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Dimeric Building Blocks with N-Cyanoguanidine Linkage for Oligonucleotide Synthesis

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Abstract: *Synthesis of dimeric nucleoside building blocks with a N-cyanoguan*idinc *linkage was performed* by *reaction of 3' with S,S-dimethyl-N-cyanodithioimidocarbonate followed by reaction of the obtained isothiourea with 5'-amino-2',5'-dideoxynucleosides. These dimers were protected in the 5'-position* and *converted* into *their phosphoramidites. Oligonucleotides were synthesized using these building blocks.*

The synthesis of oligonucleotides with modified nucleosides is of great current interest because of their potential use as antitumoral and anti-infectious agents (antisense and antigene approach)[1]. Natural constructs suffer from two serious drawbacks : their instability against enzymatic degradation and their poor cellular uptake (and bioavailability) due to their polyanionic character. On the other hand, synthetic efforts are directed to develop oligonucleotides which are uncharged, enzymatically stable, achiral and still able to hybridize strongly and selectively with the target sequence[2,3].

We tried to solve this problem is by synthesizing a.o. oligonucleotides with an *N*-cyanoguanidine linkage. This functionality is uncharged (at physiological pH), achiral and enzymatically stable. One idea which stimulates us to select this substituted guanidine is that the entropy driven free energy change due to restricted rotation around the intemucleotide linkage during double helix formation would be minimal starting from a conformational rigid molecule[3].

The synthesis of the dimeric building blocks which we used for oligonucleotide synthesis and which are presented here, were optimalized in order to obtain sufficient material for carrying out oligonucleotide synthesis on a larger scale. The reactions were first investigated for the synthesis of the thymine-thymine dimer : N^1-cyano-N^2-(5'-deoxythymidin-5'-yl)-N^3-(3'-deoxythymidin-3'-yl)-guanidine (5d).

Reaction of 3'-amino-3'-deoxythymidine[4] (1) with *S,S*-dimethyl-*N*-cyanodithioimidocarbonate (2) in ethanol at room temperature gives 1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea (3) which could be isolated directly from the reaction mixture in 80% yield[5]. The cyanoguanidine derivative can be obtained from this compound by a substitution reaction with 5'-amino-5'-deoxythymidine[6] (4d). This reaction was carried out in a mixture of triethylamine and dimethylformamide (1:1) as solvent and using 1.2 equivalent silver nitrate. The yield of this reaction is 80%.

For the synthesis of the thymine-thymine dimer (5d) no protecting group is needed. Also the

thymine-adenine dimer (6a) can be synthesized without the use of a protecting group. The yield is, however, better when the 6-amino group of adenine is protected with a dimethoxytrityl group. Protection of the base moiety is necessary to obtain good yield of the thymine-cytosine and thymine-guanine dimers. First the ammo group in the 4-position of the cytosine base and the amino group in the 2-position of the guanine base were protected with a monomethoxytrityl group[7]. Surprisingly, this protecting group was quite stable at the dimeric level and could not be removed without concommitant hydrolysis of the cyano function.

A dimethoxytrityl group seemed to be a better choice. Starting from N^4-dimethoxytrityl-5'-amino-2',5'-dideoxycytidine (4c), N^2-dimethoxytrityl-5'-amino-2',5'-dideoguanosine (4b) and N^6-dimethoxytrityl-5'-amino-2',5'-dideoxyadenosine (4a), the thymine-cytosine (5c), thymine-guanine (5b) and thymine-adenine (5a) dimers were obtained respectively. The dimethoxytrityl protecting groups were removed with 50% acetic acid for 3 min on a steam bath (6a-c).

This internucleoside linkage withstands all protection, deprotection and activation steps necessary to incorporate them into oligonucleotides. As an example the thymine-thymine dimer was protected in the S-position with a dimethoxytrityl group and phosphitylated in the 3'-position with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite. This building block (7) was used to synthesize oligonucleotides[8] (8).

The synthesis of the protected starting materials, the assembly of the dimers to oligonucleotides and their biological and physicochemical properties will be presented in a full paper.

Experimental

1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea (3)

A solution of 1.2g (5 mmol) of 3'-amino-3'-deoxythymidine (1) in 30 mL ethanol was added dropwise to a solution of 2.2g (15 mmol) of S,S-dimethyl-N-cyanodithioimidocarbonate (2) in 10 mL of ethanol. The reaction mixture was stirred for 24 h at room temperature. The white precipitate was collected by filtration giving 1.352 g (4 mmol, 80% yield) of the title compound (3). Elem. anal. ($C_{17}H_{17}N_5SO_4$): calculated C 46.01%, H 5.05%, N 20.64%; found C 45.80 %, H 5.08 %, N 20.58 %.

N^1-cyano-N^2-(5'-deoxythymidin-5'-)-N^3-(3'-deoxythymidin-3'-yl)-guanidine (7)

850 mg (5 mmol) of silver nitrate was added to a solution of 1.7 g (5 mmol) of 1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea (3) and 1.3 g (5.4 mmol) of 5'-amino-5'-deoxythymidine (4d) in 100 mL of a mixture of Et_3N/DMF (1: 1). The reaction mixture was stirred at room temperature for 16 h, evaporated, coevaporated with xylene and purified by column chromatography (CH_2Cl_2-MeOH 85:15) giving 2.13 g (4.01 mmol, 80% yield) of the title compound (7) as a solid. Elem. anal. ($C_{22}H_{28}N_8O_6.H_2O$): calculated C: 48.00%, H : 5.49%, N : 20.35%. Found : C : 48.07%, H : 5.52%, N : 19.86%.

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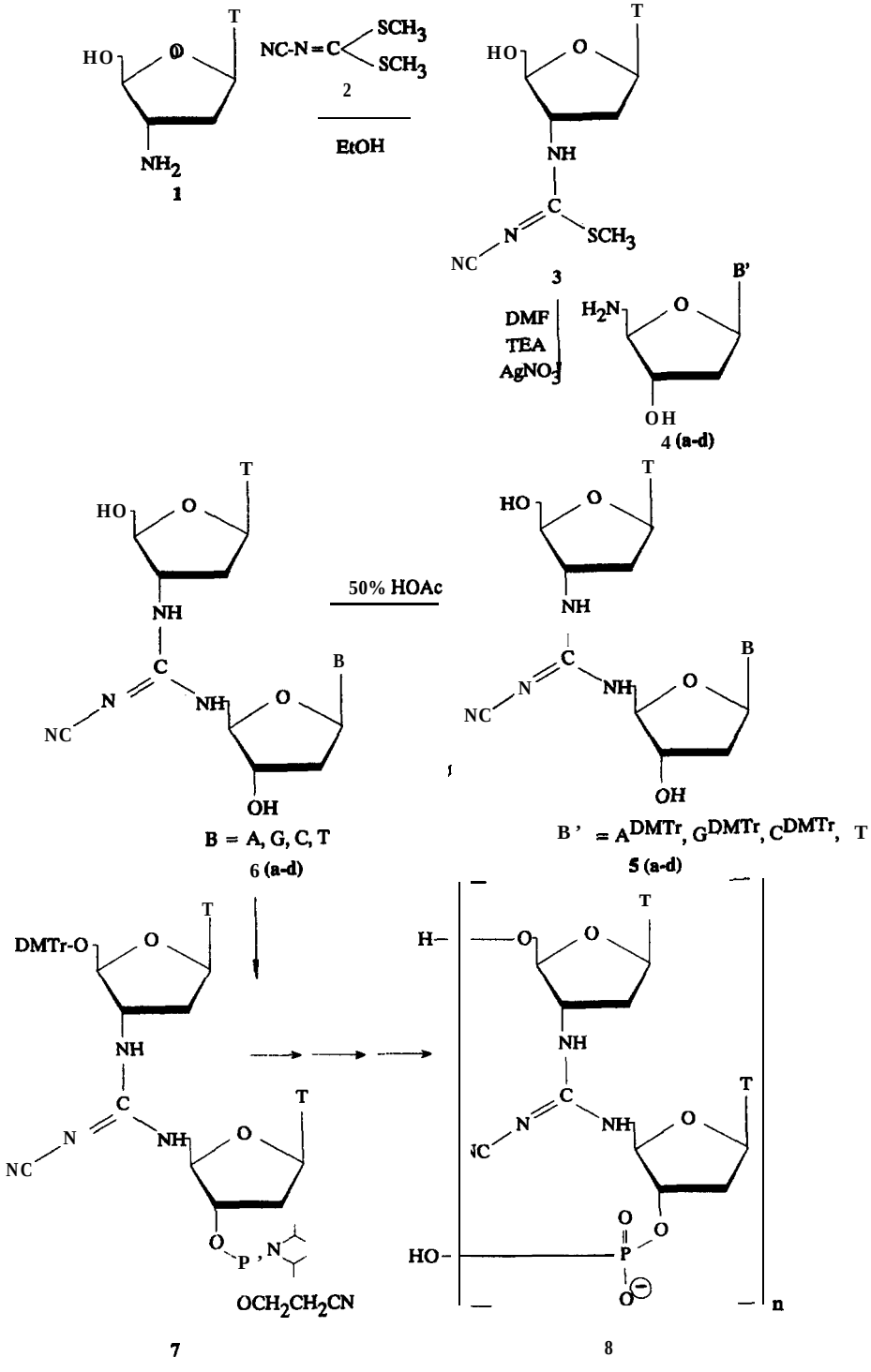

A = adenine, C = cytosine, G = guanine, T = thymine, DMTr = dimethoxytrityl.

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