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FIRST SYNTHESIS OF CARBOCYCLIC OLIGOTHYMIDYLATES§

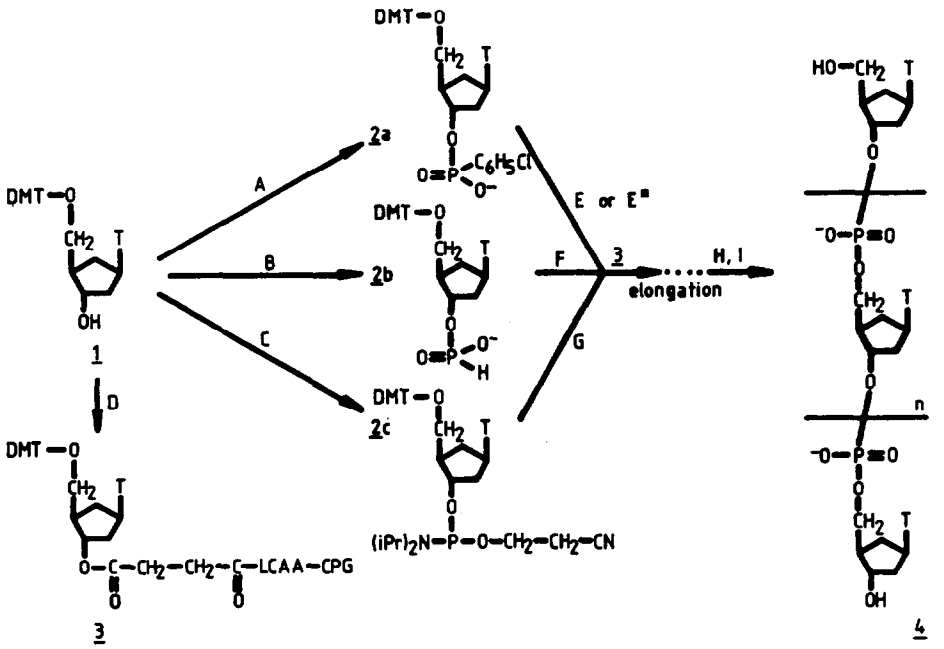
A. Szemző*, J. Szécsi, J. Ságí and L. Ötvös

Central Research Institute for Chemistry, Hung. Acad. Sci.,
H-1525 Budapest, P.O. Box 17, Hungary

Summary: 5'-O-(dimethoxytrityl)-(+)-carbocyclic thymidine 1 is converted into different 3'-substituted intermediates 2a-c following standard procedures. From these compounds stereochemically pure carbocyclic oligothymidylates 4 are obtained using solid phase synthetic methods.

Carbocyclic nucleoside isosteres[1] in which the furanose oxygen of the sugar part of nucleosides has been replaced by a methylene group could be considered as a novel type of building blocks for antisense oligomers[2]. Complications due to chirality[3] can be avoided by making use of the stereospecific synthesis of (+)-carbocyclic thymidine (c-dT)[4]. Carbocyclic oligothymidylates [$c(dT)_n$] have been chosen as model compounds for the study of bioorganic properties of hitherto unknown carbocyclic oligonucleotides. Since carbocyclic nucleotides are not substrates for polymerase enzymes[5] we had to resort to chemical polymerization. Solid phase synthesis has been applied due to its known advantages.

The modified phosphotriester approach[6] has already been successfully used for synthesis of short oligodeoxynucleotides. According to standard procedures[7] we prepared 2a[8] and 5'-DMT-(+)-c-dT-3'-succinyl-LCAA-CPG functionalised support 3. It was found that the coupling efficiency of the carbocyclic analogue was much lower (72%) than that of the natural thymidine (90%) (Table 1). In order to increase efficiency of assembly we tried to use more intensive conditions for coupling[9] but only slight improvement could be achieved (E* in Table 1). This low reactivity of the carbocyclic monomer was unexpected although some 2'-substituted ribonucleosides show similar behaviour[10]. Decreased reactivity of carbocyclic nucleosides *(vide infra)* can be attributed to conformational and/or stereoelectronic differences between carbocyclic[11] and "natural" nucleosides. To increase the very low overall yield of carbocyclic oligomers we searched for other methods of oligonucleotide synthesis. The H-phosphonate method[12] resulted in a moderate coupling efficiency (Table 1), however, it was suitable for the synthesis of short (n = 1-3) carbocyclic oligomers.



A/ o-chlorophenyl pyridinium phosphate, rt, 45 min, 90%
B/ PCl_3, imidazole, MeCN, 2 hr, 94%
C/ 2-cyanoethyl-bis(N,N-diisopropyl)amino phosphite, diisopropyl-amine hydrotetrazolide, dichloroethane, rt, 4 hr, 95%
D/1 succinic anhydride, DMAP, acetone, rt, 5 hr, 90%
D/2 DCC, LCAA-CPG (Pierce®), DMF, rt, 24 hr, 35%
E/ 3(1μmol), **2a**(10μmol), MSNT(50μmol), MeIm(120μmol), rt, 15 min
E*/ 3(1μmol), **2a**(15μmol), MSNT(50μmol), BuIm(120μmol), 55°C ,45 min
F/ 3(1μmol), **2b**(30μmol), pivaloyl chloride(150μmol), rt, 4 min
G/ 3(1μmol), **2c**(10μmol), tetrazole(100μmol), rt, 3 min
H/ 30% NH_4OH, 55°C, 5 hr
I/ 5% aq. trifluoracetic acid, rt, 2 min;

Table 1.

METHODS		$(dT)_{3-20}$ coupling efficiency (%)	$c(dT)_{3-20}$[a] coupling efficiency (%)
E/	Standard phosphotriester	90	72
E*/	Intensive phosphotriester	97	78
F/	H-phosphonate	96	93
G/	Phosphoramidite	98	95

[a] oligonucleotides containing carbocyclic monomers are as follows: $c(dT)_3$, $c(dT)_{10}$, $c(dT)_{12}$, $c(dT)_{20}$, $(dT)_9c(dT)$, $(dT)_5c(dT)_2(dT)_5$

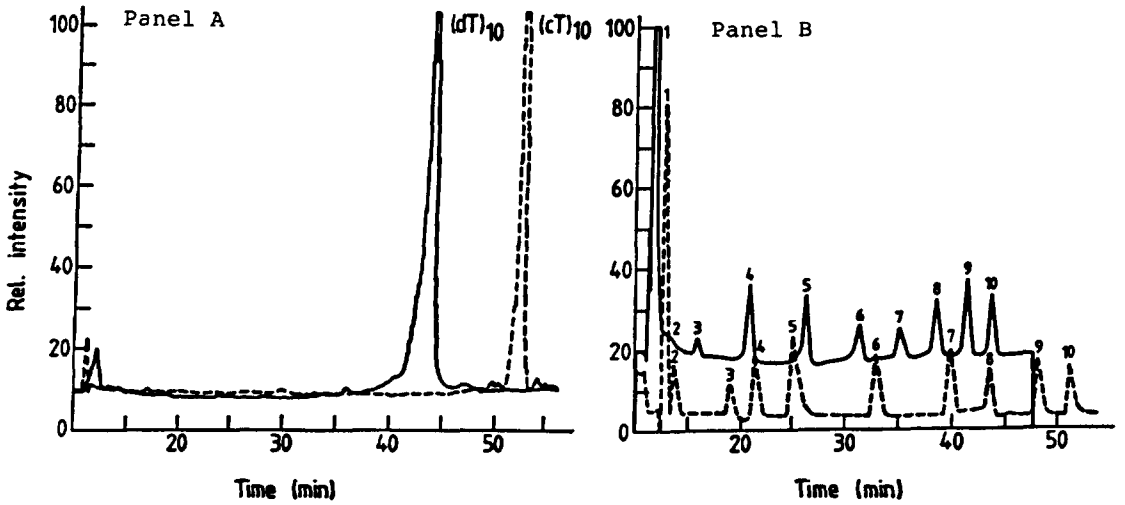


Figure 1. HPLC profile of decathymidylate (——) and (+)-carbocyclic decathymidylate (....). Panel A: after purification; Panel B: digested by snake venom phosphodiesterase enzyme. Experimental details are described in ref. 18.

Monomer 2b[8] was prepared by the reaction of 1 and tris-(imidazolyl)phosphite[13]. Finally, using a DNA synthesizer (Pharmacia Gene Assembler) the phosphoramidite route[14] has been applied. Carbocyclic monomer 2c[8] was produced by the reaction of 1 and 2-cyanoethyl-bis-(N,N-diisopropylamino) phosphite in the presence of diisopropylamine hydrotetrazolide[14]. Slightly diminished reactivity of this monomer was also observed[16] (Table 1), nevertheless, this methodology proved to be suitable for the synthesis of longer (n=18) oligomers.

Carbocyclic oligothymidylates were cleaved from the support and deprotected in a single step by treatment with 30% NH_4OH (50°C, 5 hr). Purification of the products was achieved by NENSORB PREP® disposable columns for nucleic acid purification and standard gel electrophoresis followed by reversed-phase HPLC analysis[6]. A typical HPLC chromatogram is shown in Figure 1. Sequence analysis of oligothymidylates was carried out according to standard protocols[17] which were not suitable to prove the structure of carbocyclic analogues because of the chemical stability of their C-N "glycosidic" bond and poor template properties in DNA polymerase reactions[18] used in sequence analysis. However, they proved to be substrates for snake venom phosphodiesterase. HPLC analysis of the

products of partial hydrolysis verified the degree of polymerization (see e.g. for $c(dT)_{10}$ and $(dT)_{10}$ in Figure 1, panel B). Preliminary results of examination of carbocyclic and "mixed" oligothymidylates as controllers of gene expression are promising[18]. Work is in progress to optimize conditions which result in a better coupling efficiency.

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References and Notes

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