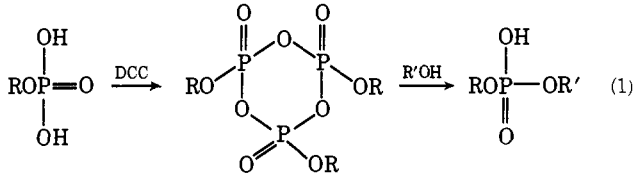
R. Taking a constant value of 110 for all the acids one obtains the estimates for the electron affinities shown in Table Ib. All values are quite high and lead one to expect an affinity of over 100 kcal for CF_3COO. Measurements involving fluoroacetic and other organic acids are presently in progress.

R. Yamdagni, P. Kebarle*
Chemistry Department, University of Alberta
Edmonton, Alberta, Canada T6G 2G2
Received February 28, 1973

An Improved Method for the Study of Reaction Intermediates. The Mechanism of Peptide Synthesis Mediated by Carbodiimides

Sir:

Carbodiimides continue to be the most versatile dehydrating agents for the synthesis of peptides and nucleotides, yet their mechanism of action remains incompletely understood. During phosphodiester synthesis with *N,N*-dicyclohexylcarbodiimide (DCC) an exceedingly complex reaction occurs in which Khorana[1] was able to identify the trimetaphosphate (**1**) as the initial phosphorylating agent (eq 1). The structure of the corresponding (acylating) agent during peptide synthesis with DCC (eq 2) has yet to be established despite considerable research.[2]

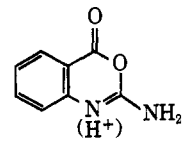


1

$$RCO_2H \xrightarrow{DCC} ? \xrightarrow{R'NH_2} RCONHR' \quad (2)$$

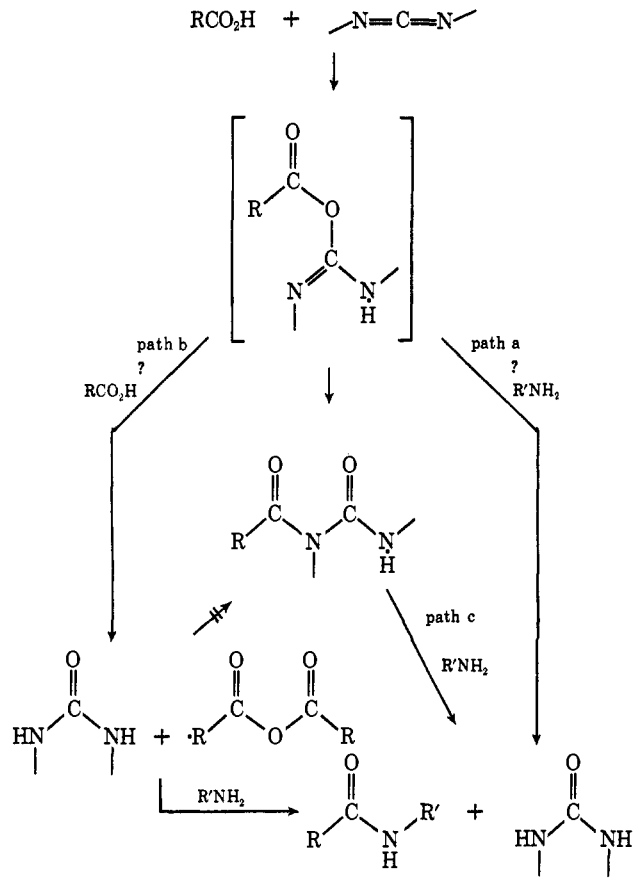
Scheme I formulates the mechanistic question, does amide formation proceed directly from the *O*-acylisourea (path a) or *via* the symmetrical anhydride (path b)? The *N*-acylurea (path c) has already been excluded as the acylating agent.[3]

Evidence for the two paths may be summarized as follows. In the absence of amine nucleophiles DCC smoothly converts carboxylic acids to their anhydrides; subsequent aminolysis occurs at a rate compatible with the anhydride's intermediacy during peptide synthesis.[3-5] Evidence supporting the *O*-acylisourea was first obtained by Weetall[6] through the DCC-mediated acylation of amines by polymer-bound carboxylic acids, a system which virtually precludes anhydride formation. More recently, Bruice[7] has shown that the model compound **2** (and its protonated form) readily acylates amines in the absence of carboxylic acids. Since each path has been demonstrated only in systems which



2

Scheme I



physically exclude the other, the evidence for either path is necessary but not sufficient, and the mechanism during the actual conditions of peptide synthesis remains unknown.

Similar mechanistic problems are posed by a variety of existing peptide reagents such as ethoxyacetylene,[8] isonitriles,[9] and redox systems,[10] which share with DCC the ability to convert carboxylic acids to their anhydrides. The mechanistic distinction is an important one since peptide synthesis is more likely to benefit from new acylating agents than from new methods of generating symmetrical anhydrides. Herein we report evidence that bears on this question for DCC-mediated peptide synthesis and propose an improved technique which appears applicable to the study of a variety of reaction intermediates.

We have determined the product distributions for the reaction of three amines with the anhydride, $(Z\text{-}Gly)_2O$, of benzyloxycarbonylglycine *and* with the Z-Gly-OH-DCC intermediate *under identical reaction conditions*. The results, tabulated in Table I for two reaction temperatures, indicate that the anhydride cannot entirely account for behavior of the initial acylating

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(2) A summary may be found in E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York, N. Y., 1965, p 108.
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(10) T. Mukaiyama, *Syn. Commun.*, **2**, 243 (1972).

Table I

Temp, °C	Product	$^{14}C/^{3}H$	Yield,[a] % *Via* anhydride	Yield,[a] % *Via* DCC
5	**3**	1.18	66	59
	4	1.19	20.5	18
	5	0.84	13	21
			Total 99.5	98
30	**3**	1.14	57	51
	4	1.34	23	17
	5	0.90	15	19
			Total 95	87

[a] By isotope dilution and scintillation counting.

agent of the DCC reaction. A maximum of 55% (5°)

$$^{14}C(\text{Z-Gly})_2O^{11} + \text{DCC} + {}^{3}H\text{Z-Gly-OH} +$$
(1 equiv) (1 equiv) (30 equiv)

$$\text{Gly-OEt} + C_6H_5CH_2NH_2 + C_6H_5NH_2$$
(10 equiv) (10 equiv) (10 equiv)

$$\xrightarrow[10\text{ hr}]{CH_2Cl_2}$$

$$\underset{\mathbf{3}}{\text{Z-Gly-Gly-OEt}} + \underset{\mathbf{4}}{\text{Z-Gly-NHC}_6H_5} + \underset{\mathbf{5}}{\text{Z-Gly-NHCH}_2C_6H_5}$$
(both labels)

to 60% (30°) can be calculated as the extent of the anhydride's participation during peptide synthesis in solution with DCC. Delayed addition of the amine component,[2] or solid phase peptide synthesis, during which the amine component is anchored to an insoluble support is expected to decrease the importance of path a and increase the extent of anhydride participation. Experiments on the solid phase are in progress.

While this technique borrows heavily from classical product distribution analyses, several unique advantages arise through the use of distinctly labeled reactants for the study of systems which can be generalized as in Scheme II. (1) The reactions for which a com-

Scheme II

$$\begin{matrix} {}^{3}HRX \\ {}^{14}CRY \end{matrix} \xrightarrow[rds_2]{rds_1} ? \xrightarrow{\text{fast}} P_1 + P_2 + P_3\cdots$$
reactants intermediate products

mon intermediate is suspected may be run simultaneously, *in the same solution*, thus maintaining identical conditions for both reactions. (2) The scope of manageable product distributions is considerably expanded through the determination of products by isotope dilution. (3) The relative rates, rds_1/rds_2, of formation for any product can be assessed directly from the $^{3}H/^{14}C$ activity ratio of that product. The tremendous range of available tritium activities, *ca.* 10^8, can be exploited to ensure that these ratios are readily measurable even if the slower reaction attains only a few per cent completion. (4) The $^{3}H/^{14}C$ ratios of the products reveal the presence (or absence) of a common intermediate.

Although the present study used an isolable intermediate (the anhydride) directly, the method is of potential utility for the study of such reactive species as carbenes, carbenoids,[12] and "dark" *vs.* photochemical intermediates.[13]

Acknowledgment. Generous financial support of this work by the Eli Lilly Co. is gratefully acknowledged.

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Julius Rebek,* David Feitler[14]
Contribution No. 3092
Department of Chemistry, University of California
Los Angeles, California 90024
Received February 21, 1973

A Tetranuclear Copper(I) Complex. The Crystal and Molecular Structure of the 2:1 Derivative of Copper(I) Iodide and Bis(diphenylphosphino)methane

Sir:

Copper(I) halides react with bis(diphenylphosphino)-methane (DPM) and 1,2-bis(diphenylphosphino)ethane (DPE) giving compounds with several stoichiometries, $(CuX)_nL_m$ (L = DPM, DPE and X = Cl, Br, I). The preparation and characterization of these complexes have recently been reported.[1] The crystal and molecular structure of $(CuCl)_2DPE_3$ have also been determined,[2] revealing binuclear centrosymmetric molecules with tetrahedral copper atoms bridged by one DPE molecule, the other two of which act as chelate ligands. However, many obscurities still remain in the structure and behavior of this series of complexes. Therefore we have undertaken a systematic study of the structure of such compounds by single-crystal X-ray analysis. We report here the preliminary structural results on $(CuI)_2DPM$.

The prismatic crystals, elongated along [100], were found to be orthorhombic, with cell parameters a = 17.128 ± 0.009, b = 18.306 ± 0.009, and c = 16.508 ± 0.008 Å. Space group *Pbca* was indicated by systematic absences. The density 1.94 ± 0.01 g/cm^3, as determined by flotation, is in agreement with the calculated value of 1.960 g/cm^3 with eight formula units of $(CuI)_2DPM$ in the cell.

Intensity data ($\theta \leqslant 30$) were collected on a Siemens automated diffractometer with Mo Kα-filtered radiation using the θ–2θ scan technique. Independent reflections (1336) with $I > 3\sigma_I$ were corrected for Lorentz and polarization factors. Although the adsorption coefficient is 43 cm^{-1}, no correction was applied because of the small size of the crystal (nearly a cylinder of 0.15 mm of diameter), the adsorption correction factor A^* ranging from 1.65 to 1.64 in the θ range of interest. The structure was determined by the heavy-atom method and refined to a present value of the conventional R index of 0.065, by the full-matrix least-squares method with anisotropic temperature factors for the iodine and copper atoms.

The crystals consist of discrete centrosymmetric dimetric units of $[(CuI)_2DPM]_2$ arranged around the crystallographic symmetry center to form a parallelogram

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(2) V. Albano, P. L. Bellon, and G. Ciani, *J. Chem. Soc., Dalton Trans.*, 1938 (1972).