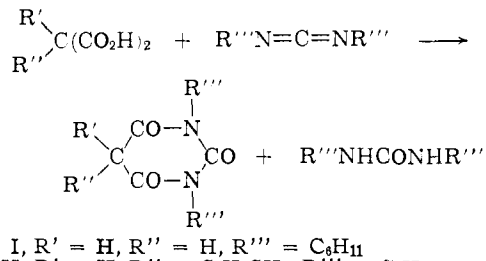
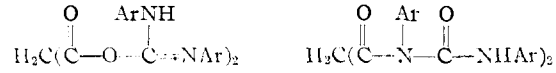


I, R′ = H, R″ = H, R‴ = C_6H_{11}
II, R′ = H, R″ = $C_6H_5CH_2$, R‴ = C_6H_{11}
III, R′ = Et, R″ = Et, R‴ = C_6H_{11}
IV, R′ = H, R″ = H, R‴ = *i*-Pr
V, R′ = Et, R″ = Et, R‴ = *p*-tolyl

Substituted malonic acids led to substituted barbiturates on reaction with N,N′-dicyclohexylcarbodiimide and N,N′-diisopropylcarbodiimide. Some of the compounds prepared by this method are: II, m.p. 120–121°; III, m.p. 160–162°; IV, m.p. 128–129°.

Malonic acid and di-*p*-dimethylaminophenylcarbodiimide have been reported to react in pyridine medium to give a diacylurea but the fate of malonic acid on reaction with di-*p*-tolylcarbodiimide has not been established.[1] We found that the reaction of malonic acid with N,N′-di-*p*-tolylcarbodiimide in tetrahydrofuran solution afforded N,N′-di-*p*-tolylurea and a compound that melted at 141–142°, solidified at about 150° and remelted at 225–230°. *Anal.* Calcd. for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21; mol. wt., 548.6. Found: C, 72.63; H, 5.68; N, 9.93; mol. wt., 570; λ_{max}^{nujol}: 3.05–3.20μ (weak), 5.76μ (strong), 5.95μ (weak), 6.00μ (weak); n.m.r. peaks,[2] 7.68 (6H), 7.58 (6H), 6.77 (2H), 2.78 (center of multiple peak, 16H), −0.87 (2H).

On the basis of infrared and n.m.r. spectra, we prefer structure VI to the acylurea structure VII for this compound. On heating above the melting point, VI was converted into the di-*p*-toluidide of malonic acid. Analogous results were obtained on using ethylmalonic acid. When, however, diethylmalonic acid was allowed to react with N,N′-di-*p*-tolylcarbodiimide the barbiturate V, m.p. 168–168.5°, was formed readily.

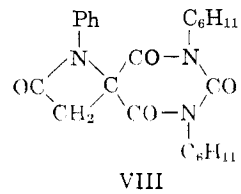


VI

VII

The difference between aromatic and aliphatic carbodiimides in the reaction with malonic acid is striking. It is also remarkable that diethylmalonic acid yields a barbiturate on reaction with di-*p*-tolylcarbodiimide but ethylmalonic and malonic acids do not. The role of steric and electronic factors in these reactions is under investigation.

The usefulness of this novel reaction of carbodiimides was demonstrated by the synthesis of β-lactam-substituted spirobarbiturates [for example, VIII, m.p. 234–235°] which could not be prepared by the usual methods of barbituric acid synthesis.



VIII

We are thankful to J. Zuclich[3] and J. Pelosi[3] for valuable technical assistance and to Dr. E. R. Malinowski and R. S. Magee[3] for the n.m.r. spectra and their interpretation. This investigation was supported in part by a research grant (MY-3930) from the National Institute of Mental Health, U. S. Public Health Service, for which grateful acknowledgement is made.

(1) F. Zetzsche and H. Lindlar, *Ber.*, **71**, 2095 (1938).

(2) Determined in dilute deuterated chloroform solution using tetramethylsilane as an internal standard. The first number indicates the τ value and the figure in parentheses denotes the number of equivalent protons under the peak.

(3) National Science Foundation undergraduate research participant.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
STEVENS INSTITUTE OF TECHNOLOGY AJAY K. BOSE
HOBOKEN, NEW JERSEY SHEILA GARRATT

RECEIVED FEBRUARY 23, 1962

COMPLEX FORMATION IN OLIGONUCLEOTIDES AND ITS APPLICATION TO THE SEPARATION OF POLYNUCLEOTIDES

Sir:

Recent studies[1] on oligonucleotides have shown that they are capable of complexing with themselves in buffered salt solutions. The complex formation appears to be of the "Watson-Crick" type and depends on the chain length, temperature and nature of the nucleotide bases present. For example, a mixture of thymidine dodecanucleotide,[2] $(pT)_{12}$ and 2 mole equivalents of deoxyadenosine hexanucleotide, d-$(pA)_6$ at 0° in 1 *M* NaCl, pH 7 displays an 11% decrease in optical density at 259 and 267 mμ. The complex melts over the range 4–30° with a melting point (T_m) at *ca.* 16°. A mixture of $(pT)_6$ and d-$(pA)_6$, however, shows no significant hypochromicity under these conditions.

These results suggested the possibility of devising a polynucleotide-cellulose column which would separate polynucleotides not only on the basis of their nucleotide composition, but also by virtue of their nucleotide sequence. A column of cellulose on which polynucleotide chains had been incorporated by bonding at one end of the chains, was expected to be the most efficient, as this type of column should allow maximum interaction with the polynucleotides to be separated.

Thymidine 5′-phosphate (2 mmole) in dry pyridine (3 ml.) was mixed with dicyclohexylcarbodiimide (4 mmole) and shaken with glass balls for 5 days. The mixture was added to dry cellulose (5 g.) dicyclohexylcarbodiimide (2 g.) in pyridine (50 ml.) and shaken for 5 days. The filtered cellulose was washed with pyridine and allowed to stand in aqueous pyridine overnight and then washed with ethanol and water. Analysis of the combined washings showed an incorporation of the nucleotide of more than 50%. The cellulose prepared in this way was expected to contain chains of thymidine polynucleotides (of up to *ca.*

(1) P. T. Gilham, unpublished.

(2) For preparation of oligonucleotides see H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961); R. K. Ralph and H. G. Khorana, *ibid.*, **83**, 2926 (1961).

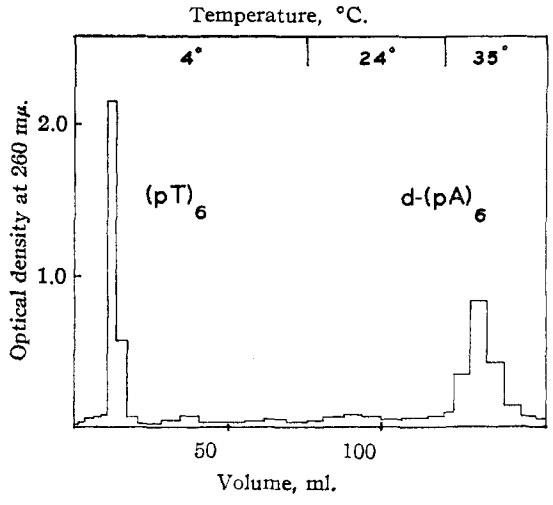


Fig. 1.

20 units long) connected at their 5′ phosphoryl ends to the cellulose by phosphodiester linkages. The cellulose was packed into a column (20 cm. × 1 cm. diam.) and washed extensively with 1 *M* NaCl–0.01 *M* NaH_2PO_4 (pH 7) solution. Ten optical density units each of $(pT)_6$ and d-$(pA)_6$ were passed slowly through the column in this salt solution at 4°. The elution diagram shows that the d-$(pA)_6$ was selectively bound to the column and could not be eluted until the temperature of the column had been raised to 35°. The separation was complete and the recovery quantitative. Preliminary experiments with *E. coli* amino acid transfer RNA on this column have resulted in a selective adsorption of 6% of the RNA. This fraction is now being examined for the presence of a consecutive adenosine sequence and for its specificity in accepting amino acids.

This work was supported by research grants (No. RG8817, C5178) from the National Institutes of Health.

INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN P. T. GILHAM
MADISON, WISCONSIN

RECEIVED FEBRUARY 20, 1962

YLID INTERMEDIATE IN THE REACTION OF TRIPHENYLPHOSPHINE WITH CARBON TETRACHLORIDE

Sir:

We wish to report an extremely simple, high yield route to prepare the ylid, triphenylphosphine dichloromethylene[1] [by the direct reaction of carbon tetrachloride with triphenylphosphine] and a convenient one-step synthesis for β,β-dihaloölefins.

When a concentrated solution of triphenylphosphine in carbon tetrachloride is allowed to stand at room temperature for 48 hours or is heated at 60° for 2–3 hours and then hydrolyzed, no triphenylphosphine is recovered.[2] This reaction was considered responsible for these observations

$$CCl_4 + 2(C_6H_5)_3P \longrightarrow (C_6H_5)_3PCl_2 (I) + (C_6H_5)_3P{=}CCl_2 (II)$$

In order to test this hypothesis, an equimolar amount of benzaldehyde was added to a mixture of triphenylphosphine in excess carbon tetrachloride which had been heated for 3 hours at 65°. Gas chromatographic analysis of the resulting solution indicated that most of the benzaldehyde was consumed and two new products, identified as β,β-dichlorostyrene and benzal chloride appeared. These lend support to the proposed intermediacy of triphenylphosphine dichloromethylene and triphenylphosphine dichloride.

$$(C_6H_5)_3P{=}CCl_2 + C_6H_5CHO \longrightarrow (C_6H_5)_3PO + C_6H_5CH{=}CCl_2$$

$$(C_6H_5)_3PCl_2 + C_6H_5CHO \longrightarrow (C_6H_5)_3PO + C_6H_5CHCl_2$$[3]

For synthetic utility it is convenient to conduct the entire reaction in one step by adding the carbonyl component to the initial mixture. For example, when a solution of 72.0 g. (0.274 mole) of triphenylphosphine and 29.0 g. (0.274 mole) of benzaldehyde in 150 ml. of carbon tetrachloride was heated at 60° for 2 hours, gas chromatographic analysis revealed that the benzaldehyde disappeared rapidly while benzal chloride and β,β-dichlorostyrene formed in equal quantities. Addition of 200 ml. of low boiling petroleum ether to the reaction mixture precipitated 69 g. (90%), m.p. 153–156°, of triphenylphosphine oxide. Fractional distillation of the filtrate gave less than 1 g. of unreacted benzaldehyde, 15.1 g. (72%) of benzal chloride, b.p. 121–3° (63 mm.) and 16.0 g. (72%) of β,β-dichlorostyrene,[4] b.p. 135–7° (63 mm.).

A careful study of the reaction of 32.7 g. (0.125 mole) of triphenylphosphine and 70 ml. of carbon tetrachloride in the presence of 22.8 g. (0.125 mole) of benzophenone at 60° was conducted and followed by quantitative gas chromatography. After 4 hours, the ratio of the peak area of the product, 1,1-diphenyldichloroethylene, to that of the benzophenone became constant. The solution contained 9.65 g. (0.052 mole) of benzophenone and 12.2 g. (0.049 mole) of 1,1-diphenyldichloroethylene (78% based on the stoichiometry):[5]

$$2(C_6H_5)_3P + 2(C_6H_5)_2CO + CCl_4 \longrightarrow 2(C_6H_5)_3PO + (C_6H_5)_2C{=}CCl_2 + (C_6H_5)_2CCl_2$$

However, *no* diphenyldichloromethane was observed, demonstrating that the triphenylphosphine dichloride produced in this reaction does not exchange with benzophenone. Addition of benzaldehyde to an aliquot of the reaction mixture produced benzal chloride almost immediately but, as anticipated, no β,β-dichlorostyrene was formed.

This shows that under special conditions and depending on the carbonyl component, considerable control of the product may be obtained. This type of reaction thus provides a potentially convenient route to otherwise difficultly accessible 1,1-dihaloölefins.

(1) This ylid was first described by A. J. Speziale, G. L. Marco and K. W. Ratts, *J. Am. Chem. Soc.*, **82**, 1260 (1960).

(2) In the 60° case, 76% of triphenylphosphine oxide was obtained. An additional 12% of the triphenylphosphine was combined in a water soluble unidentified crystalline solid, m.p. 239–241° with an empirical formula of $C_{19}H_{18}Cl_2P$.

(3) L. Horner, H. Oediger and H. Hoffmann, *Ann.*, **626**, 26 (1959).

(4) *Anal.* Calculated for $C_8H_6Cl_2$: C, 55.5; H, 3.46; Cl, 41.0. Found: C, 55.9; H, 3.65; Cl, 41.23.

(5) The reaction mixture was conveniently worked up taking advantage of the insolubility of triphenylphosphine oxide in ethyl ether and the much greater solubility of benzophenone in methanol than that of 1,1-diphenyldichloroethylene. The latter, m.p. 78.0–78.5° was identified by comparison with an authentic sample.