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ATTACHMENT OF CHOLESTEROL TO AMINO-LNA: SYNTHESIS AND HYBRIDIZATION PROPERTIES

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□ *Here, we present our synthesis of amino-LNA with a C6-linker and hybridization studies of these. A cholesterol moiety was attached at the end of the C6-linker. This resulted in drastic drops against DNA of the modified oligonucleotide.*

Keywords LNA; amino-LNA; cholesterol

Oligonucleotides modified with LNA[1] monomers (Figure 1) have demonstrated an unprecedented high affinity toward complementary DNA and RNA with increases in the melting temperature (T_m) up to 10°C per modification. The amino-LNA (Figure 1) monomers have demonstrated similar hybridization properties.[2] The secondary amino group of amino-LNA can be regarded as a handle for the attachment of various groups. The attachment of cholesterol to miRNA knockdown probes has resulted in increased activity of those.[3] We wanted to explore the opportunity of introducing several cholesterol units to knockdown probes by utilizing the handle of amino-LNA. In order to have the effect of the cholesterol unit this was introduced to the amino-LNA via a C6 linker (Figure 1).

The known nucleoside **1**[2] (Scheme 1) was alkylated with phtalimidohexanal[4] in the presence of $NaCNBH_3$ to nucleoside **2** in 51% yield. Nucleoside **3** was obtained by protection of the primary hydroxy group with a DMT group using DMTCl in pyridine in 56% yield. Subsequently the phtalimide group was removed by treatment with hydrazine affording nucleoside **4** in 70% yield having a primary amino group ready for functionalization. The cholesterol group was introduced by formation of amide **5** in 53% yield by a chemoselective reaction with cholesteryl chloroformate in the presence of pyridine. Amide **5** was transformed into phosphoramidite **6** using standard conditions in a yield of 42%. Key intermediate **4** was also transformed into nucleoside **7** using ethyl

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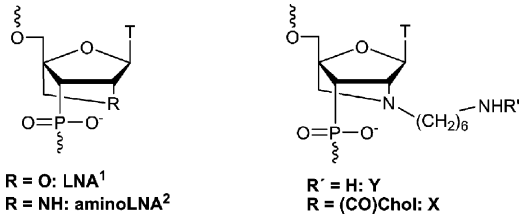
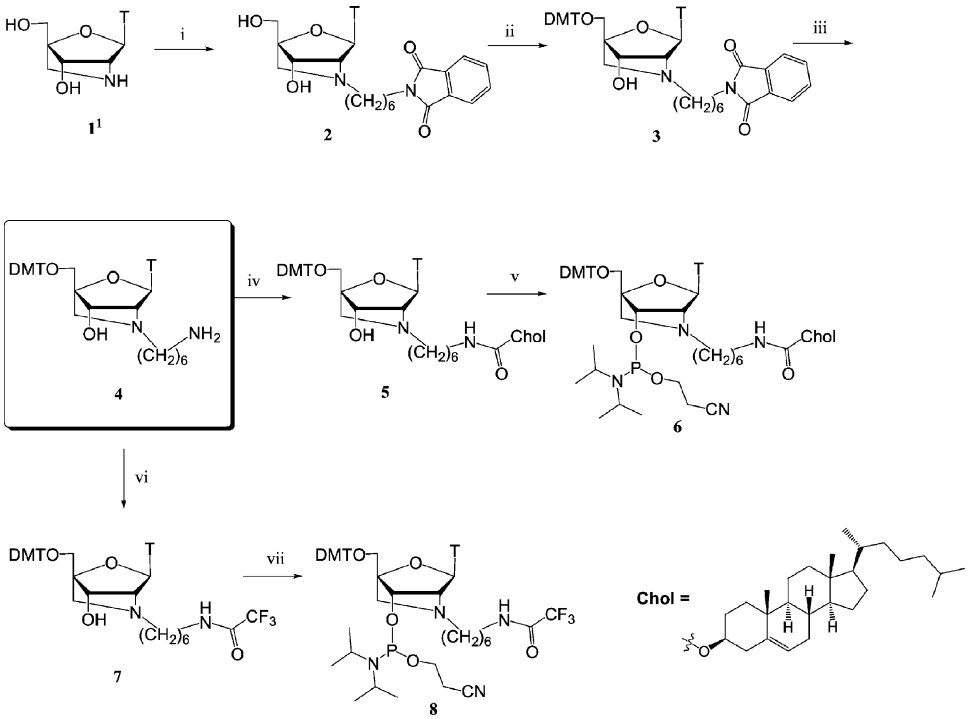


FIGURE 1 LNA and analogs.

trifluoroaceate. This was subsequently transformed into phosphoramidite **8** using the same conditions as for phosphoramidite **6**. Phosphoramidite **6** and **8** gave monomers **X** and **Y** when incorporated in oligonucleotides *vide* infra.

The synthesis of monomers **X** and **Y** was achieved. The hybridization studies of ONs modified with monomer **Y** show that this modification induced an increased towards complementary DNA resulting in ΔT_m/mod. between 1 and 9°C (Table 1). These results are similar to those of amino-LNA. Introduction of the cholesterol group did, however, resulted in dramatic decreases in T_m of the modified ONs when hybridized toward



SCHEME 1 i) 6-Phtalimidohexanal, $NaCNBH_3$, MeOH; ii) DMTCl, pyridine; iii) H_2NNH_2, EtOH, pyridine, acetic acid; iv) Cholesteryl chloroformate, CH_2Cl_2, pyridine; v) $((iPr)_2N)_2PO(CH_2)_2CN$, DCI, CH_2Cl_2 vi) CF_3COOEt, Et_3N; vii) $((iPr)_2N)_2PO(CH_2)_2CN$, DCI, CH_2Cl_2.

TABLE 1 Thermal denaturation temperatures measured as the maximum of the first derivative of the melting curve (A_{260} versus temperature; 5°C to 80°C with an increase of 1°C/minute) recorded in medium salt buffer (100 mM NaCl, 10 mMNaH_2PO_4 0.2 mM EDTA, pH 7.0)

	5′-d(GATAGCGAAGA)	
	T_m °C	ΔT_m/mod. °C
5′-d(TCTTCGCTATC)	34.2	ref.
5′-d(TCTTCGCTA**X**C)	32.6	−1.6
5′-d(TCTTCGCTA**Y**C)	38.5	+4,3
5′-d(**X**CTTCGCTATC)	30.2	−4.0
5′-d(**Y**CTTCGCTATC)	35.4	+1.2
5′-d(TC**X**TCGCTATC)	30.2	−4.0
5′-d(TC**Y**TCGCTATC)	37.6	+3.4
5′-d(TCTTCGC**X**ATC)	32.2	−2.0
5′-d(TCTTCGC**Y**ATC)	43.1	+8.9
5′-d(TCT**X**CGC**X**ATC)	<10	>−12.1
5′-d(TCT**Y**CGC**Y**ATC)	44.8	+5.3
5′-d(**X**CT**X**CGC**X**ATC)	<10	>−8.1
5′-d(**Y**CT**Y**CGC**Y**ATC)	44.8	+3.5
5′-d(**X**CTTCGCTA**X**C)	<10	>−12,1
5′-d(**Y**CTTCGCTA**Y**C)	38.6	+2.2
5′-d(**X**CT**X**CGCTATC)	<10	>−12,1
5′-d(**Y**CT**Y**CGCTATC)	36.1	+1.0

complementary DNA. The incorporation of more than 2 **X** monomers in an 11-mer led to T_m's lower than 10°C. This effect can be contributed to a steric effect of the cholesterol groups, having the cholesterol groups interfering with the nucleobases. We, therefore, conclude that this construct was unsuited for the use in knock-down probes.

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