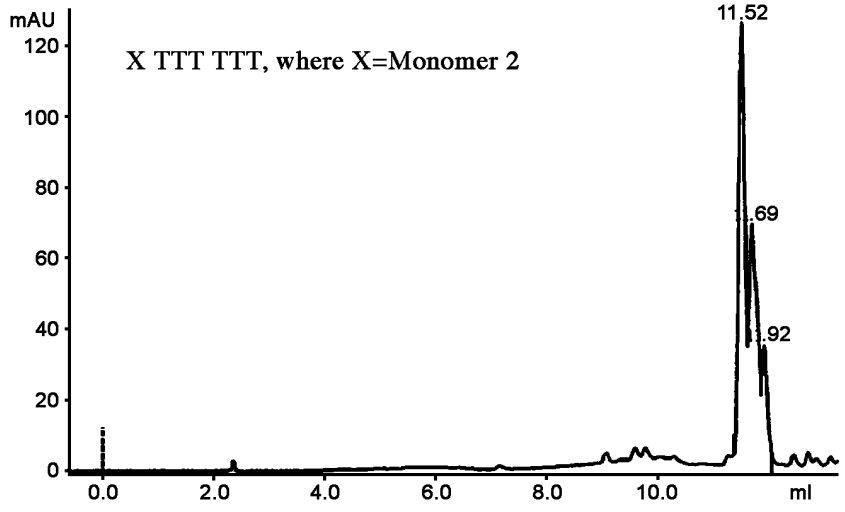


FIGURE 1

CONCLUSIONS

A variety of furan-bearing phosphoramidite monomers have been synthesized and efficiently incorporated into oligonucleotides. Furan-modified oligonucleotides enter into Diels-Alder reactions with maleimido-fluorophores to yield fluorescently labelled products. This methodology is a promising route to multiply labeled oligonucleotides and is orthogonal to amine-based strategies.