Tetrahedron Letters No.50, pp. 4579-4584, 1965. Pergamon Press Ltd.
Printed in Great Britain.

THE SYNTHESIS OF DINUCLEOSIDE PHOSPHATES OF NATURAL LINKAGES BY THE "ANHYDRONUCLEOSIDE METHOD"*

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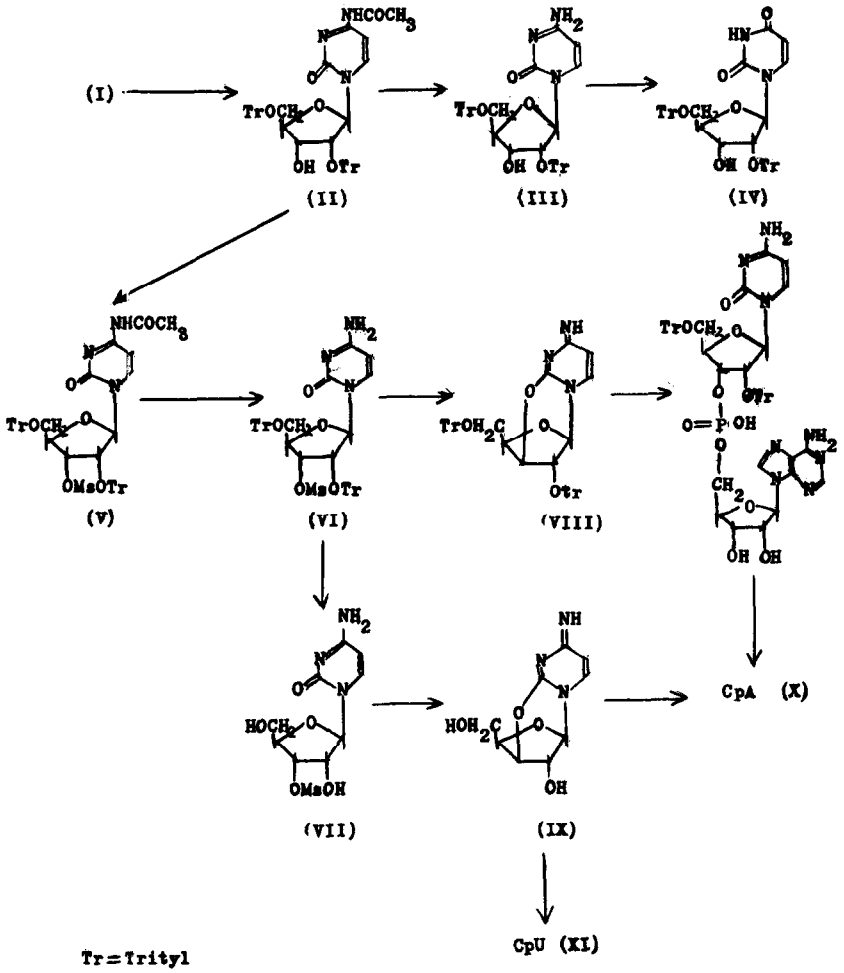
(Received 19 October 1965)

The use of anhydronucleosides in the synthesis of the nucleotides and oligonucleotides was reported from four laboratories (1-5). In principle, the synthesis of the initial dinucleoside phosphate intermediates by the "anhydronucleoside method" can be achieved by either (a) the reaction of 2,5'-anhydronucleosides with nucleoside-3' phosphates or (b) the reaction of 2,3'-anhydronucleoside with nucleoside-5' phosphates. It was anticipated that the approach (b) possesses advantages over the shortcomings (3) inherent in approach (a). However, for approach (b) to the synthesis of oligonucleotides, 2,3'-anhydro-xylofuranosyl pyrimidines are necessary which are sufficiently reactive toward weak nucleophiles (e.g. phosphate anions). Though methods for the synthesis

* "Anhydronucleoside method" is tentatively refered to as the synthetic method for the preparation of nucleotides and oligonucleotides by use of anhydronucleosides as key intermediates (1-4).



Tr=Trityl

Ms=Mesyl

of 2,3'-anhydro-xylofuranosyluracils from 2',5'-O-trityluridine have been reported (9), these 2,3'-anhydrouracil nucleosides were not satisfactory as intermediates in approach (b).**

In this communication, we report the synthesis of 2,3'-anhydro-1-(β-D-xylofuranosyl)cytosine (IX) as a candidate for approach (b) and the successful synthesis of a dinucleoside phosphate from (IX) bearing the natural 3',5'-phosphodiester linkage. Treatment of N^4-acetylcytidine (I) (6) with a three fold excess of triphenylmethylchloride in pyridine according to the procedure of Yung and Fox (8) yielded, after fractional crystallization, the 2',5'-di-O-trityl derivative II (Anal. Calcd. for $C_{49}H_{43}N_3O_6$: C, 76.46; H, 5.59; N, 5.46. Found: C, 76.36; H, 5.66; N, 5.47. M.p. 180° (prior sintering at 168°). Tlc (7) in silica gel ($CHCl_3$-EtOH 35:5), Rf 0.77 (a single spot) in 20% yield. To the product the structure (II) has been assigned on the basis of the following sequence of reactions. Deacetylation of (II) with methyl alcohol saturated with ammonia at 0° gave (III) (Anal. Calcd. for $C_{47}H_{41}N_3O_5$: C, 77.55; H, 5.64; N, 5.77. Found: C, 77.49; H, 5.65; N, 5.71. λ_{max}^{MeOH} 270 mμ, λ_{min}^{MeOH} 252 mμ. M.p. 178-180° (prior sintering at ca. 160°) in 70% yield. Treatment of (III) with isoamyl nitrite and glacial acetic acid in DMSO for 2 hours at room

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** Attempted synthesis of uridine-3' phosphate via approach (b) by use of 2,3'-anhydro-xylosyluracil (9) as the key intermediate has failed (2). To our best knowledge, 2,3'-anhydro-xylocytosines have not been prepared.

temperature led to the formation of (IV), m.p. 224-225° (recrystallized from a mixture of benzene and ether). Rf in tlc in silica gel (ethyl acetate)*** : 0.62; Rf in chloroform-ethyl alcohol (35:5)*** : 0.70. The product was identical with authentic 2',5'-di-O-trityluridine (8) on the basis of criteria of admixture melting point, Rf and IR. Mesylation of (II) in pyridine by standard procedure**** afforded a mesylated product (V); IR 1170 $cm.^{-1}$ (sulfonate). Anal. Calcd. for $C_{50}H_{45}N_3O_8S$: C, 70.83; H, 5.31; N, 4.95. Found: C, 70.68; H, 5.52; N, 5.17. Treatment of (V) with saturated (at 0°) methanolic ammonia afforded (VI) as needles, m.p. 180-183°. Anal. Calcd. for $C_{48}H_{43}N_3O_7S$: C, 71.55; H, 5.34; N, 5.34. Found: C, 71.80; H, 5.54; N, 5.45. Treatment of (VI) with 80% acetic acid at 100° for 30 min. afforded 3'-O-methansulfonylcytidine (VII) (Anal. Calcd. for $C_{10}H_{15}N_3O_7S$: C, 37.38; H, 4.64; N, 13.08. Found: C, 37.40; H, 4.58; N, 13.15. M.p. 214-217°(dec.) in 76% yield. Treatment of (VI) with 1.5 equiv. amount of sodium tert-butoxide in DMF afforded 2',5'-di-O-trityl-2,3'-anhydro-β-D-xylofuranosylcytosine (VIII), (Anal. Calcd. for $C_{47}H_{39}N_3O_4$: C, 79.54; H, 5.50; N, 5.92. Found: C, 79.55; H, 5.57; N, 5.89. M.p. 210°, recrystallized from ethyl alcohol and ether) in 56% yield. Treat-

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In these solvent systems (ethyl acetate and a mixture of chloroform -ethyl alcohol 35:5), Rf-values of 3',5'-di-O-trityluridine are 0.43 and 0.81, respectively.

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For instance, see ref. 8 (mesylation of 2',5'-di-O-trityluridine.

ment of (VII) with 1.5 equivalent amount of sodium tert-butoxide at 100° for 3 hours afforded (IX) in 84% yield. Anal. Calcd. for $C_9H_{11}N_3O_4$: C, 45.77; H, 4.88; N, 18.66. Found: C, 45.67; H, 4.49: N, 18.62. M.p. 134°, recrystallized from ethyl alcohol. These (VIII and IX) are the first example of 2,3'-anhydronucleosides of cytosine series. Treatment ***** of the anhydronucleoside (IX) with adenylyl-5' benzoic anhydride (9) or similar treatment of the anhydronucleoside (VIII), followed by de-blocking (98% formic acid at room temperature for 20 min.) gave cytidine-(3'-5')-adenosine (X) in 60% and 54% yield, respectively. The dinucleoside phosphate (X) was almost (ca. 91%) hydrolyzed by pancreatic RNase (10) to cytidine-3' phosphate (11) and adenosine. Starting from uridylyl-5' phosphoric benzoic anhydride and (IX), a similar series of reactions afforded cytidylyl-(3'-5')-uridine (ca. 97%) to cytidine-3' phosphate (11) and uridine.

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The reaction conditions were essentially similar to those for the reaction of 2,5'-anhydro-2',3'-di-O-isopropylideneuridine with benzylphosphoric benzoic anhydride for the synthesis of uridine-5' phosphate (1).

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