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SYNTHESIS OF A NEW CLASS OF ACYCLIC 2',5'- AND 3',5'-OLIGONUCLEOTIDE ANALOGS BASED ON 9-[1,5-DIHYDROXY-4(S)-HYDROXYMETHYL-3-OXAPENT-2(R)-YL]-ADENINE

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The acyclic analogs of 2',5'- and 3',5'-oligoadenylates possessing all functional groups of the natural compounds were prepared on the basis of "oxydized-reduced" adenosine.

The recent discovery of a new low-molecular-weight regulator (2',5')pppApApA [1,2] greatly stimulated the interest in short-chained oligoribonucleotides. Many oligonucleotides on the basis of natural nucleosides and their analogs were prepared [3,4].

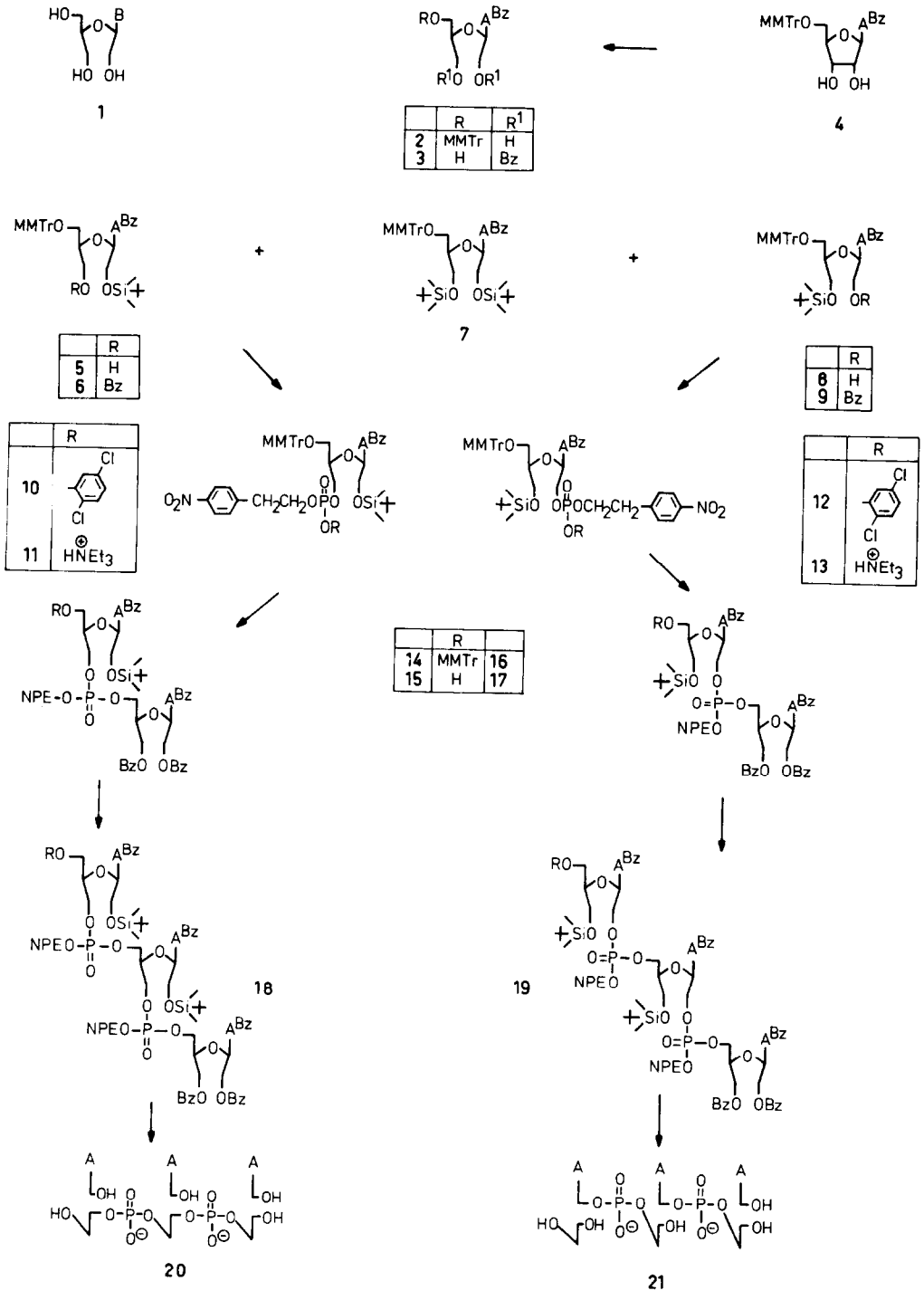
In a continuation of these investigations we present the synthetic strategy to a new class of acyclic oligonucleotide analogs containing all of the functionalities of the natural compounds on the basis of 9-[1,5-dihydroxy-4(S)-hydroxymethyl-3-oxapent-2(R)-yl]-adenine. The so-called "oxydized-reduced" nucleosides (1) are known for more than two decades also as their 5'-phosphates [5-7]. For the preparation of the oligonucleotides on this basis different blocking of the various primary hydroxyl groups has to be achieved. The 5'-hydroxyl group has to be blocked before subsequent oxidation and reduction in order to preserve chirality at C-4'.

The developed strategy for the synthesis of such oligonucleotides is demonstrated in the following synthesis of the acyclo-2',5'- and 3',5'-oligoadenylates. Readily available N^6-benzoyl-5'-O-monomethoxytrityl-adenosine (4) [8] was used as a starting compound. After oxidation with $NaIO_4$ in a water-dioxane mixture followed by reduction with $NaBH_4$ partially blocked 2 was obtained in high yield.

The reaction of compound 2 with 1.2 equiv. of t-butyldimethylsilylchloride in pyridine led to a mixture of the corresponding bis- 7 and mono-O-butyldimethylsilyl derivatives 5 and 8, which was separated by means of silica gel chromatography. The structure of these key-intermediates for oligonucleotide synthesis was proven by PMR spectroscopic decoupling experiments. In order to simplify the PMR-spectra and to get further evidence for the structural assignment also the O-benzoyl derivatives 6 and 9 were prepared and studied. The terminal building block 3 was obtained in overall good yield by subsequent benzoylation (BzCN-dioxane-NEt_3) and detritylation (1 % F_3CCOOH in $CHCl_3$) of the acyclic nucleoside 2.

The preparation of the acyclic 2',5'- and 3',5'-oligonucleotide trimers was achieved by a general method recently developed [9,10]. Phosphorylation of the nucleosides 5 and 8 respectively with 2,5-dichlorophenylphosphoro ditriazolide and subsequent addition of p-nitrophenylethanol yielded the blocked nucleotides 10 and 12 in 80-85 % yield. These compounds were converted into the corresponding phosphodiesters 11 and 13 (4-nitrobenzaldoxime NEt_3-water-dioxane 1:1:1, 40-50 min at 20°C) and then condensed in the presence of triisopropylbenzenesulfonyl chloride (TPSCl) and N-methylimidazole [11,12] in dichloroethane with the terminal nucleoside 3 to give the dimers [13] 14 and 16 in 80-90 % overall yields. After detritylation (1 % F_3CCOOH in $CHCl_3$, 10 min at 20°C) compound 15 an 17 respectively were used as nucleoside components in the analogous synthesis of the trimers 18 and 19 under the same conditions.

Both oligomers 18 and 19 were deblocked using first 0.5 M DBU in pyridine for 16 h at 20°C, then semisaturated ammonia in methanol for 24 h at 20°C, followed by 1 M Bu_4NF in THF for 24 h at 20°C and finally 80 % acetic acid for 6 h at 20°C. The resulting products were purified on a DEAE-cellulose column using a linear gradient of 0.0 → 0.2 M ammonium bicarbonate buffer to give 20 and 21 in 90-95 % yield. The purity of all products has been checked by chromatographical means and the various structures have been proven by UV, CD and NMR spectra as well as elementary analyses.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Physical Data of Acyclic 2',5'- and 3',5'-Adenosine Trimers

Compound	NMR - Spectra in D_2O 8-H and 2-H			1'-H (J)			UV - Spectra in MeOH / pH 7 λ_{max} (nm)	lg ε
18							230 [260] 278	4.91 [4.72] 4.88
19							230 [260] 278	4.91 [4.71] 4.87
20	8.40s 8.21s	8.38s 8.16s	8.35s 8.25s	6.01t (5.6)	5.97t (5.5)	5.94t (5.2)	259	4.60 (pH 7)
21	8.30s 8.15s	8.26s 8.10s	8.20s(2H)	6.00t (5.6)	5.93t (5.5)	5.92t (5.2)	259	4.60 (pH 7)

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The method is limited to the preparation of dimers. No details of chemical synthesis have been published.

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