Nucleosides and Nucleotides. Part 9. Synthesis of Dinucleoside Monophosphates Containing 2'-Deoxycytidine and 1-(2'-Deoxy-β-D-ribofuranosyl)-2(1H)-pyridone [1].

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Dedicated to Dr. Ken'ichi Takeda at the Occasion of his Seventieth Birthday

Condensation of 1-(5'-O-monomethoxytrityl-2'-deoxy-β-D-ribofuranosyl)-2(1H)-pyridone ((MeOTr)T_d, 4) and N^4-anisoyl-3'-O-acetyl-2'-deoxycytidine-5'-phosphate (pC_d^{an} (Ac), 5) using an excess of dicyclohexylcarbodiimide (DCC) in absolute pyridine yielded the dinucleoside monophosphate (MeOTr)$T_d pC_d^{an}$ (6). Successive removal of the protecting groups of compound 6 led to the partially protected dinucleoside phosphates (MeOTr)$T_d pC_d$ (7) and $T_d pC_d^{an}$ (8), and to the free dinucleoside monophosphate $T_d pC_d$ (9). The enzymatic degradation of 9 by the phosphodiesterases I and II was complete and gave T_d and pC_d, and $T_d p$ and C_d respectively with the expected ratio of 1:1.

1) Part 8, cf. [1].

Modified nucleosides and nucleotides, which differ from the natural compounds by structural modification of the sugar or base moiety, exhibit various interesting biological activities [2]. Our interest is mainly directed to nucleoside and nucleotide analogs which, by modification of the structure of their bases, have lost partially or completely the capacity to form specific hydrogen bonds with the natural bases according to the Watson and Crick base pairing concept. Oligo- and polynucleotides containing such a modified base could contribute to a better understanding of the replication or transcription of deoxyribonucleic acids. Such considerations have motivated Séquin & Tamm to synthesize the modified nucleoside 1-(2'-deoxy-β-D-ribofuranosyl)-2(1H)-pyridone (Π_d, 1) [2] and its 5'-phosphate $p\Pi_d$ (2) [3]. On the basis of these investigations Gregor et al. have studied the reactivity of the nucleoside 1 under the standard conditions of oligonucleotide synthesis (i.e. introduction and removal of protecting groups, stability in the presence of condensing and phosphorylating agents [1]). Their successful synthesis of the dinucleoside monophosphate 1-(3'-O-phosphoryl-2'-deoxy-β-D-ribofuranosyl)-2(1H)-pyridone-(3'-5')-thymidine ($\Pi_d pT_d$, 3a) and of its derivatives 3b - 3e, has demonstrated that it is possible to join the unnatural

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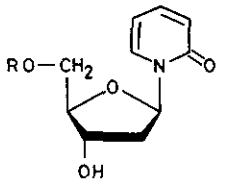
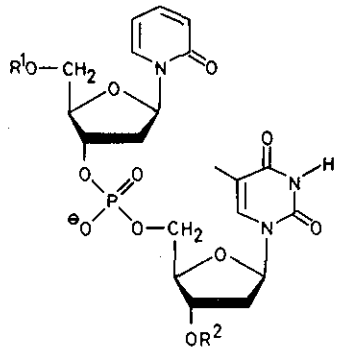
2) For abbreviations cf. [3].





1 R = H
2 R = Phosphoryl

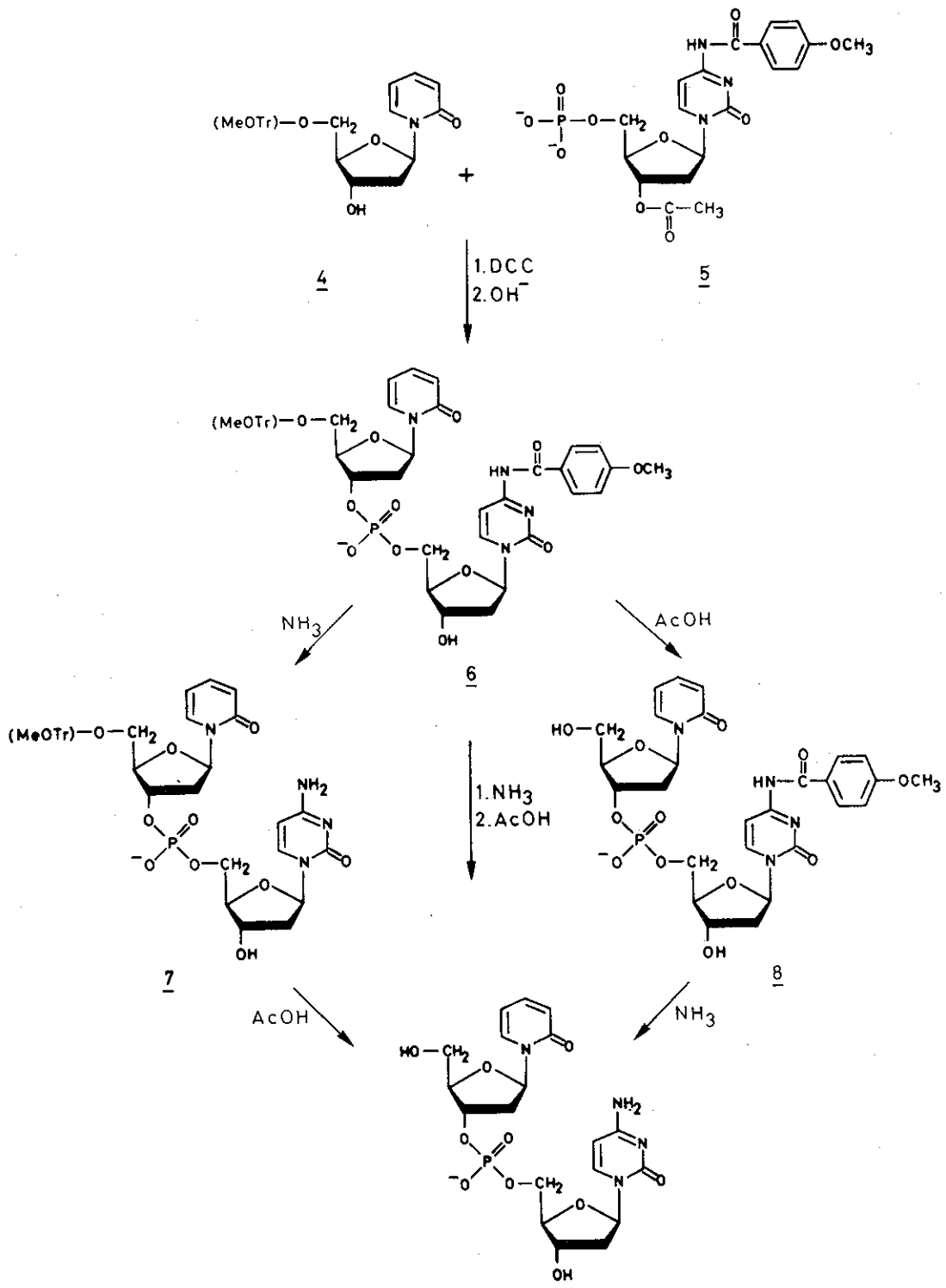
3a $R^1 = R^2 = H$
3b R^1 = p-Methoxytrityl, R^2 = Acetyl
3c R^1 = p-Methoxytrityl, $R^2 = H$
3d R^1 = Phosphoryl, $R^2 = H$
3e $R^1 = H$, R^2 = Phosphoryl

nucleoside 1 to a natural nucleotide via chemical reactions if specially mild conditions are used. Thymidine was chosen because it has no amino group and thus facilitates protecting.

In continuation of these studies we now report the synthesis of 1-(3'-O-phosphoryl-2'-deoxy-β-D-ribofuranosyl)-2(1H)-pyridone-(3'-5')-2'-deoxycytidine ($\pi_d pC_d$, 9) and of its derivatives 6, 7 and 8. We applied the commonly used procedure by which a 5'-nucleoside phosphate is condensed with the free 3'--hydroxy group of a protected nucleoside (cf. [4] [5]). Equimolar amounts of 1-(5'-O-monomethoxytrityl-2'-deoxy-β-D-ribofuranosyl)-2(1H)-pyridone ((MeOTr)π_d, 4) [3] and N^4-anisoyl--3'-O-acetyl-2'-deoxycytidine-5'-phosphate (pC_d^{an} (Ac), 5 [4]

3) For details of the synthesis cf. [1].

4) Prepared according to Khorana et al. [5] [6].



MeOTr = p-Methoxytrityl

were condensed in the presence of a 5 to 7-fold excess of dicyclohexylcarbodiimid (DCC) in absolute pyridine at room temperature. After 5 to 7 days the excess condensing reagent was hydrolysed and the dicyclohexyl urea formed removed by filtration and extraction with ether. These ether extracts also contained up to 33 per cent of unreacted (MeOTr)T_d (4). The selective removal of the 3'-O-acetyl group was carried out by using a 1:1 mixture of 2N aqueous sodium hydroxide and 50 per cent aqueous pyridine at 0°. After 12 - 13 minutes the reaction was stopped by the addition of pyridinium Dowex 50. The separation of unreacted mononucleotide, which still contained the protecting anisoyl group, and the further purification and isolation of the desired product 6 was achieved in two ways: (1) Preparative thin layer chromatography with precoated silica gel plates and chloroform-methanol 1:1 or (2) ion exchange chromatography on DEAE-Sephadex using two subsequent linear gradients of solvent: (a) 0 - 0.15 M aqueous ammonium hydrogen carbonate (elution of the mononucleotide) and (b) 0 - 0.1 M ammonium hydrogen carbonate in 30 per cent aqueous ethanol (elution of product 6, which was lyophilized immediately from an aqueous solution). The yields of pure (MeOTr)$T_d p C_d^{an}$ (6) ranged between 50 and 60 per cent, whereby up to 30 per cent of the starting materials 4 and 5 were recovered. UV spectrum

(1:1 mixture of 0.1 M phosphate buffer, pH 7, and ethanol): λ_{max} (ε) 301 nm (26'400); 228 nm (shoulder) (27'800). The absorption maximum at 301 nm of compound 6 appears practically at the same position as those of (MeOTr)TT$_d$ (λ_{max} (ε) 303 nm (6560), in ethanol) [1] and of pC$_d^{an}$ (λ_{max} (ε) 302 nm (24'890), in water). The difference between the sum of the extinction coefficients of the single components and the value measured for compound 6 is probably due to some kind of base stacking interaction [5] (hypochromous effect, cf. [7]).

Removal of the anisoyl group of (MeOTr)TT$_d$pC$_d^{an}$ (6) was carried out by treatment with conc. ammonia containing 25 per cent of pyridine for 24 hours at 23°. The dinucleoside monophosphate (MeOTr)TT$_d$pC$_d$ (7) was precipitated by dropwise addition of a solution of 7 in absolute pyridine to an excess of absolute ether. The product 7 was isolated in a yield of 84 per cent and was almost pure according to t.l.c. [6]. It contained only

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5) The quantitative determination of this effect in substances of type 6 is under investigation.

6) Solvent systems (v/v): A) chloroform/methanol 1:1; B) 1-butanol/glacial acetic acid/water 5:2:3; C) 2-propanol/conc. NH_3/water 7:1:2; D) ethanol/1 M aqueous NH_4HCO_3, pH 7.5, 7:3; E) 1-propanol/conc. NH_3/water 6:1:3.

a trace of a tritylated impurity. The UV absorption maximum was observed at (ε) 277 nm (12'700) (50 per cent aqueous ethanol) mainly due to the nonprotected cytidine moiety. In addition, the spectrum exhibited two shoulders at (ε) 299 nm (7780) and 224 nm (26'400).

For the preparation of $T_dpC_d^{an}$ (8) the p-methoxytrityl group of compound 6 was removed by treatment with a mixture of glacial acetic acid/pyridine 7:3 for 30 to 40 minutes at 100° according to Gregor et al. [1]. Removal of trityl alcohol and acetic acid was achieved by partitioning the reaction mixture between water and ether. The crude product was purified by chromatography on DEAE-Sephadex. Substance 8 (yield 74 per cent) was eluted by 0.03 to 0.035 M ammonium hydrogen carbonate. UV spectrum (0.1 M phosphate buffer, pH 7): λ_{max} (ε) 300 nm (27'500) and 216 nm (22'400).

The free dinucleoside monophosphate T_dpC_d was obtained either by detritylation of compound 7 (yield 87 per cent) or by removal of the anisoyl group in the product 8 (yield 85 per cent) or even directly from the derivative 6 which contains two protecting groups (yield 72 per cent). The methods used were those described above. The corresponding crude products were purified on DEAE-Sephadex columns. Compound 9 was eluted by 0.018 to 0.021 M ammonium hydrogen carbonate. UV spectrum

(0.1 M phosphate buffer, pH 7): λ_{max} (ε) 274 nm (10'100) with shoulders at 300 nm (5420) and 226 nm (12'100).

The dinucleoside monophosphate 9 was characterized further by the phosphodiesterases I (snake venom phosphodiesterase) and II (spleen phosphodiesterase) according to known methods [8] [9]. Treatment with phosphodiesterase I yielded the nucleoside T_d and the nucleotide pC_d in a ratio of 1:0.98. Similar degradation with phosphodiesterase II gave the 3'-phosphate T_dp along with the nucleoside C_d in a ratio of 1:1.00. With both enzymes cleavage of the substrate 9 was complete.

Conclusion: The successful synthesis of T_dpC_d (9) and of its derivatives 6, 7 and 8 has clearly demonstrated that it is possible to join the unnatural nucleoside T_d not only to thymidine derivatives (Gregor et al. [1]) but also to a natural nucleotide containing an amino group. The anisoyl blocking group, necessary for the protection of the amino group during internucleotide bond synthesis, could be removed under conditions which did not attack the labile glycosidic bond of the pyridone nucleoside moiety of the corresponding compounds.

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