Oligonucleotide Synthesis on Polar Polymer Supports. The Use of a Polypeptide Support

By TOBY M. CHAPMAN* and DENNIS G. KLEID

(*Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania* 15213)

Summary The solid-phase synthesis of a trinucleotide phosphate, pTpTpT, using a polar polypeptide support is described.

THE use of polymer supports has proved to be of great importance in the synthesis of peptides;[1] their use in the synthesis of oligonucleotides has yet to reach this potential.[2] The major problem is the lack of a simple, high yield phosphorylation reaction in the construction of the internucleotide phosphate ester linkage. Another problem may be the incompatibility of the highly polar oligonucleotide with the non-polar polymers usually used as supports, mostly derivatized polystyrenes; indeed, the use of polymers containing polar backbone structures has hardly been investigated. Anfinsen[3] proposed the use of polypeptide supports for peptide synthesis and recently Köster[4] has investigated the use of polysaccharide and polyethylene glycol supports for the synthesis of oligonucleotides. We report the synthesis of a trinucleotide on a polypeptide support.

Poly-L-lysine hydrobromide (**1**) (mol. wt. 80,000) was modified so as to permit an aromatic phosphoramidate linkage between the support and the oligonucleotide. *p*-Trifluoroacetaminobenzamide linkages to polymer (**1**) were produced with *p*-trifluoroacetaminobenzoic isobutylcarbonic anhydride (**2**) in dimethylformamide (DMF) and triethylamine; further reaction with phenyl isocyanate[5] to block unreacted aliphatic amine gave polymer (**3**). The amidation reaction went in 61% yield based on fluorine analysis. Anhydride (**2**) (m.p. 95—95·5°) was prepared from *p*-trifluoroacetaminobenzoic acid[6] and isobutylchloroformate. Treatment of (**3**) with saturated methanol–ammonia (12 h) liberated the aromatic amine; cross-linking with hexamethylenedi-isocyanate (2·4 mole %) gave polymer (**4**). The polymer swells in DMF and pyridine. The arylaminopolymer (**4**) was mixed with the dipyridinium salt of 5′-phosphoro-3′-acetylthymidine (**5**) and dicyclohexylcarbodi-imide (DCC), in pyridine to give the nucleotide phosphoramidate (**6**) (15%). The method of Blackburn[7] was used to remove the 3′-acetyl group from (**6**) and the product was further elaborated by reaction with (**5**) and tri-isopropylbenzene sulphonyl chloride (TPS). After reaction with 1-naphthylisocyanate[5] to block unreacted mononucleotide, the 3′-acetate was removed and the product extended with (**6**) and TPS, followed by isocyanate treatment and removal of 3′-acetate.

Cleavage of the product from the polymer was effected using isoamyl nitrite in pyridine-acetic acid (1 : 1 v/v).[2] Paper chromatography of the product gave four u.v. absorbing spots, corresponding to pT-OH, pTpT-OH, pTpTpT-OH, and $pTOCONHC_{10}H_7$. The R_F values obtained were in good agreement with literature values[2,8] and were also checked against authentic samples kindly provided by Dr. K. L. Agarwal and Dr. H. G. Khorana. U.v. analysis showed 19 μmol of pT-OH, 4·3 μmol of pTpT-OH, and 3·3 μmol of trinucleotide pTpTpT-OH. This corresponds to a 14% yield of trinucleotide from phosphoramidate polymer (**6**), with the conversion of dinucleotide to desired product occurring in at least 43% yield.

We thank the Health Research and Services Foundation, Pittsburgh, Pennsylvania for support of this work and the University of Pittsburgh Department of Chemistry for providing an assistantship to D.G.K.

(*Received, 12th January* 1973; *Com.* 039.)

[1] R. B. Merrifield, *J. Amer. Chem. Soc.*, **1964**, **86**, 304.
[2] E. Ohtsuka, S. Morioka, and M. Ikehara, *J. Amer. Chem. Soc.*, **1972**, **94**, 3229 and references therein.
[3] C. B. Anfinsen, in 'Chemistry of Natural Products', I.U.P.A.C., Butterworths, London, **1968**, p. 461.
[4] H. Köster, *Tetrahedron Letters*, **1972**, **1531**, 1535.
[5] K. L. Agarwal, A. Yamazaki, P. J. Cashion, and H. G. Khorana, *Angew. Chem. Internat. Edn.*, **1972**, **11**, 451.
[6] F. Weygand and E. Leising, *Chem. Ber.*, **1954**, **87**, 248.
[7] G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem. Soc.*, **1967**, 2438.
[8] H. G. Khorana and J. P. Vizsoly, *J. Amer. Chem. Soc.*, **1961**, **83**, 675; S. A. Narang, J. J. Michniewicz, and S. K. Dheer, *ibid.*, **1969**, **91**, 936.