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SOLID PHASE SYNTHESIS OF CYCLIC OLIGODEOXYRIBONUCLEOTIDES.

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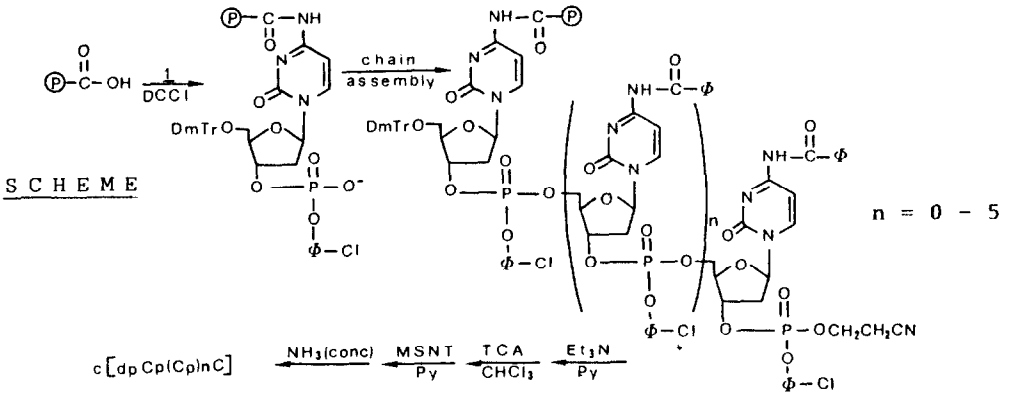
Abstract. - The polymer supported synthesis of cyclic oligomers of deoxycytidilic acid is described, by a method based on an oligonucleotide-solid phase linkage through the amino group of the base.

In conjunction with our studies on the chemical synthesis of polydeoxyribonucleotides[1], we have planned to develop a method for obtaining cyclic oligomers, whose biochemical and physico-chemical properties have not been so far extensively studied. We wish to report here that the goal was successfully reached performing the solid-phase synthesis in very high yields of cyclic oligodeoxycytidilic acids containing up to 7 units, c[dpCp(Cp)$_{0-5}$C], through the cyclization of the corresponding linear oligomers anchored to a polymeric support through the exocyclic amino group of the base.

The resin (commercially available polyacrylamido acryloilsarcosinemethylester, 0.35 meq/g) was prefunctionalized with ethylendiamine and then was reacted with succinic anhydride (50 eq) in dry pyridine for 15 hr at room temp.. The latter reaction was quantitative as deduced from the Kaiser test[2] performed on a sample of resin. The resulting succinylated polymer was left in contact with a solution of 5'-O-(4,4'-dimethoxytriphenylmethyl)-2'-deoxycytidine-3'-O-(2--chlorophenyl)phosphate (1) (4 eq) in the presence of DCCI (40 eq). After washing, the amount of the nucleotide attached to the resin was estimated by spectroscopic measurement (500 nm, ε 8700) of 4,4'-dimethoxytriphenylmethyl cation released by acidic treatment (70% $HClO_4$-EtOH, 3:2, v/v) and resulted to be 0.20 meq/g. The synthesis of the dimer was performed by reaction of N^4-benzoyl-2'-deoxycytidine-3'-O-(2-chlorophenyl-2-cyanoethyl)phosphate (2) with the phospho-diester group on the resin according to the reported procedure[1]. At this point a further elongation of the chain can be performed in both 3'-5' and in 5'-3' direction, by removal of the 5'-trityl or of 3'-ß-cyanoethyl protecting group respectively[3].

In order to obtain the cyclic dimer we removed both the above protecting groups and, after appropriate washing steps, we added a solution of MSNT (15 eq) in dry pyridine. After 3 hr the resin was exhaustively washed with pyridine and treated with conc. ammonia (50°C, 6 hr) in order to remove the protecting groups and detach the nucleotidic material from the resin (see scheme). HPLC analysis (Partisil 10 SAX column, eluted with a gradient of KH_2PO_4, KCl, 5% EtOH, pH 6.8, from 1 mM to 0.25 M at room temp.)[1] clearly indicated the nearly quantitative conversion of the linear dimer into a product with a shorter retention time. This compound was collected, rechro-



(P) = Polimeric support; DCCI = N,N'-dicyclohexylcarbodiimide; DmTr = 4,4'-dimethoxytriphenylmethyl; Et_3N = triethylamine; TCA = trichloroacetic acid; MSNT = mesitylenesulphonyl-3-nitro-1,2,4-triazole.

matographed on a μ-Bondapak C_{18} column (eluent: linear gradient from 0 to 30% of CH_3CN in 0.1 M triethylammonium acetate, pH 7.0) and repeatedly liophylized. Starting from 100 mg of succinylated resin 9.8 mg of pure c(dpCpC) were obtained. FAB mass [m/z 579 $(MH)^+$], ^1H NMR [250 MHz, D_2O, δ 7.75 (d, J= 7.5 Hz, H-6); 6.06 (dd, J= 6.0 and 4.0 Hz, H-1'); 5.88 (d, J= 7.5 Hz, H-5); 4.65 (m, H-3'); 4.01 (m, H-4'); 3.46 (dd, J= 11.5 and 4.5 Hz, H_A-5'); 3.36 (dd, J= 11.5 and 6.0 Hz, H_B-5'); 2.45 (m, H_2-2')] and ^{31}P-NMR [80.95 MHz, D_2O, δ -0.30 (85% H_3PO_4 ext. ref.)] spectra clearly showed it to be the cyclic dimer c(dpCpC).

Analogously the cyclization of the linear dpCp$(Cp)_{1-5}$C obtained by elongation of the chain in the 5'- to 3'-end direction[4] and, therefore, anchored to the resin through the $-NH_2$ group of the first nucleotide, afforded the corresponding cyclic oligomers (see scheme), in almost quantitative yields[5] as resulted from HPLC analyses. Spectral data confirmed the cyclic nature of the products. Particularly, the NMR spectra showed that in the cyclic oligomers all the nucleotides are equivalent since only a signal for each type of nucleus was observed.

Further evidence for the cyclic structures was provided by enzymatic tests. All the synthesized compounds resulted to be unaffected by exonuclease I. Moreover enzymatic digestion (performed on the cyclic hexamer and heptamer) with micrococcal or S1 endonucleases afforded, as expected, dCp or pdC, respectively, as the sole products.

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4. Each cycle comprises two reactions, coupling and deprotection. In the coupling step the resin is left in contact with a solution of 2 (5 eq) and MSNT (15 eq) for 30 min. at 50°C in dry pyridine. The removal of the 3'-phosphate protecting group (ß-cyanoethyl) is performed by treatment with triethylamine/pyridine (1:1, v/v, 30 min. at 50°C). Coupling yields are consistently in the order of 90-95% (by HPLC).
5. Our attempts to synthesize cyclcic oligomers (up to 4-mer) of deoxycytidilic acid in solution afforded the desired products in much lower yields, even at high dilution.

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