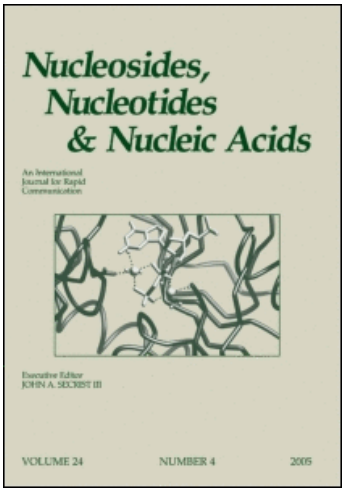

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Conversion of Unprotected Amino-Link Oligonucleotides into Their Tetramethylguanidinium Derivatives

B. Greiner[a]; E. Uhlmann[a]
[a] Hoechst Marion Roussel, Frankfurt a. M., Germany

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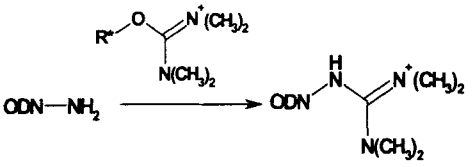
CONVERSION OF UNPROTECTED AMINO-LINK OLIGONUCLEOTIDES INTO THEIR TETRAMETHYLGUANIDINIUM DERIVATIVES

B. Greiner and E. Uhlmann
Hoechst Marion Roussel, Chemical Research G 838, D-65926 Frankfurt a. M., Germany

ABSTRACT: The preparation of tetramethylguanidinium oligodeoxynucleotide (ODN) derivatives by reaction of the corresponding aminoalkyl-ODN with the uronium salts HBTU, TBTU or HATU, respectively, is described. The binding affinity of the new tetramethylguanidinium ODN derivatives was determined.

Modified oligonucleotides are important tools in molecular biology [1]. For effective antisense oligomers, the basic requirements are sufficiently strong hybridisation with the RNA sense strand, stability against enzymatic degradation and sufficient cellular uptake. Here, we report the introduction of the tetramethylguanidinium group into ODN aiming at the enhancement of binding affinity by partial charge compensation, stabilisation against exonucleases and improvement of cell penetration.

The uronium salts HBTU, TBTU and HATU are activators for the amide coupling reaction in peptide chemistry. It has been reported that peptide coupling is accompanied by the formation of a Schiff' base between the tetramethyluronium cation of the activator and phenylalanine by nucleophilic attack of the amino group at the positively charged uronium carbon [2].



ODN = 5'-R^1-ACA TCA TGG TCG-R^2-3'

	R^1	R^2	
1	AM	OH	$O\text{-}PO_3(CH_2)_6NH_2$
2	OH	AM	
3	AM	AM	AM
4	TMG	OH	$OPO_3\text{-}(CH_2)_6\text{-}NH$
5	OH	TMG	
6	TMG	TMG	TMG

In this study, we describe the use of this side reaction for post-synthesis conversion of aminolink-ODN **1**, **2** and bis-(aminolink)-ODN **3** into the corresponding tetramethyl-guanidinium (TMG) derivatised ODN **4** - **6**. Unprotected 5'-aminoalkyl- **1**, 3'-aminoalkyl- **2** or 3',5'-bis-aminoalkyl derivatised ODN **3** (10 OD) in DMF/water/0.5 M aq. Na_2CO_3 (160:130:10µl) were reacted with 0.1 M HBTU, TBTU or HATU, respectively, in the presence of 0.2 M DIPEA in DMF. Purified ODN derivatives were analysed by HPLC (RP-18; LiChrospher™ WP300 column; E. Merck, Darmstadt; Germany) and Negative Ion Electrospray-MS.

R^1	R^2	T_m(I)	T_m(II)	T_m(III)	T_m(IV)
OH	OH	49.9	50.3	52.8	50.2
AM	OH	50.7	-	50.4	-
TMG	OH	51.3	-	49.9	-
OH	AM	52.4	52.8	-	-
OH	TMG	51.7	52.9	-	-
AM	AM	52.6	-	-	50.9
TMG	TMG	52.9	-	-	51.2

5'-R^1-ACA TCA TGG TCG-R^2-3'

(I) 3' TGT AGT ACC AGC 5'

(II) TGT AGT ACC AGC AGT

(III) AGT TGT AGT ACC AGC

(IV) AGT TGT AGT ACC AGC AGT

T_m [°C ± 0.3]: 260 nm (140 mM KCl, 10 mM NaH_2PO_4, 0.1 mM Na-EDTA, pH 7.4)

The UV melting curve experiments show that the TMG group increases the T_m value of the corresponding blunt-end duplexes by about 1.4 °C when incorporated at the 5'-position, 1.8 °C when incorporated at the 3'-end, and has an additive effect of about 3 °C when incorporated at the 3' and 5'-position.

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