Table IV. Characterization of S-Ethyl Derivatives of Oligonucleotides

Compound	Base T	C	A	G
EtS-dpTpT	2			
EtS-dpTpC	1	1.03		
EtS-dpCpT	1	1.06		
EtS-dpTpA	1		1.05	
EtS-dpCpA		1	1.11	
EtS-dpTpTpT	3			
EtS-dpTpApG	1		1.09	1.09
EtS-dpTpCpA	1.11	1	1.14	
EtS-dpCpTpA	1	1.39	0.78	

and after storage overnight the solution was evaporated, dissolved in water (5 ml), and extracted with ether (three 5-ml portions). The aqueous layer was treated overnight with an equal volume of concentrated aqueous ammonia, and evaporated to dryness. The residue was dissolved in water (25 ml), and purified by passage through a DEAE cellulose column (60 × 2.3 cm, bicarbonate form), which was eluted with triethylammonium bicarbonate, pH 7.5. A linear gradient of 0.005–0.35 *M* was used, and the required product was obtained at a buffer molarity of 0.25 *M*, 501 OD_{260} units (69%).

Self-Condensation of 1. The pyridinium salt of **1** (0.97 mmol) was dried by evaporation of pyridine (three 10-ml portions), and treated with iodine (1.29 g) in dry pyridine (5 ml) for 70 hr at room temperature. Water (5 ml) was added, and after 2 hr the solution was evaporated to dryness, dissolved in water (10 ml), and extracted with ether (three 10-ml portions). The aqueous layer was evaporated to dryness, and treated with pyridine (10 ml) and acetic anhydride (5 ml) overnight. The solution was cooled to 0°, and water was added with cooling. After 5 hr at 0° the solution was evaporated to dryness and treated with concentrated aqueous ammonia (30 ml) for 16 hr. The product was evaporated to dryness, dissolved in water, and applied to a DEAE cellulose column (55 × 3.4 cm, bicarbonate form). The column was eluted with a linear gradient of 4 l. of triethylammonium bicarbonate buffer, pH 7.5 (0.005 *M*) in the mixing vessel and 4 l. of the same buffer (0.5 *M*) in the reservoir. The appropriate fractions were combined, evaporated to dryness, and the products were identified by paper chromatographic comparison with authentic samples,[19] and by their susceptibility or resistance to snake venom diesterase. The products are listed in Table I.

Table V. Paper Chromatographic Properties of Oligonucleotide Derivatives

Compound	R_f (solvent A)	R_f (solvent B)
EtS-dpT	0.68	0.64
EtS-dpC	0.60	0.61
EtS-dpTpT	0.45	0.54
EtS-dpTpC	0.38	0.51
EtS-dpTpA	0.34	0.50
EtS-dpCpT	0.41	0.51
EtS-dpCpA	0.34	0.37
EtS-dpTpTpT	0.33	0.43
EtS-dpTpCpA	0.19	0.29
EtS-dpTpApG	0.19	0.15
EtS-dpCpTpA	0.15	0.31
MeO-dpTpCpA	0.20	0.29
MeO-dpTpApG	0.16	0.25
MeO-dpCpTpA	0.14	0.31
d-pT	0.46	0.39
d-pTpCpA	0.18[a]	0.11
d-pCpTpA	0.18	0.12
d-pTpApG	0.08	0.11
d-pppTpT	0.17	0.26
d-pppTpTpT	0.10	0.20
d-pTpTpT	0.28	0.23
r-pA		

[a] This, and the remaining R_f values in this column, are calculated with respect to d-pT.

Characterization of Oligonucleotides. Samples of each oligomer were digested with snake venom diesterase, and the products were separated by paper chromatography in system A or C. The appropriate spots were cut out, eluted with water, and measured spectrophotometrically. Samples containing both d-pA and d-pC were not well separated by these systems. In these cases, the original sample was hydrolyzed to its constituent bases by treatment with 80% formic acid for 1 hr at 175°, and the products were separated by paper chromatography in acetonitrile/0.1 *M* ammonium acetate/concentrated ammonium hydroxide/*n*-butyl alcohol 6:2:1:1 (v/v). The appropriate spots were cut out and assayed as before. The results are summarized in Table IV. In Table V are given paper chromatographic properties of oligonucleotide derivatives.

Secondary Isotope Effects in Reactions Catalyzed by Yeast and Muscle Aldolase[1]

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Abstract: The aldolase-catalyzed condensation of (1*R*)-[1-^{3}H]dihydroxyacetone-P with D-glyceraldehyde-3-P to give [3-^{3}H]fructose-1,6-diP was found to go more slowly than normal under conditions which previously indicated that the proton abstraction step is rate determining. Furthermore, slower reaction of fructose-diP containing ^{3}H at C-3 or C-4 is characteristic of both the yeast and muscle enzymes, indicating that the C–C cleavage step (kinetic effect) or glyceraldehyde-P release (equilibrium effect) is the rate-limiting step for the formation of the first product, glyceraldehyde-3-P.

The enzyme-catalyzed aldol cleavage of fructose-1,6-diP (FDP)[4] is known to occur in an ordered sequence in which C–C bond cleavage and release of D-glyceraldehyde-3-P (G3P) precede the protonation of the

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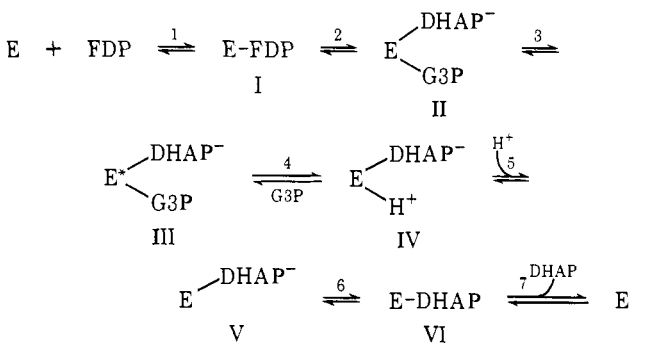
enzyme-bound anion of dihydroxyacetone-P (DHAP), which is here designated E-DHAP$^-$. The formal



mechanism represented by the six enzyme–intermediate complexes could be extended with additional isomerized forms, an example of which is III, for the purpose of considering other possibilities for rate-determining steps. It has been established, however, by isotope-exchange studies that an isomerization of E-DHAP$^-$ (to E*-DHAP$^-$) cannot be a kinetically important step in the reaction pathway for either yeast or rabbit muscle aldolases.[4]

The reaction with muscle aldolase involves Schiff's base formation [complexes I and VI] as shown by inactivation with BH_4^- in the presence of substrate[5] and by ^{18}O-exchange studies.[6] Therefore, more than simple association complexes should be implied for I and VI when considering the muscle enzyme. The yeast enzyme is thought not to proceed in this way, but to carry out an enolization, perhaps assisted by the Zn^{2+} cofactor required for the reaction.[7] Both enzymes have the same specificity with respect to the hydrogen activated (the pro-S hydrogen[8]) on DHAP.[9,10] Although there is no direct evidence relevant to the question, it seems probable that the proton addition and abstraction reactions involve proton transfer between DHAP and an amino acid residue acting as a base, step 5. Indirect evidence for this in the case of the muscle enzyme derives from the fact that a number of treatments of the enzyme result in severe inhibition of prototropy (IV → DHAP) with little change in transaldolase rate (FDP ⇄ IV).[4,11,12] Thus, for carboxypeptidase-treated muscle aldolase, although net FDP cleavage is decreased 20-fold,[13] the rates of FDP ⇄ G3P exchange[4] and [2-^{18}O]FDP ⇄ H_2O exchange[6] are unaltered, whereas the exchanges FDP ⇄ DHAP[4] and DHAP ⇄ ^3H_2O[14] are greatly diminished. The observation of a large ^2H_2O effect on the FDP cleavage rate and of a sevenfold decrease in the condensation rate of (1*S*)-[1-^{2}H]DHAP with glyceraldehyde catalyzed by the carboxypeptidase-treated enzyme[4] implies that step 6[15] is rate determining in formation of DHAP from FDP and in the condensation reaction. In the formation of FDP by yeast aldolase, (1*S*)-[1-^{3}H]DHAP is used at $^1/_7$ the rate of normal DHAP at pH 8, whereas no discrimination is evident at pH 6 where detritiation exceeds the rate of condensation with D-G3P.[4] Thus, presumably, at alkaline pH, step 6[15] may be rate determining with the yeast enzyme.

The present study concerns the use of secondary hydrogen isotope effects to identify and characterize rate-limiting steps in the aldolase reactions. If the force constant to a hydrogen not directly involved in the reaction differs in the reactant and transition state, a kinetic secondary isotope effect results that may be described as due to inductive or steric factors.[18] Vibrational energy differences between hydrogen that is bonded to a tetrahedral as compared with a trigonal carbon result in equilibria more favorably for the sp^2 state product with H than ^{2}H by about 30–40%.[19] Similar but smaller kinetic effects are seen in going to a partially carbonium- or carbanion-like transition state.[19,20] To this date, only a few examples of the use of secondary isotope effects for enzyme studies can be cited.[21]

Experimental Section

Materials. **(1*R*)-[1-^{3}H]DHAP.** The stereospecific tritiation at C-1 occurs in the reaction of D-G3P with triose-P isomerase.[10] The mixture of triose-P's (90% DHAP) was isolated on Dowex-1-Cl$^-$ by elution with 0.02 *N* HCl, concentrated, neutralized, and allowed to react with muscle aldolase (free of triose-P isomerase) in order to convert the G3P to FDP. The DHAP was reisolated on Dowex-1-Cl$^-$ as before.

[3-^{3}H]FDP. The condensation of (1*R*)-[1-^{3}H]DHAP (prepared above) with excess D-G3P was effected by muscle aldolase and the FDP isolated on Dowex-1-Cl after removing the triose-P's (with 0.03 *N* HCl) by elution with 0.1 *N* HCl.

[4-^{3}H]FDP. [4-^{3}H]Glucose was kindly provided by Dr. O. Gabriel. This was converted quantitatively to FDP by reaction with ATP by the three enzymes, hexokinase, P-glucose isomerase, and P-fructokinase, and isolated as above.

[1-^{3}H]D-G3P. [4-^{3}H]FDP was converted with muscle aldolase and glycerol-P dehydrogenase to a mixture of α-glycerol-P plus 1-^{3}H-D-G3P, and the enzymes inactivated with $HClO_4$. This solution was supplemented with DHAP and added unlabeled D-G3P for studies of the synthesis of FDP with yeast or muscle aldolase. In the absence of enzyme no radioactivity was found in the FDP region of the ion-exchange separation.

Muscle aldolase was obtained from Sigma Chemical Co., and was freed of triose-P isomerase on DEAE.[22] Carboxypeptidase treatment[12] resulted in a 95% decrease in the maximum rate of FDP cleavage as measured under standard conditions.

Yeast aldolase was prepared by the method of Richards and Rutter[23] and was free of triose-P isomerase. Other enzymes were obtained commercially. FDP was assayed by reaction with aldolase, triose-P isomerase, and glycerol-P dehydrogenase, coupled to DPNH oxidation. Triplicate assays and duplicate countings were made of peak tubes isolated by column elution as soon after they were obtained as possible. Ion-exchange separations and main-

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Table I. Secondary Isotope Effects in (1*R*)-[1-^{3}H]DHAP + D-G3P → [3-^{3}H]FDP

Aldolase	pH	Specific activity of FDP, cpm/μmol × 10^{-3} Early FDP	(%)[a]	Equilibrium FDP	$k_H/k_{^3H}$[b]
Yeast	6.0	32.1 ± 0.3	(30)	31.9 ± 0.2	0.99 ± 0.01
	8.0	3.44	(10)	4.34	1.28
	9.0[c]	21.9 ± 0.4	(22)	25.3 ± 0.3	1.18
Native, muscle	7.5	7.85 ± 0.05	(26)	8.10 ± 0.04	1.04
C.P., muscle[d]	7.3	26.6 ± 0.9	(21)	30.2 ± 0.7	1.15
C.P., muscle	7.3	54.4 ± 1.0	(25)	60.6 ± 2	1.14

[a] Per cent of the equilibrium concentration of FDP present at the time of sampling. [b] Calculated from Figure 1 of Collins and Lietzke.[25] [c] At pH 9, (1*S*)-[1-^{3}H]DHAP is used at 20% the rate of normal DHAP. [d] Carboxypeptidase treated rabbit muscle aldolase.

tenance of the samples were at 3°. Glycerol-P was separated from FDP on Dowex-1-Cl$^-$ by elution with 0.02 *N* HCl and assayed in duplicate by oxidation with glycerol-P dehydrogenase in the presence of hydrazine.[24]

Replicate spectrophotometric assays of FDP and glycerol-P agreed to within 1–3%. All counting was of 0.5 ml of the isolated peak tube in a mixture of 5 ml of ethanol, 10 ml of toluene, and scintillator.[9] All samples were counted under identical conditions of temperature and acidity, and for an identical period following the preparation of the sample. Counting rates were greater than 10 times background and replicates agreed to within 1–3%. The data are reported as average specific activity ± the maximum deviation obtained. The apparent isotope effects obtained by comparison of specific activities of the "initial" and terminal product were corrected to the true initial effect according to Collins and Lietzke.[25]

Methods. (1*R*)-[1-^{3}H]DHAP + G3P → 3-^{3}H-FDP (Table I). Yeast Aldolase. A typical incubation for Table I contained DHAP (2 m*M*), G3P (2 m*M*), $ZnSO_4$ (1.5 m*M*), cysteine (1 m*M*), EDTA (0.5 m*M*), K-acetate (50 m*M*), buffer (100 m*M* of cacodylate, triethanolamine, or N,N-bis(2-hydroxyethyl)glycine at pH 6, 8, or 9), and a small amount of (1*R*)-[1-^{3}H]DHAP to provide the desired specific activity. Yeast aldolase (0.01–0.03 unit) was added to the incubation at 35° and samples of 0.03 ml were transferred to 2 m*M* EDTA to stop the aldolase reaction. These were assayed for remaining DHAP and samples were taken at 10–30% reaction for the isolation of FDP. At this time excess yeast aldolase was added to the remainder of the reaction mixture in order to provide the sample that had established isotopic equilibrium.

Muscle Aldolase. The 35° incubations contained equimolar triosephosphates, EDTA (0.5 m*M*) and triethanolamine-HCl buffer (200 m*M*, pH 7.5). After the addition of native or carboxypeptidase-treated aldolase, samples placed in dilute HCl were assayed for DHAP until about 25% reaction was achieved. The remaining sample was brought to equilibrium by excess aldolase.

All samples were placed on columns of Dowex-1-Cl$^-$ (1 × 3 cm). No radioactivity was detected in the breakthrough and water wash of the column, indicating that labelization did not occur in any of the incubations. The columns were eluted with 0.02 *N* HCl to remove triose-P's and then with 0.1 *N* HCl for FDP. The effluent was monitored for radioactivity.

FDP Cleavage Reactions (Table II). The reactions with carboxypeptidase-treated aldolase of muscle contained labeled FDP (0.5 or 1.7 m*M*), Na-cacodylate (0.1 m*M*, pH 6.0), EDTA (1 m*M*), serum albumin (1 mg/ml), DPNH (1.4 m*M*), glycerol-P dehydrogenase (3.6 units/ml), and triose-P isomerase (0.05 unit/ml). Reactions were terminated at completion or at 30% of completion as indicated by the change of absorbance at 340 mμ. The yeast aldolase reactions containing [3-^{3}H]FDP (0.5 or 1.7 m*M*), glycylglycine buffer (0.1 *M*, pH 7.0 or 8.0), $ZnSO_4$ (4 m*M*), cysteine (1 m*M*), dithiothreitol (1 m*M*), serum albumin (1 mg/ml), K-acetate (0.1 *M*), and DPNH, glycerol-P dehydrogenase, and triose-P isomerase (as above) were followed to 100% and 30% at 26°.

In these reactions the glycerol-P dehydrogenase activity was in great excess of the aldolase and isomerase activities to prevent these enzymes from labelizing tritium derived from [4-^{3}H]FDP and [3-^{3}H]FDP, respectively.

[1-^{3}H]G3P + DHAP → [4-^{3}H]FDP (Table III). These experiments were similar to those of Table I with the exceptions that DHAP was in twofold excess of the [1-^{3}H]G3P and that a single equilibrated sample was used for comparison with the two early time incubations. The incubation temperature was 26°.

Results and Interpretations

The effect of isotope substitution at a bond that is not broken in the reaction generally will be most accurately measured by the method of isotope competition. This is particularly true for enzyme-catalyzed reactions where impurities carried with one form of the substrate might have a differential effect in a study of comparison of rates. In the present study the competition between ^{3}H and ^{1}H forms of substrate is evaluated from the specific activity of product formed after 10–30% of the substrate has reacted with that formed after complete conversion of substrate to product in the case of FDP as substrate or after equilibrium has been established in case FDP is product. The use of product only, in making the calculations, avoids effects of nonreactive isotopic impurities in the substrates since the product is well separated from substrate in the ion-exchange isolation.

In the formation of FDP catalyzed by the yeast enzyme at pH 6.0, it was observed that detritiation of (1*S*)-[1-^{3}H]DHAP exceeded the rate of condensation by almost twofold,[4] indicating that either condensation or product release are rate determining. In agreement with this, no effect of α-^{3}H substitution in the 1 pro-R position was detected (Table I). In the case that condensation, step 2, was rate limiting, the equilibrium constant, (II)/(DHAP)(E), would be less for the tritiated species of DHAP. Since $k_{-2}^H/k_{-2}^T < 1$ is expected for a transition state having significant sp^3 character at the developing bond, the two effects would cancel. In the case that product release, step 1, was rate limiting, the preequilibrium, I ⇄ E + DHAP, would not involve a change in bond hybridization and hence no discrimination against the tritiated species of DHAP is expected. On the other hand, at pH 8, where condensation with D-G3P to form FDP is 7-fold greater than detritiation from the pro-S position[4] and at pH 9, where it was found to be 5.5-fold greater, normal secondary isotope effects were observed. A similar correlation is observed with muscle aldolase; the native enzyme shows little discrimination with either position substituted, whereas the carboxypeptidase-treated enzyme has a 20-fold primary tritium effect and 13% slower rate with the pro-R ^{3}H-DHAP. Limitation in the proton abstraction, step 6 would lead to both the primary and secondary effects that are noted.

Little is known to identify the rate-limiting step in the cleavage of FPD by either the yeast or muscle enzymes. Studies of Model and Rittenberg[6] of [2-^{18}O]-FPD exchange with H_2O catalyzed by muscle aldolase

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Table II. Isotope Effects in the Cleavage of [3-^{3}H]FDP and [4-^{3}H]FDP

Substrate	Enzyme	pH	Specific activity of glycerol-P, cpm/μmol $\times 10^{-3}$ 30% product	Complete product	k_H/k^3_H
[3-^{3}H]FDP	C.P., muscle	6	11.0 ± 0.8	14.15 ± 0.5	1.33
	Yeast	8	4.9 ± 0.5	6.58 ± 0.5	1.43
		7	5.1 ± 0.5		1.33
[4-^{3}H]FDP	C.P., muscle	6	2.92 ± 0.1	3.90 ± 0.1	1.37

indicated a rate of exchange 3.3-fold greater than the net cleavage rate for the native enzyme. Since the aldol cleavage mechanisms of both enzymes involve the liberation of G3P as the first product, a process unaltered by carboxypeptidase treatment in the case of the muscle enzyme,[4] one may look for secondary isotope effects with these enzymes as a test of rate limitation in the C–C cleavage step. Since the formation of the first product, G3P, is made irreversible by the presence of triose-P isomerase and glycerol-P dehydrogenase in the reaction mixture, the occurrence of an even slower step, such as step 6, would not prevent the expression of isotope discrimination in steps 1–4. This is true for the present isotope competition kind of experiment, but would not be true if one compared the reaction rate of deuterated and normal substrates since in this case the rate of free-enzyme, E, regeneration would be important. The results of Table II indicate that FDP substituted with tritium in either the C3 or C4 positions is used less well in competition with the protonated species. Isotope substitution remote from the site of reaction results in rate effects that are much smaller than those observed.[26] Thus, steps prior to C–C cleavage concerned with the C2 position of FDP, such as those involved in Schiff's base formation, cannot be rate limiting. This result indicates that the slow step in formation of free G3P could be either, (a) the C–C cleavage itself, step 2, leading from an sp^3 ground state to a partial sp^2 transition state for both the developing DHAP anion and the developing G3P, or (b) a later step, 3 or 4, related to the dissociation of G3P, since the equilibrium isotope effect attendant on the C–C cleavage reaction would result in a lower tritium-specific activity for II than for I or the starting FDP (3-^{3}H or 4-^{3}H).

In an effort to distinguish between these possibilities, [1-^{3}H]D-G3P was tested for an isotope effect in the condensation with DHAP using either yeast or muscle enzyme. Again, although deprotonation of DHAP might limit the net formation of FDP, it is feasible that discrimination between the two forms of D-G3P, resulting in an inverse isotope effect, could be demonstrated if step 2 were limiting for the utilization of G3P and if, and *only* if, the transition state for this step had significant sp^3 character at the C-1 of the G3P component. On the other hand, rate limitation in the development of complex II from G3P should not lead to discrimination unless a change of orbital hybridization of C-1 of G3P exists between the free and bound forms. In this case an equilibrium isotope effect would be observed. The relevant data are given in Table III. The failure to observe an inverse isotope effect prevents the differentiation between these possibilities. After these experiments were completed, however, it was learned that proton nmr studies of G3P indicate the presence of large amounts of dimer form with little free carbonyl form in aqueous solution.[27] It is therefore possible that the observed isotope effect is a combination of the isotope effect on the $(G3P)_2 \rightleftarrows 2(G3P)$ equilibrium and the kinetic isotope effect in the condensation. These will oppose each other.[28]

Table III. Isotope Effects in the Reaction [1-^{3}H]G3P + DHAP → [4-^{3}H]FDP

Enzyme	pH	% of G3P condensed	Specific activity of FDP, cpm/μmol $\times 10^{-3}$	k_H/k^3_H
Yeast	8	34	22.7	1.06
C.P., muscle	6	30	23.5	1.03
Native, muscle	6	97[a]	24.2	

[a] Equilibrium established with excess native muscle aldolase.

Since the exchange between D-G3P and FDP with carboxypeptidase-treated aldolase is many times greater than the rate of formation of DHAP, it was possible in the presence of D-G3P to measure the net kinetic secondary isotope effect in the conversion, [3-^{3}H]-FDP → (1*R*)-[1-^{3}H]DHAP under conditions of equilibrium between FDP and G3P plus species IV. When this was done at pH 6.2 (0.1 *M* maleate) and 35°, the DHAP (as α-glycerol-P) formed at 20% conversion of FDP with limiting carboxypeptidase-treated aldolase had a specific activity of 12,300 cpm/μmol, whereas that formed by complete reaction with excess native aldolase was 15,600 cpm/μmol. This corresponds to a normal isotope effect of $k^H/k^T = 1.37$. This is similar to the effect seen with [3-^{3}H]FDP in Table II in which the presence of triose-P isomerase with the glycerol-P dehydrogenase caused the rapid removal of G3P as it was formed, making step 4 irreversible. Evidently, although steps 1–5 are at equilibrium and step 6 is rate determining, the isotope discrimination is determined by step 2, *i.e.*, the secondary equilibrium isotope effect, which favors a lower specific activity for intermediates IV and V than for FDP, offsets any inverse secondary kinetic isotope effect due to step 6. If may be easily shown that under these conditions the inverse secondary isotope effects in the reverse of step 2 and in step 6 will tend to cancel each other leaving the aldol cleavage reaction to be the major factor in determining the discrimination.

This example serves to emphasize that the interpretation of kinetic secondary isotope effect data is generally

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made ambiguous by the likelihood that equilibrium effects may be at least as large as kinetic effects, unlike the case for the primary isotope substitution. This is particularly true for isotope substitution at the carbon undergoing reaction since the orbital state of the bond to the isotope is altered, and hence an equilibrium effect is to be expected.

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Preparation of a *t*-Alkyloxycarbonylhydrazide Resin and Its Application to Solid Phase Peptide Synthesis[1]

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Abstract: A new type of resin with *t*-alkyloxycarbonylhydrazide functional groups was prepared: H_2NNH-$COOC(CH_3)_2CH_2CH_2C_6H_5$ polymer. With this resin as solid support, a procedure was developed which should be useful for preparation of protected peptide hydrazides that can be purified, converted to the azides, and then condensed to other fragments to yield longer polypeptides. Combination of the conventional and solid phase approaches with retention of the best features of each is now possible. The procedure was tested by the synthesis of the crystalline tetrapeptide Z-Phe-Val-Ala-Leu-$NHNH_2$.

In solid phase peptide synthesis[3] the anchoring bond holding the peptide chain to the resin support has usually been a benzyl ester. The combination of this benzyl ester linkage with α-Boc[4] amino protection has allowed the convenient stepwise synthesis of a number of biologically active peptides by this method.[5] However, in order to release the desired peptide chain from the resin after completion of the synthesis treatment with strong acids such as HBr–TFA or anhydrous HF[6] is required and these reagents also remove most of the side chain protecting groups that are commonly used. It has, therefore, been difficult to obtain protected peptides that could be purified and then coupled to other peptide chanis by the fragment condensation method to yield larger polypeptides. The opportunity for isolation and purification of the small peptides at intermediary stages during the synthesis is an important advantage of the fragment approach. Efforts to combine the conventional and solid phase approaches, with retention of the best features of each, have been made by Anfinsen, *et al.*,[7] and by Weygand.[8] The former employed hydrazinolysis of the α-benzyl ester linkage to prepare peptide hydrazides while the latter made use of sulfhydryl-sensitive phenacyl esters to anchor the peptide to the resin. Though both of these methods are attractive, they are not without some undesirable complications and limitations and a more generally applicable method is desirable.

Recent progress in the development of acid labile urethan amino protecting groups in peptide chemistry[9] has enabled us to consider the application of *t*-alkyloxycarbonyl hydrazides as the anchoring bonds in solid phase peptide synthesis. In the following, the preparation of a new type of substituted styrene–divinylbenzene copolymer resin, $H_2NNHCOOC(CH_3)_2CH_2CH_2$–resin, is described.

Peptides can be synthesized stepwise on this type of polymer support by using the 2-(*p*-biphenyl)isopropyloxycarbonyl (Bpoc) group[10,11] for α-amino protection.

The Bpoc group can be removed at each cycle of the synthesis with very mild acid under conditions where the anchoring bond is stable. On the other hand the final peptide chain can be released as the hydrazide under more acidic conditions where side chain protecting groups remain unaffected, thus providing fragments suitable for further condensations. The feasibility of this approach was demonstrated by the synthesis of the protected tetrapeptide hydrazide, Z-Phe-Val-Ala-Leu-$NHNH_2$. The product was obtained in good yield in crystalline form with satisfactory amino acid and elementary analyses.

As indicated in Scheme I, a ketone functional group $CH_3COCH_2CH_2$– was introduced into copolystyrene–2% divinylbenzene resin beads (I) by a Friedel–Crafts reaction with methyl vinyl ketone[12] using HF as catalyst. The ketone-containing resin (II) showed an intense carbonyl absorption at 1725 cm^{-1} in the ir spectrum (see Figure 1). This functional group was then converted to a tertiary alcohol by a Grignard reaction. The absorption band at 1725 cm^{-1} disappeared completely at this stage. To prepare the *t*-alkyloxycarbonyl

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