

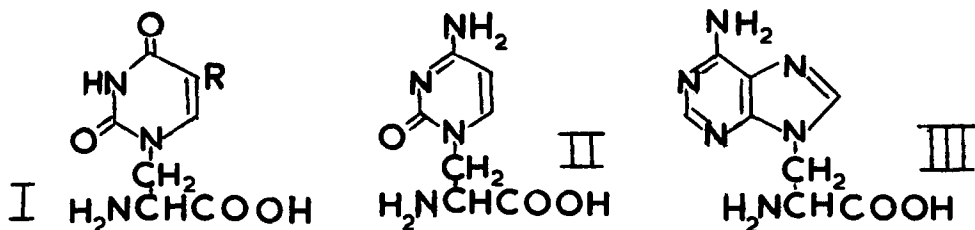
AN APPROACH TO THE SYNTHESIS OF PEPTIDE ANALOGUES  
OF OLIGONUCLEOTIDES (NUCLEOPEPTIDES)

M. T. Doel, A. S. Jones and N. Taylor

Chemistry Department, The University of Birmingham, Birmingham 15.

(Received in UK 24 April 1969; accepted for publication 9 May 1969)

The synthesis of analogues of polynucleotides in which the phosphodiester linkages are replaced by carboxymethyl linkages has been reported in previous papers (1). Another suitable linkage is the peptide linkage and, if in the analogue the sugar residue of the nucleoside is dispensed with, then suitable monomers would be  $\alpha$ -amino acids in which the side chains would be natural purine and pyrimidine residues. Oligo- and poly-peptides from these amino acids might be expected to interact by means of hydrogen bonds with natural polynucleotides. Other workers have made  $\alpha$ -amino acids with purine and pyrimidine side chains i. e. DL- $\beta$ -(2, 4-dihydroxypyrimidin-5-yl) alanine (2, 3), L- $\epsilon$ -(purin-6-yl) lysine (3) and DL- $\beta$ -(2, 4-dihydroxypyrimidin-1-yl) alanine (I, R=H) (DL-willardiine) (4). L-Willardiine is a naturally-occurring compound (5).



The present work reports the synthesis of the thymine and cytosine analogues of willardiine (I, R=CH<sub>3</sub> and II respectively) and DL- $\beta$ -(6-aminopurin-9-yl) alanine (III).

Initially a study was made of the synthesis of DL-willardiine which had been accomplished by Dewar and Shaw (4). A key intermediate is 1-(2, 2-diethoxyethyl) uracil which is obtained by the alkylation of uracil with 1-bromo-2, 2-diethoxyethane as described

by Martinez and Lee (6). We repeated this work but found that by using a suspension of anhydrous potassium carbonate in dimethylformamide (7) instead of sodium hydride a simplified procedure could be used and a better yield obtained. This modified procedure was used to obtain the cytosine and adenine derivatives.

Alkylation of thymine with 1-bromo-2,2-diethoxyethane gave 1-(2,2-diethoxyethyl) thymine as the major product (some 1,3 dialkylated thymine was also produced). Acid hydrolysis gave 2-(2,4-dihydroxy-5-methylpyrimidin-1-yl) ethan-1-al as the crystalline monohydrate. Treatment of this with sodium cyanide and ammonium chloride in the Strecker Synthesis gave DL- $\beta$ -(2,4-dihydroxy-5-methylpyrimidin-1-yl) alanine.

To obtain the 1-substituted cytosine derivative it was necessary to alkylate 4-N-acetylcytosine. A single product was obtained which was not isolated but directly deacetylated with methanolic ammonia to give 1-(2,2-diethoxyethyl) cytosine (identified by ultraviolet spectroscopy). Hydrolysis of this acetal with 4N-hydrochloric acid gave the corresponding aldehyde which was directly converted, by means of the Strecker Synthesis, into DL- $\beta$ -(4-amino-2-hydroxypyrimidin-1-yl) alanine.

Alkylation of adenine with 1-bromo-2,2 diethoxyethane gave several products but the major one was established by ultraviolet spectroscopy to be the 9-substituted adenine. This was isolated in pure form, hydrolysed to the corresponding aldehyde, which by means of the Strecker Synthesis was converted into DL- $\beta$ -(6-aminopurin-9-yl) alanine.

These compounds had ultraviolet absorption spectra typical of a 1-substituted thymine, 1-substituted cytosine or 9-substituted adenine and gave colours with ninhydrin characteristic of amino acids.

The synthesis of the corresponding guanine derivative and of oligo- and poly-peptides from these amino acids is proceeding.

#### Experimental

##### Reaction of 1-bromo-2,2-diethoxyethane with pyrimidines and adenine.

(a) For uracil and thymine the conditions of Martinez and Lee (6) were used. Uracil gave 1-(2,2-diethoxyethyl)uracil, m. p. 89° in 25% yield (Found: C, 52.3; H, 7.0; N, 11.9.

Calc. for  $C_{10}H_{16}N_2O_4$ : C, 52.6; H, 7.0; N, 12.3%.  $\lambda_{\max}$  267m $\mu$  (pH 1); 265m $\mu$  (pH 12).

Thymine gave 1-(2,2-diethoxyethyl)thymine, m. p. 76-78° in 30% yield (increased to 60% by using twice the amount of sodium hydride). (Found: C, 54.4; H, 7.1; N, 11.6.  $C_{11}H_{18}N_2O_4$  requires C, 54.5; H, 7.1; N, 11.6%);  $\lambda_{\max}$  271m $\mu$  ( $\epsilon$ ,  $8.6 \times 10^3$ ) pH 1; 269m $\mu$  ( $\epsilon$ ,  $6.5 \times 10^3$ ) pH 12.

(b) For uracil, 4-N-acetylcytosine and adenine the following procedure was used. The base was suspended in dry dimethylformamide (25ml/g of base; 100ml/g of base in the case of the acetylcytosine), dry  $K_2CO_3$  (1 molecular proportion), 1-bromo-2,2-diethoxyethane (1 molecular proportion) added and the mixture heated to 130° with stirring for 14 h, and the solution concentrated in vacuo to dryness. The uracil derivative was extracted with chloroform from aqueous solution and finally obtained in 60% yield by trituration with ether, as a crystalline solid identical to that obtained as in (a). The 4-N-acetylcytosine derivative was also extracted with chloroform, deacetylated by treatment with methanolic ammonia overnight at room temperature to give 1-(2,2-diethoxyethyl)cytosine, m. p. 232-4° in 57% yield (Found: C, 52.9; H, 7.4; N, 18.6.  $C_{10}H_{17}N_3O_3$  requires C, 53.0; H, 7.5; N, 18.5%);  $\lambda_{\max}$  283m $\mu$  ( $\epsilon$ ,  $13.4 \times 10^3$ ) pH 1; 273m $\mu$  ( $\epsilon$ ,  $8.65 \times 10^3$ ) pH 12. The adenine derivative was crystallised from ethanol to give 9-(2,2-diethoxyethyl)adenine, m. p. 212° in 47% yield (Found: C, 52.5; H, 6.6; N, 27.8.  $C_{11}H_{17}N_5O_2$  requires C, 52.4; H, 6.8; N, 27.9%);  $\lambda_{\max}$  259m $\mu$  ( $\epsilon$ ,  $14.0 \times 10^3$ ) pH 1; 261m $\mu$  ( $\epsilon$ ,  $14.1 \times 10^3$ ) pH 12.

Hydrolysis of the 2,2-diethoxyethyl derivatives to aldehydes. Hydrolysis of the uracil derivative was carried out with ammonium chloride as described by Martinez and Lee (6).

The thymine and adenine derivatives were hydrolysed with N HCl at 100° for 1 h. 2-(2,4-Dihydroxy-5-methylpyrimidin-1-yl)ethan-1-al, decomp.  $> 300^\circ$ , was obtained in 79% yield as the monohydrate (Found: C, 45.0; H, 5.5; N, 15.1.  $C_7H_{10}N_2O_4$  requires C, 45.1; H, 5.4; N, 15.1%);  $\lambda_{\max}$  270m $\mu$  ( $\epsilon$ ,  $9.72 \times 10^3$ ) pH 1; 267m $\mu$  ( $\epsilon$ ,  $6.74 \times 10^3$ ) pH 12. 2-(6-Aminopurin-9-yl)ethan-1-al was obtained as the hydrochloride dihydrate (m. p.  $> 300^\circ$ ) in 69% yield (Found: C, 33.8; H, 4.8; N, 28.5; Cl, 14.3.  $C_7H_{12}ClN_5O_3$  requires C, 33.8; H, 4.8; N, 28.2; Cl, 14.2%);  $\lambda_{\max}$  259m $\mu$  ( $\epsilon$ ,  $13.8 \times 10^3$ ) pH 1; 261m $\mu$  ( $\epsilon$ ,  $14.1 \times 10^3$ ) pH 12. The cytosine

derivative was hydrolysed with 4 N HCl at 100° for 1 hr. but the aldehyde was not isolated but converted directly into the amino acid.

Conversion of the aldehydes into DL-alanine derivatives. This was carried out by the Strecker Synthesis as described by Dewar and Shaw (4) for the synthesis of DL-willardiine and the following products obtained: DL-β-(2,4-dihydroxy-5-methylpyrimidin-1-yl)alanine

(43% yield) (Found: C, 45.3; H, 5.3; N, 19.7.  $C_8H_{11}N_3O_4$  requires C, 45.1; H, 5.2; N, 19.7%);

$\lambda_{\max}$  269m $\mu$  ( $\epsilon$ ,  $9.72 \times 10^3$ ) pH 1; 271m $\mu$  ( $\epsilon$ ,  $7.45 \times 10^3$ ) pH 12; DL-β-(4-amino-2 hydroxy-

pyrimidin-1-yl)alanine (49% yield) (Found: C, 36.0; H, 5.9; N, 24.2.  $C_7H_{14}N_4O_5$  requires C, 35.9; H, 6.0; N, 23.9%);  $\lambda_{\max}$  279m $\mu$  ( $\epsilon$ ,  $12.0 \times 10^3$ ) pH 1; 275m $\mu$  ( $\epsilon$ ,  $8.52 \times 10^3$ ) pH 12;

DL-β-(6-aminopurin-9-yl)alanine (53% yield) (Found: C, 43.1; H, 4.3; N, 37.5.  $C_8H_{10}N_6O_2$

requires C, 43.3; H, 4.5; N, 37.8%);  $\lambda_{\max}$  257m $\mu$  ( $\epsilon$ ,  $14.7 \times 10^3$ ) pH 1; 262m $\mu$  ( $\epsilon$ ,  $15.0 \times 10^3$ ) pH 12.

The authors thank Professor M. Stacey for his interest, the Medical Research Council for a Research Grant and the Science Research Council for Research Studentships (to M. T. D. and N. T.).

#### References

- (1) M. H. Halford and A. S. Jones, Nature, 217, 638 (1968); M. H. Halford and A. S. Jones, J. Chem. Soc. (C), 2667 (1968).
- (2) Yu. P. Shvachkin and I. K. Shprunka Vestn. Mosk. Univ. Ser. II Khim., 19, 72 (1964).
- (3) A. Vincze, C. Lackman and S. Cohen, Israel J. Chem., 6, 641 (1968).
- (4) J. H. Dewar and G. Shaw, J. Chem. Soc., 583 (1962).
- (5) A. Kjaer, A. Knudsen and P. Olesen Larsen, Acta Chem. Scand., 15, 1193 (1961).
- (6) A. P. Martinez and W. W. Lee, J. Org. Chem., 30, 317 (1965).
- (7) c. f. J. A. Montgomery and H. J. Thomas, J. Org. Chem., 30, 3235 (1965).