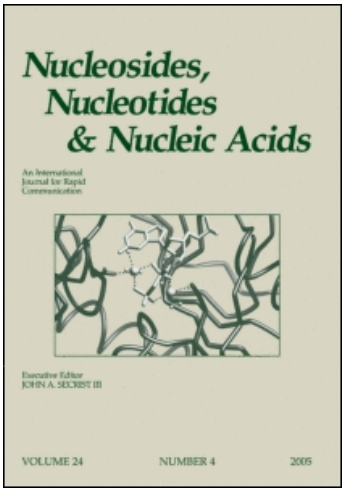

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Synthesis of Thymine-Modified Oligonucleotides

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ABSTRACT

Oligonucleotide-resins containing *N*-nitrothymidine residues yield *N*3-thymine modified oligonucleotides by reaction with a variety of amines followed by the standard ammonia treatment.

Key Words: Modified oligonucleotides; Convertible nucleotides; *N*-Nitronucleosides; *N*-nucleotides.

The preparation of nucleobase-modified oligonucleotides can be accomplished using two different approaches. One alternative is the incorporation of specifically modified phosphoramidite building blocks into the growing oligonucleotide chain, which may require developing special protection/deprotection schemes. The second approach involves the use of "convertible nucleoside" building blocks[1] that may be transformed into a range of differently modified nucleosides at the latest steps of the synthesis.

N-Nitrothymidine has been described to react with ammonia and primary amines to yield *N*3-modified nucleosides through a ring-opening/ring-closing mechanism.[2] As this reaction seemed a suitable alternative for the preparation of

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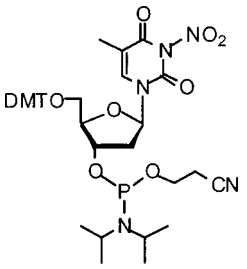
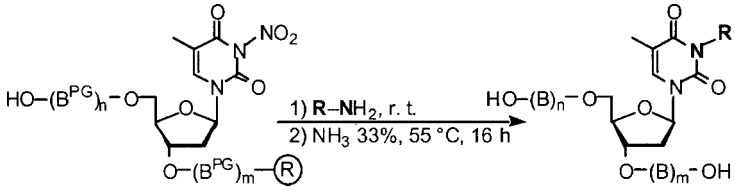
Table 1. *N*-Nitrothymidine-containing oligonucleotide-resins prepared.

		Oligonucleotide-resin
 1	**1**	T-T-T-$\mathbf{T^{NO_2}}$-T-T-T-suc-PEG-PS
	2	T-$\mathbf{T^{NO_2}}$-T-T-T-T- $\mathbf{T^{NO_2}}$-T-suc-PEG-PS
	3	T-T-T-$\mathbf{T^{NO_2}}$-T-T-T-succ-sarc-PEG-PS
	4	T-G^{iBu}-A^{Bz}-$\mathbf{T^{NO_2}}$-C^{Bz}-A^{Bz}-T-suc-PEG-PS
	5	$\mathbf{T^{NO_2}}$-G^{iBu}-A^{Bz}-$\mathbf{T^{NO_2}}$-C^{Bz}-A^{Bz}-T-suc-PEG-PS
	6	G^{iBu}-C^{Bz}-A^{Bz}-$\mathbf{T^{NO_2}}$-G^{iBu}-C^{Bz}- suc-CPG

thymine-modified oligonucleotides, the phosphoramidite derivative **1** was prepared[3] and successfully incorporated into a range of model oligonucleotide-resins following standard phosphite triester procedures (Table 1). No evidence of degradation of the *N*-nitrothymidine moiety was found during the subsequent elongation cycles.

The modification step was carried out by treating the oligonucleotide-resins **1**–**4** with primary amines or hydrazine. As shown in Table 2, reactions were straightforward and could be carried out efficiently under a variety of conditions. In the case of resins bearing a succinyl linker (**1**, **2**, **4**), this treatment also cleaved to some extent the oligonucleotide from the resin and removed some protecting groups. In contrast, no concomitant cleavage was observed in resins bearing the more stable sarcosyl-succinyl linker.[4] In any case, a subsequent overnight treatment with concentrated

Table 2. Modification of resins **1**–**4**.



Resin	R-NH_2 treatment	Oligonucleotide	R
1	$MeNH_2$ (33% in H_2O), 1 h	T-T-T-$\mathbf{T^R}$-T-T-T	Me
1	$BuNH_2$ (neat), 1 h	T-T-T-$\mathbf{T^R}$-T-T-T	Bu
1	$H_2N(CH_2)_3NH_2$ (neat), 1 h	T-T-T-$\mathbf{T^R}$-T-T-T	$(CH_2)_3NH_2$
2	$MeNH_2$ (33% in H_2O), 1 h	T-$\mathbf{T^R}$-T-T-T-T- $\mathbf{T^R}$-T	Me
3	$BuNH_2$ (1M in ACN), 2 h	T-T-T-$\mathbf{T^R}$-T-T-T	Bu
3	Dansyl-$NH(CH_2)_8NH_2$ (1M in DMF), 24 h	T-T-T-$\mathbf{T^R}$-T-T-T	$(CH_2)_8$NH-dansyl
3	NH_2NH_2 (0.01M in ACN), 1 h	T-T-T-$\mathbf{T^R}$-T-T-T	NH_2
4	$PhCH_2NH_2$ (0.25 M in ACN), 4 days	T-G-A-$\mathbf{T^R}$-C-A-T	CH_2Ph
4	$H_2N(CH_2)_3NH_2$ (0.1 M in ACN), 24 h	T-G-A-$\mathbf{T^R}$-C-A-T	$(CH_2)_3NH_2$
4	$HO(CH_2)_5NH_2$ (0.14 M in ACN), 48 h	T-G-A-$\mathbf{T^R}$-C-A-T	$(CH_2)_5OH$

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aqueous ammonia at 55°C ensured completeness of all cleavage and deprotection processes. Sarcosyl-succinyl based resins proved to be superior to the standard succinyl-resins in reactions involving either complex amines, such as the dansyl derivative that yields a fluorescent product, or hydrazine, that affords *N*-aminated thymines.

In summary, phosphoramidite **1** is a good building block for the synthesis of *N*3 thymine-modified oligonucleotides by means of the "convertible nucleoside" approach. As modifications are done at the latest steps of the synthesis, modifying reagents may contain diverse functional groups or even can include non-protected hydroxyl or amine groups. In addition, preliminary results show that this methodology can be also applied to the preparation of specifically ^{15}N-labelled oligomers.

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