Nmr Spectra of *cis*-1 and *trans*-1 with Eu(tfac)$_3$.—Solutions of *cis*-1 (18 mg) and *trans*-1 (26 mg), each in 0.35 ml of CCl_4 containing TMS, were prepared, and solutions of Eu(tfac)$_3$ (Willow Brook Laboratories) in CCl_4 were prepared such that 0.01 ml would contain 0.05 molar equiv of the shift reagent. Nmr spectra (100 MHz) of the esters were recorded after each addition of 0.01- or 0.02-ml aliquots of the shift reagent solution. The esters were recovered from the resulting solutions by column chromatography on silica gel, eluted with benzene. (The shift reagent could not be recovered by this procedure.)

3,3,4,4-Tetramethylcyclobutane-*trans*-1,2-dicarboxylic Acid (*trans*-4) from *cis*-1.—A mixture of *cis*-1 (16 mg, 0.07 mmol, recovered from the shifted nmr sample as described above) and 3 ml of a solution of 10% KOH in 80% EtOH was heated under reflux for 2 hr and diluted with H_2O. The basic solution was extracted with CH_2Cl_2 and the organic layer discarded, acidified with concentrated HCl, and extracted continuously with CH_2Cl_2 for 29 hr. The CH_2Cl_2 solution was evaporated to give 11 mg (79%) of crude *trans*-4, mp 210–211.5°, mmp with authentic *trans*-4 212–213°. A 2-mg sample of acid was esterified in MeOH solution with ethereal diazomethane to give only *trans*-1, identical with an authentic sample by glpc and ir.

Acknowledgment.—I wish to thank Mr. Don Schifferl for the mass spectra and for assistance with the 100-MHz nmr spectra.

Registry No.—*trans*-1, 42151-26-8; *cis*-1, 42151-27-9; 2, 42151-28-0; 3, 42151-29-1; *trans*-4, 42151-30-4; 5, 42151-31-5; dimethyl maleate, 624-48-6; 2,3-dimethyl-2-butene, 563-79-1; acetyl chloride, 75-36-5.

N-Acylation during the Addition of Carboxylic Acids to *N-tert*-Butylacylketenimines and the Use of the Reagent *N-tert*-Butyl-5-methylisoxazolium Perchlorate for Peptide Synthesis

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Diacylamide precursors of amide impurities have been detected in spectral tests of the addition of carboxylic acids to *N-tert*-butylacylketenimines. Variations in the product distribution with an inefficient acylketenimine in media of differing acidity suggest that diacylamide formation involves a second intermediate adduct that does not convert rapidly to the desired enol ester. A high free acid concentration results in interception of the intermediate to give acid anhydrides. Partial deuterium incorporation at the vinylic position of the enol esters indicates that intramolecular *O,O*-acyl migration is relatively slow for the adducts of *N-tert*-butylacylketenimines, and possible substituent influences are discussed. The preparation of β-acyloxy-*N-tert*-butylcrotonamide enol ester acylating agents from *N-tert*-butyl-5-methylisoxazolium perchlorate succeeds with unprotected hydroxyl groups and the carboxamide function of glutamine. However, amide dehydration was observed in the case of asparagine and competing azlactone formation was detected with benzoylleucine. Crystalline esters were not obtained with Z-Ala-OH, Z-Tyr-OH, and Z-Met-OH. Test couplings have established compatibility of the enol esters with unprotected hydroxyl groups in the amine component but results are not markedly improved relative to *N*-ethyl-5-phenylisoxazolium 3′-sulfonate. A new side reaction, condensation of the amine component with the coupling by-product, is shown to be a likely source of impurities in the use of the esters of hindered carboxylic acids. The original zwitterionic isoxazolium salt reagent is much less susceptible to the side reaction.

Since the discovery of the facile conversion of carboxylic acids to enol ester acylating agents upon reaction with 3-unsubstituted isoxazolium salts,[1] there have been continuing attempts to obtain isoxazolium cations with superior properties for application as reagents in peptide synthesis. Following the development of the zwitterion *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (NEPIS),[2] structural modifications have centered on the substituent on nitrogen,[3,4] benzisoxazolium cations,[5,6] and other ring-fused isoxazolium salts.[7] The issues of concern underlying these efforts have been the avoidance of rearrangement of the enol esters to diacylamides, the efficiency of enol ester formation, and the prevention of racemization *via* azlactones during the formation and reactions of enol esters of N-protected peptide acids. In contrast to the *N*-aryl heterocycles,[3] the *N-tert*-butyl compounds (**1**) were found to stabilize the enol esters relative to rearrangement.[4] However, examination of the reagent *N-tert*-butyl-5-methylisoxazolium (**1a**) perchlorate revealed *N-tert*-butylamides (**7**) as a side reaction product.[4] Our further examination of the *N-tert*-butyl system has led to the partial elucidation of the side reaction, the discovery of new complications in the reaction of the *N-tert*-butylacylketenimine intermediates (**2**) with carboxylic acids, and a definition of the limits of synthetic utility for **1a**.

A likely explanation of the side reaction observed in the *N-tert*-butyl series would involve *N-tert*-butyldiacylamides (**6**) as precursors of the amides **7**. Since the diacylamides were not themselves detected in the previous study, they would have to be relatively labile compounds. Consistent with this possibility, an attempt to force thermal rearrangement of the enol ester **4a** (R_3 = $ZNHCH_2$) of carbobenzoxyglycine to the corresponding diacylamide **6a** gave only the decomposition fragments amide **7** and *N-tert*-butylacetoacetamide (**5a**).[8] Spectral data in support of the proposed

(1) R. B. Woodward and R. A. Olofson, *J. Amer. Chem. Soc.*, **83**, 1007 (1961); *Tetrahedron, Suppl.*, **7**, 415 (1966).

(2) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Amer. Chem. Soc.*, **83**, 1010 (1961); *Tetrahedron Suppl.*, **No. 8**, 321 (1966).

(3) R. B. Woodward, D. J. Woodman, and Y. Kobayashi, *J. Org. Chem.*, **32**, 388 (1967).

(4) D. J. Woodman and A. I. Davidson, *J. Org. Chem.*, **35**, 83 (1970).

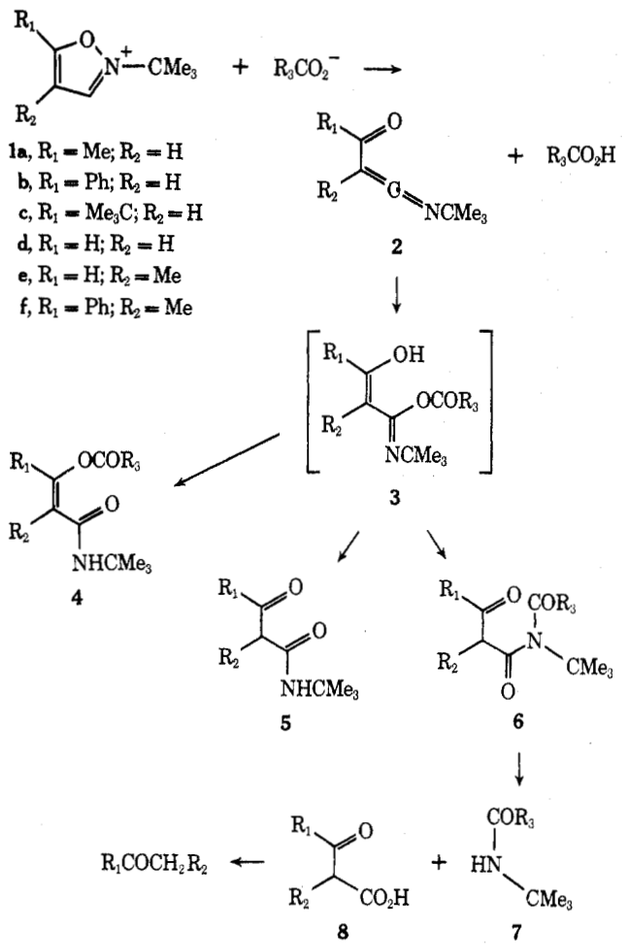
(5) D. S. Kemp, Ph.D. Thesis, Harvard University, Cambridge, Mass., 1964.

(6) D. S. Kemp and S. W. Chien, *J. Amer. Chem. Soc.*, **89**, 2743 (1967).

(7) R. A. Olofson and Y. L. Marino, *Tetrahedron*, **26**, 1779 (1970).

(8) Other workers[9] have reported that the enol ester of 3,5-diamino-6-chloropyrazine-2-carboxylic acid similarly gives the *N-tert*-butylamide on treatment with triethylamine, but a compound assigned the diacylamide structure was obtained with methoxide in polar media. If the latter structure is correct, it remains unclear why fragmentation took place only in the former medium and what special factors account for imide stability in the latter experiment.

(9) K. L. Shepard, W. Halczenko, and A. J. Cragoe, Jr., *Tetrahedron Lett.*, 4757 (1969).



decomposition of the *N-tert*-butyldiacylamides was obtained from the addition of formic acid to the isolated intermediate acylketenimine **2a**. Combination of the reactants resulted in considerable effervescence, as in the dehydration of formic acid to carbon monoxide upon reaction with carbodiimides.[10] In addition to appreciable (52%) dehydration product **5a**, the nmr spectrum of the product mixture revealed signals for the formate enol ester **4a** (R_3 = H, 34%) and the HCO, Me_3C, and $COCH_2CO$ protons of the *N*-formyl diacylamide **6a** (12%). The formate ester **4a** proved unstable, decomposing completely within a few hours to give additional keto amide **5a** and diacylamide **6a** in roughly equal amounts. On longer standing, the peaks attributed to the diacylamide also disappeared, as corresponding amounts of acetone and the amide **7** were observed to form. On the basis of these spectral assignments, fragmentation to amide **7** appears the dominant mode of diacylamide decomposition, and the lifetime of diacylamide in this instance may reflect greater stability associated with the less crowded formyl derivatives.[11]

In contrast to the above reaction with formic acid, in the previous study it was found that enol esters of other acids did not rearrange to diacylamides or decompose to give the amide impurities under the reaction conditions.[4] In those cases, it would be necessary that the unstable diacylamides arise directly from the initial adduct **3**[12] *via* an iminoanhydride rearrangement[13] in competition with conversion to enol ester **4**. Proof of the formation of **6** by such a pathway was achieved using an acylketenimine (**2f**) that was found to give an adduct especially prone to side reactions.

A survey of the outcome of the addition of carbobenzoxyglycine to a variety of acylketenimines showed that small amounts of amide **7** (R_3 = $ZNHCH_2$) resulted with the *N-tert*-butylcumulenes **2a–c** from different 5-substituted isoxazolium salts **1a–c**. The quantities of amide from **2d** and **2e** were below the limits of nmr detection. While the lesser severity of the side reaction could be an indication that these latter formylketenimines might provide a more efficient route to enol esters, the formyl substituted structures would have little synthetic utility owing to the greater tendency of the coproducts **5d** and **5e** to condense with the amine components of coupling reactions (discussed below). Extension of the survey to the disubstituted ketenimine **2f** led to a surprising result, the formation of the anhydride of carbobenzoxyglycine. Up to now the detection of intramolecular side reactions has provided the only experimental support for the existence of the postulated high-energy intermediate **3** in the reaction leading to enol esters,[4,5,14] and the present finding of interception by unconsumed carboxylic acid constitutes the first case of intermolecular trapping of **3**. In addition, a large amount (32%) of amide **7** was produced when the acid was added slowly to **2f** to keep anhydride formation to a minimum.

Further examination of the ineffective ketenimine **2f** with formic acid again provided an observable diacylamide. When **2f** was added to excess formic acid, dehydration once more was encountered, leading exclusively to keto amide **5f**. With the reverse order of addition of formic acid to excess **2f**, the product mixture nmr spectrum was in accord with a 3:1 ratio of diacylamide **6f** (R_3 = H) and enol ester **4f**, along with some keto amide **5f**.[15] In this case, the diacylamide proved sufficiently stable for partial purification and confirmation of the nmr peak assignments. The infrared spectrum also revealed no NH or OH absorption, in accord with the diacylamide structure **6f**. Control experiments with mixtures of different proportions of diacylamide **6f** and ester **4f** in the presence of acid or base showed no rapid interconversion. Therefore, this diacylamide cannot have been formed by rearrangement of enol ester and must have arisen directly from an intermediate. The two detectable formyl imides **6a** and **6f** thus provide confirmation of the postulated origin and decomposition of *N-tert*-butyldiacylamides proposed as the source of amide impurities.

Additional evidence for diacylamide intervention in a more representative case was obtained from the study of the reaction of ketenimine **2f** with acetic acid. Addition of **2f** to excess acid again gave keto amide **5f** (about 50%) together with an equivalent amount of acetic anhydride. The nmr spectrum was consistent

(10) I. Muramatsu, M. Itoi, M. Tsuji, and A. Hagitani, *Bull. Chem. Soc. Jap.*, **37**, 756 (1964).

(11) In the series of salicylamide esters from the *N*-ethylbenzisoxazolium cation, the lesser steric requirements of the formyl group permits *O,N*-acyl migration.[5]

(12) The adduct is represented for simplicity as **3**, the form presumed[1] to be produced initially.

(13) D. Y. Curtin and L. L. Miller, *J. Amer. Chem. Soc.*, **89**, 637 (1967).

(14) R. B. Woodward and D. J. Woodman, *J. Org. Chem.*, **34**, 2742 (1969).

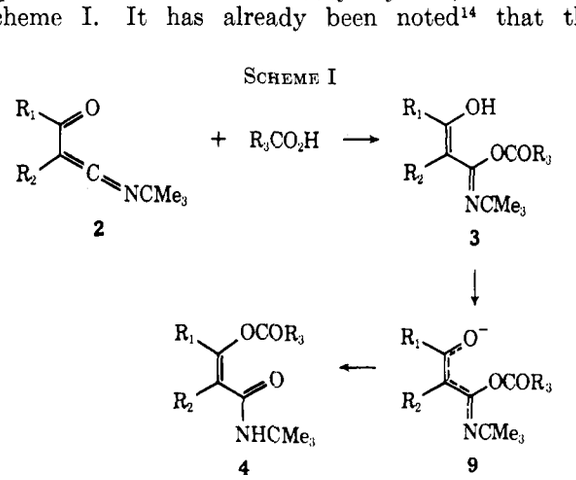
(15) The dependence of the outcome on the order of addition suggests that dehydration of formic acid involves the anhydride, following interception of the intermediate **3f** by formic acid.

with about a 2:1 ratio of the noninterception products, ester **4f** (R_3 = Me) and amide **7**, and the second diacylamide cleavage product **8f** was isolated from the reaction mixture.[16] Moreover, a transient *N-tert*-butyl peak was detected during nmr monitoring of the reaction. The complexity of the spectrum prevented a complete structural assignment on the basis of the nmr data, but the main change as the transient signal faded was the development of the amide *N-tert*-butyl peak, as would be expected for the diacylamide **6f**.

The occurrence of diacylamide formation with *N-tert*-butylacylketenimines presents the ironic result that the same structural factor which suffices to block *O,N*-acyl migration for most enol esters results in N-acylation as a side reaction of an earlier intermediate. Decomposition of an intermediate by N-acylation is only one example of the general situation that enol ester formation is less rapid relative to alternative reactions of the adducts from acylketenimines bearing the *N-tert*-butyl group. Previously it was found that azlactone formation was especially severe in the reaction of **2a** and hippuric acid.[14] The present results with formic acid and **2a** are consistent with a further new intermolecular reaction to give the acid anhydride. With the disubstituted ketenimine **2f**, both anhydride formation and increased N-acylation are clearly established.

The increased extent of side reactions in the *N-tert*-butyl series at least in part may be rationalized on the basis of the slow rate of the addition step and the accessibility of the transition state for rearrangement to enol esters. In the proposed[1] mechanism for the reaction of isoxazolium salts with carboxylate anions, it was argued that the most favorable direction of addition to the acylketenimines would give an adduct **3**[12] with the proper geometry for *O,O*-acyl migration. The simplest interpretation of a later finding,[5,14] that the intramolecular side reaction with peptide acids leading to azlactones was diminished by bases, would be favored conversion of the intermediate *via* its anion **9** to enol ester **4**. Accepting both these reasonable speculations, the most rapid pathway to enol ester, represented for the *N-tert*-butyl system, reduces to Scheme I. It has already been noted[14] that the

SCHEME I



relatively slow addition of carboxylic acids to the *N-tert*-butylcumulenes **2** gives rise to a medium effect. The intermediates from **2** undergo decomposition in the presence of a relatively high (drifting) concentration of unconsumed free acid, so that the environment may be less favorable for rearrangement to enol ester *via* the anion. In addition, the stability of the anion **9** relative to its conjugate acids would be reduced by the *N-tert*-butyl group. Both factors would tend to increase the importance of side reactions of **3** or its tautomers. These arguments could be extended to account for the still greater severity of side reactions with the disubstituted ketenimine **2f**, although an additional factor in that case might be the introduction of an unfavorable steric interaction in the anion **9**.

The simple picture of increased side reactions involving the conjugate acids of **9** is supported by partial isotope incorporation at the vinylic position of the enol ester **4a** (R_3 = $ZNHCH_2$) prepared in media containing exchangeable deuterium. In the original study of the reactions of isoxazolium salts,[1] the absence of such exchange established that the rearrangement was more rapid than equilibration with the CH tautomer of the initial adduct. The opposite result here shows that the *N-tert*-butyl substituent of **2a** does permit more favorable access to at least the CH tautomer prior to enol ester formation. The substituent influences cited above, then, probably are of general validity, but further scrutiny of the N-acylation side reaction has established that the foregoing analysis does not fully account for the behavior of the *N-tert*-butyl intermediates.

The extent of side reactions proceeding *via* the conjugate acids of **9**, according to Scheme I, should be subject to control by adjusting the acidity of the medium, as is dramatically true for azlactone formation.[14] However, testing this approach in the reaction of acetic acid with the ineffective ketenimine **2f** resulted in only a minor increase in the yield of enol ester when either excess ketenimine or even excess **2f** and 1 equiv of pyridine was employed.[17] At the same time the product distributions (Table I) for various conditions

TABLE I

Stoichiometry	Ester **4f**, %	Amide **7**, %	Keto amide **5f**, %
1.0 **2f** + 3.0 HOAc	35	9	56
1.0 **2f** + 2.0 HOAc	37	14	49
2.0 **2f** + 1.0 HOAc	46	40	14
2.0 **2f** + 1.0 HOAc + 1.0 pyridine	48	45	7

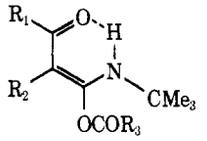
reveal a striking reversal of the proportions of amide **7** and keto amide **5f**. Although the magnitude of the side reactions is enhanced with the disubstituted ketenimine **2f**, the susceptibility of the N-acylation reaction to diversion to keto amide is not unique to this system. Previously a selective channeling of the less severe side reaction with cumulene **2f** was inferred from the increase of keto amide at the expense of amide as water was added to the reaction mixture.[4]

We consider the most likely explanation of these results to be that both by-products **3** and **7** are formed from a common intermediate that does not readily convert to enol ester **4**. The new results with **2f** make it unlikely that the intermediate in question is the

(16) While the involvement of adventitious moisture could lead to **8** directly, the spectral data show that the second fragment of diacylamide cleavage is the mixed anhydride of **8f** and acetic acid which undergoes hydrolysis during the work-up procedure.

(17) With **2a** and hippuric acid, the use of 1 equiv of pyridine results in a twofold decrease in the ratio of azlactone to enol ester.[14]

diacylamide **4** itself, since the observed transient species postulated to be diacylamide is not diverted to **5f** and anhydride in the presence of excess carboxylic acid. An attractive candidate instead would be an adduct **10** with the wrong geometry for *O,O*-acyl



10

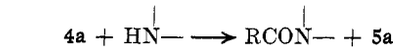
migration. The results of Table I would reflect a competition for **10** between N-acylation to give diacylamide and interception to give anhydride that shows a dependence on free acid concentration similar to the situation in the addition of carboxylic acids to carbodiimides.[18] Possible routes to **10** would include isomerization of a common initial adduct (with the same acidity dependence as for rearrangement to enol ester) or direct formation by a different mode of addition to the acylketenimine. The latter possibility would permit an interpretation of the especially unfavorable outcome with the disubstituted cumulene **2f** in terms of the rotamer of the acylketenimine undergoing addition.[19] Still, a general explanation for the unfavorable influence of the *N-tert*-butyl group on N-acylation remains obscure. Moreover, whatever factors are actually responsible for the side reaction, they can be easily overridden, as shown by the clean formation of the enol ester **4f** (R_3 = Me_3C) from the disubstituted ketenimine **2f** and pivalic acid.

Regardless of the reasons for N-acylation in the *N-tert*-butyl series, the possibility of diverting the side reaction was utilized earlier to obtain improved yields in the formation of enol esters with the reagent *N-tert*-butyl-5-methylisoxazolium (**1a**) perchlorate. In our further study, we have examined the utility of **1a** in comparison with the original isoxazolium peptide reagent NEPIS.

For the preparation of enol esters (activation step), **1a** offers the special feature of an isolable intermediate acylketenimine (**2a**)[20] of potential value in cases where careful monitoring of the progress of addition to the cumulene or the avoidance of competitive ketenimine decomposition may be important. However, the previous studies have shown that utilization of the precursor isoxazolium salt itself for making enol esters does not detract from the results, in the general synthetic recipe developed to obtain maximum yields.[4] Tests with a variety of carboxylic acids and N-protected amino acids to date have shown that difficulty in product crystallization is sometimes a problem and, in three instances (Z-Ala-OH, Z-Tyr-OH, and Z-Met-OH), solid products have not been obtained. An additional practical concern was that the lower efficiency of enol ester formation with *N-tert*-butylcumulenes might result in side reactions with polyfunctional amino acids. Examination of this point has revealed that unprotected hydroxyl groups (Z-Tyr-OH and Z-Ser-OH) and the amide function of glutamine (Z-Gln-OH) do not lead to any complications. However, with the more sensitive acid asparagine (Z-Asn-OH), partial dehydration of the amide function was observed, in contrast to the successful use of this acid in couplings with NEPIS.[1] Similarly, it appears that application of **1a** to the preparation of esters of N-protected peptide acids is hazardous, despite the definition of special conditions (2-picoline solvent) that eliminated all traces of azlactone formation with hippuric acid.[14] Using the 2-picoline procedure with Young's test acid *N*-benzoylleucine as a representative sensitive case, short-wavelength azlactone absorption was detected in the infrared spectrum of the reaction mixture.

The general applicability of the β-acyloxy-*N-tert*-butylcrotonamide enol esters **4a** as acylating agents was shown previously by the quantitative conversion of the ester of Z-Gly-OH to the benzylamide.[5] The apparent second-order rate constant for the reaction with 1 equiv of benzylamine is approximately tenfold larger than that found[5] for the corresponding stable salicylamide ester from the *N*-ethylbenzisoxazolium cation under comparable conditions. Subsequent rate comparisons have shown the latter salicylamide esters to be on the order of 100-fold less reactive than the *p*-nitrophenyl esters.[6] The indirect rate comparison indicates that the enol esters **4a** themselves are relatively placid acylating agents. This modest level of reactivity may be useful where selectivity is desired, but could be an undesirable feature in couplings of hindered carboxyl components, where competing side reactions of the amine component could become important.

As a probe of the extent of amine component decomposition, the reaction of the unhindered ester of Z-Gly-OH with the dipeptide ester H-Gly_2-OEt, which is susceptible to diketopiperazine formation, was considered. Combination of equivalents of the reactants in MeCN (~0.2 *M* in each component), evaporation after 20 hr, and by-product **5a** removal by stirring with



water left 90% of Z-Gly_3-OEt of undepressed melting point—identical with the result for the same coupling mediated by NEPIS under similar conditions. Next, the compatibility of the esters with unprotected hydroxyl groups in the amine component was demonstrated by the similar (in EtOAc) preparation of pure Z-Gly-Ser-OMe in 80% yield. Finally, for comparison purposes, two couplings (to form Phth-Gly_2-OEt and Z-Gln-Tyr-OMe) were performed which had given relatively low yields (88 and 75%, respectively) with NEPIS.[1] The use of the purified esters **4a** did result in some yield improvement (92 and 82%, respectively) under comparable conditions, but with lower product purities as reflected in melting point depressions of 2–3°.

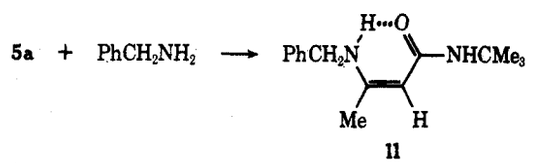
It is significant that couplings with the purified intermediate acylating agents do not provide marked improvement in contrast to the results for sequential activation and coupling with no intervening purification using NEPIS. The similarity of the outcome indicates that the extent of activation side reactions and enol ester decomposition are indeed small in the latter two-step procedure. The direct isolation of purer products with NEPIS follows from the efficiency of aqueous removal of by-products and side reaction products bearing the ionic sulfonate function. In con-

(18) D. F. Detar and R. Silverstein, *J. Amer. Chem. Soc.*, **88**, 1020 (1966).
(19) Olofson and Marino have presented arguments concerning the beneficial effects of the s-cis fused geometry on the outcome of the reaction.[7]
(20) R. B. Woodward and D. J. Woodman, *J. Amer. Chem. Soc.*, **88**, 3169 (1966).

trast, although the by-product **5a** is freely water soluble, traces of side reaction products in coupling with the esters **4a** may not be removed in the aqueous work-up.

Over-all, then, the main merit of the new reagent **1a** would be in cases where isolation of the intermediate enol esters is desirable for special reasons of convenience or for conducting couplings in solvents that do not readily bring NEPIS into solution. The isolable enol esters fail to provide significant improvement in the acylation of the amine component that would be desirable for stepwise peptide synthesis, and, given the lesser efficiency of enol ester formation with **1a**, overall yields for activation and coupling are lower than with NEPIS.

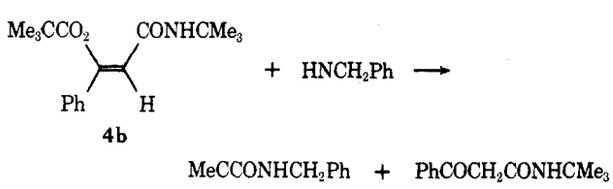
Scrutiny of possible side reactions in couplings with the enol esters also has brought to light a potential problem in applications to hindered acids. An obvious possibility would be condensation of amino acid esters with keto amide **5a**, as is well known for β-dicarbonyl compounds.[21,22] In the present case the enamine **11**



11

could be prepared in quantitative yield by evaporation of equivalents of **5a** and benzylamine in MeCN. As a measure of the severity of the side reaction, a bimolecular rate constant of approximately 5×10^{-3} l./mol min was estimated, which is roughly 3000 times smaller than for the rate of coupling with the enol ester of Z-Gly-OH. Even when coupling is 99% complete in this unhindered case, then, the desired reaction would be 30 times faster than the side reaction and only trace contamination results. At the opposite extreme, in the hindered case of pivalic acid, the reaction of the ester **4a** and benzylamine gave almost equimolar amounts of the amide and **11** after a 20-hr reaction time. On longer standing, drift toward the amide was observed as expected for the reversible condensation. Clearly, couplings with esters of hindered acids and nonselective, unhindered amine components should be avoided on account of the side reaction.

Finally, it should be stressed that the enamine side reaction is not a problem with the original isoxazolium salt reagent NEPIS. The ester **4b** (R_3 = Me_3C) was prepared as a stable model for hindered acylating agents from NEPIS. Only traces of the corresponding enamine could be detected in the coupling reaction with benzylamine. As a further test, under conditions where **5a** was 80% converted to **11**, the less reactive benzoylketo amide **5b** formed only 15% of the con-



4b **5b**

densation product in a much slower reaction. Thus, there is little likelihood that appreciable amounts of condensation product would result in the use of NEPIS and any traces of enamine would be effectively removed in the customary aqueous isolation.

Experimental Section[23]

Thermal Decomposition of the Ester 4a of Carbobenzoxyglycine.—The ester[4] was heated in a vacuum (<0.1 mm) sublimation assembly having an ice water cold finger condenser. Above the melting point (89°) of the enol ester, condensate began to form, and heating was discontinued when bumping became severe (bath temperature 110°). The nmr spectrum of the condensate was consistent with a mixture of the known[4] compounds *N-tert*-butylacetoacetamide (**5a**) and carbobenzoxyglycine *N-tert*-butylamide (**7**, R_3 = $ZNHCH_2$).

Reaction of Acetyl-*N-tert*-butylketenimine (2a) and Formic Acid.—Formic acid (0.036 ml, 0.95 mmol) was added to **2a** (0.132 g, 0.95 mmol)[19] in 0.5 ml of $CDCl_3$ over a 6-min period. Considerable effervescence (presumably CO) was observed and the nmr spectrum of the solution revealed the characteristic signals of *N-tert*-butylacetoacetamide (**5a**) at δ 3.42 (s, CH_2), 2.22 (s, Me), and 1.32 (s, CMe_3), along with peaks expected for the enol ester **4a** (R_3 = H) at δ 8.15 (s, HCO, superimposed on signal for any remaining HCO_2H), 5.60 (br s, HC=C), 2.00 (m, MeC=C), and 1.32 (CMe_3, shown by integration to be superimposed on CMe_3 signal of **5a**). In addition there were peaks at δ 8.85 (s, HCO), 3.92 (s, CH_2), 2.22 (Me, shown by integration to be superimposed on Me signal of **5a**) and 1.57 (s, CMe_3), consistent with the diacylamide **6a** (R_3 = H). The integration was consistent with approximately 52 mol % **5a**, 34 mol % **4a**, and 12% **6a**. During a few hours, the peak attributed to **4a** disappeared while the ratio of **6a** (33%) to **5a** (65%) increased.

Attempts to separate **6a** from **5a** by trituration and/or distillation were unsuccessful.

Over a 10-day period, the nmr signals of the diacylamide **6a** disappeared completely as the spectrum showed a corresponding buildup of peaks for acetone and *N-tert*-butylformamide. The acetone was also identified by ir and isolation, after distillation from the reaction mixture, as the 2,4-DNP derivative.

Tests of Addition of Carbobenzoxyglycine to Acylketenimines 2a–e.—In all cases, 1 equiv of carbobenzoxyglycine was added at 0° to a stirred 2 *M* solution of the acylketenimine in $CDCl_3$. The reactions were monitored by nmr until **2** was consumed. The benzylic doublet, at slightly higher field (δ 3.68) than the corresponding signals for the enol esters **4** and carbobenzoxyglycine, was used to assay for **7** (R_3 = $ZNHCH_2$). Small signals amounting to a few per cent were detected along with the expected enol ester signals in the additions with *N-tert*-butylacetylketenimine (**2a**), *N-tert*-butylbenzoylketenimine (**2b**),[24] and *N-tert*-butylpivalylketenimine (**2c**),[24] but not with *N-tert*-butylformylketenimine (**2d**)[24] nor *N-tert*-butylformylmethylketenimine (**2e**).[24] Additional data are reported below for the new enol esters **4b** and **4e** which were isolated.

***N-tert*-Butyl-β-Carbobenzoxyglycyloxycinnamamide (4b, R_3 = $ZNHCH_2$).**—Evaporation of the reaction mixture above (from **2b**), dissolving the residue in CH_2Cl_2, and washing the solution with 5% $NaHCO_3$ then aqueous NaCl and drying (Na_2SO_4) left the crude ester. Recrystallization from benzene–petroleum ether (bp 30–60°) and then from EtOAc–heptane gave **4b** (R_3 = $ZNHCH_2$) as colorless needles: mp 139.5–140°; ir (CH_2Cl_2) 5.62 μ; nmr ($CDCl_3$) δ 7.28 (m, 10), 6.05 (s, 1), 5.83 (br, 2), 5.05 (s, 2), 4.23 (d, 2, J = 6 Hz), and 1.24 (s, 9).

Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.29; H, 6.40; N, 6.83. Found: C, 67.14; H, 6.38; N, 6.82.

***N-tert*-Butyl-β-Carbobenzoxyglycyloxymethacrylamide (4e, R_3 = $ZNHCH_2$).**—After the same work-up as above of the reaction mixture from **2e**, trituration of the residue with petroleum

(21) E. Dane, F. Drees, P. Konad, and T. Dockner, *Angew. Chem., Int. Ed. Engl.*, **1**, 658 (1962).

(22) B. Halpern and L. B. Jones, *Nature (London)*, **202**, 592 (1964).

(23) Melting points were determined with a Mel-Temp capillary apparatus and are uncorrected. The nmr spectra were run on a Varian A-60 spectrometer, and chemical shifts are reported in τ values relative to tetramethylsilane as an internal standard. The ir spectra were recorded on a Perkin-Elmer 137 spectrophotometer and the rotations were measured with a thermostated Perkin-Elmer 141 automatic polarimeter. Elemental analyses were performed by Alfred Bernhardt Mikroanalytisches Laboratorium, West Germany.

(24) D. J. Woodman and Z. L. Murphy, *J. Org. Chem.*, **34**, 3451 (1969).

ether left an 82% yield of **4e** (R_3 = $ZNHCH_2$), mp 87.6–88°. Recrystallization from CCl_4–heptane gave material of mp 88.5–89°; ir (CH_2Cl_2) 5.62 μ; nmr ($CDCl_3$) δ 7.29 (m, 6), 6.28 (br, 1), 5.82 (br, 1), 5.10 (s, 2), 4.03 (d, 2, J = 6 Hz), 1.81 (d, 3, J = 1 Hz), 1.33 (s, 9).

Anal. Calcd for $C_{18}H_{24}N_2O_6$: C, 62.04; H, 6.96; N, 8.04. Found: C, 62.24; H, 7.06; N, 8.20.

Reaction of Carbobenzoxyglycine with *N-tert*-Butylbenzoylmethylketenimine (2f).—Repetition of the nmr assay with **2f**[24] resulted in the formation of a precipitate, while the spectrum of the supernatant showed the presence of remaining **2f**. After evaporation of the $CDCl_3$, the ir spectrum of the residue contained shoulders at 5.47 and 5.68 μ for the anhydride of carbobenzoxyglycine. Approximately 13% of **7** (R_3 = $ZNHCH_2$) was also detectable by nmr. Evaporation of the solvent and addition of petroleum ether to an EtOAc solution of the residue resulted in the precipitation of close to 20% of the crude anhydride: mp 111–115° (mp 124–125° after two further recrystallizations) (lit mp 115–116°,[25] 118–119,[26] 120.5–124°[27]); ir (MeCN), 5.45 and 5.70 μ (lit.[21] 5.44 and 5.70 μ); nmr (MeCN) δ 7.33 (s, 5), 6.00 (br, 1), 5.00 (s, 2), and 4.00 (d, 2, J = 6 Hz). As reported by other workers, addition of Et_3N to an MeCN solution led to the disappearance[21] in the ir spectrum of the 5.47-μ peak and the development of new absorptions at 5.80, 5.88, 6.05, 6.25, and 8.35 μ.

When the experiment was repeated with slow addition (45 min) of the acid to **2f**, no precipitate was observed and a larger proportion (32%) of **7** was detected by nmr.

Reaction of Formic Acid and 2f.—The usual test reaction procedure with addition of **2f** over a 15-min period to excess formic acid resulted in considerable effervescence (presumably CO) and the product nmr spectrum contained only signals for keto amide. The nmr spectrum of the product mixture was consistent with unreacted **2f**, 38% of keto amide **5f** [δ 1.28 (CMe_3), 4.25 (q, CHMe, **CH**Me masked)], 15% of enol ester **4f** (R_3 = H) [δ 1.38 ($NCMe_3$), 1.98 (MeC=, HCO masked)], and 47% of diacylamide **6f** (R_3 = H) [δ 1.50 ($NCMe_3$), 1.40 (CH**Me**), 5.37 (**CH**Me), 8.8 (HCO)].

There was no change in composition of the above reaction mixture within 24 hr.

In a modified experiment, formic acid was added in portions to **2f** until the cumulene had all been consumed. Evaporation and repeated fractional crystallization of the residue from CCl_4–heptane provided crystalline **6f** (R_3 = H) (approximately 10 mol % impurity by nmr): mp 70.5–71°; ir (CCl_4) broad imide band centered at 5.92 μ; nmr ($CDCl_3$) δ 8.83 (s, 1, HCO), 8.20–7.43 (m, 5, ArH), 5.40 (q, 1, J = 7 Hz, **CH**Me), 1.57 (s, 9, CMe_3), and 1.44 (d, 3, J = 7 Hz, CH**Me**).

Diacylamide (**6f**) rich and 1:1 diacylamide–enol ester (**4f**) mixtures from the above fractional crystallization were each allowed to stand in $CDCl_3$ solution in the presence of added base (Et_3N) and acid (HCO_2H), but no changes in composition were observed by nmr within 24 hr.

2-Benzoyl-*N-tert*-butylpropionamide (5f).—An authentic sample of keto amide **5f** was prepared by ring opening 0.316 g (1.0 mmol) of *N-tert*-butyl-4-methyl-5-phenylisoxazolium (**2f**) perchlorate with 0.14 ml (1.0 mmol) of Et_3N in 10 ml of MeCN and adding excess water to the resulting solution. After 12 hr, the solvent was evaporated and the residue was stirred for 24 hr in 12 ml of water. Digestion of the precipitate on the steam bath and filtration gave 0.213 g (91%) of colorless crystals, mp 141.5–145° (some prior softening). Recrystallization from EtOH–water gave **5f**: mp 144–145.5°; nmr ($CDCl_3$) δ 8.23–7.30 (m, 5), 6.30 (br, 1), 4.28 (q, 1, J = 7 Hz), 1.52 (d, 3, J = 7 Hz), and 1.32 (s, 9).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.06; H, 8.22; N, 6.00. Found: C, 71.96; H, 8.19; N, 5.98.

Reaction of Benzoyl-*N-tert*-butylmethylketenimine (2f) and Acetic Acid.—The usual test procedure with addition of **2f** over a 10-min period to 3 equiv of HOAc gave a solution that contained strong anhydride absorption at 5.45 μ in the ir spectrum. The nmr spectrum, in addition to the peak at δ 2.05 for excess HOAc, showed signals at δ 1.29 (s, CMe_3), 1.48 (d, J = 7 Hz, CH**Me**), and 4.30 (q, J = 7 Hz, **CH**Me) for the keto amide **5f** (56%) along with a corresponding strong peak at δ 2.18 for Ac_2O. Signals for the enol ester **4f** (R_3 = Me, 35%) were evident at δ 1.39 (s, CMe_3), 1.96 (s, MeC=C), and 2.14 (s, $MeCO_2C$). The amide **7** (R_3 = Me, 9%) was tentatively identified by its *tert*-butyl peak at δ 1.32 (s, CMe_3, **Me**CONH singlet at δ 1.87 apparent as a shoulder), and a low-field acetyl singlet at δ 2.38 was consistent with a corresponding amount of the mixed anhydride of **8f** and acetic acid. The assigned chemical shifts agreed with the nmr spectrum of an authentic solution of acetic anhydride, acetic acid, keto amide, and amide. As expected, reduced pressure evaporation of the reaction mixture removed the Ac_2O and HOAc. Further evaporation at 0.06 mm led to the partial removal of the peaks assigned to amide, and the nmr of the cold-trap condensate was the same as that of an authentic sample of amide.

Detection of an Intermediate in the Addition of Acetic Acid to 2f and Isolation of 2-Benzoylpropanoic Acid (8f).—Upon repetition of the above experiment with 2 equiv of HOAc, immediate nmr assay revealed a new *tert*-butyl peak at δ 1.15 and a methyl singlet at δ 1.88 consistent with the diacylamide **6f** (R_3 = Me) present in an amount between 1/3 and 1/2 that of the enol ester **4f** (R_3 = Me). At the outset, only a small *tert*-butyl signal for amide **7** (R_3 = Me) was evident, but within 30 min the signals attributed to **6f** disappeared as peaks for **7** increased in intensity relative to those for ester and keto amide.[28] In the same period, the acetyl methyl singlet assigned the mixed anhydride developed. Integration of the product spectrum indicated the pressure of 49% keto amide, 37% enol ester, and 14% amide.

A reaction mixture of the above stoichiometry was worked up by dilution with $CHCl_3$ and extraction with $NaHCO_3$. Acidification of the bicarbonate extract, back-extraction into $CHCl_3$, drying (Na_2SO_4) and evaporation of the organic phase, and recrystallization of the residue from benzene–petroleum ether gave 2-benzoylpropanoic acid (**8f**): mp 77–78.5° (lit.[29] mp 82–83°); nmr ($CDCl_3$) δ 8.80 (s, 1), 8.21–7.28 (m, 5), 4.43 (q, 2, J = 7 Hz), 1.49 (d, 3, J = 7 Hz). The acid was decarboxylated by heating on the steam bath for 20 min and passed through a short column of neutral alumina with ether. The residue upon evaporation of the solvent was identified as propiophenone by nmr and ir.

Addition of Acetic Acid to Excess 2f.—The usual test procedure with addition of HOAc over a 10-min period to 2 equiv of **2f** resulted in qualitatively the same outcome as above. A larger transient signal (roughly equivalent to the enol ester *tert*-butyl peak) was observed on rapid monitoring, and the proportion of keto amide and amide (and the corresponding coproducts) in the final product changed to 14 and 40%, respectively, together with 46% of enol ester.

Repetition of the experiment with an added 1 equiv of pyridine gave 7% keto amide, 45% amide, and 48% enol ester.

Formation of Enol Ester 4a (R_3 = $ZNHCH_2$) in the Presence of D_2O.—To a solution of 0.213 g (1.0 mmol) of carbobenzoxyglycine and 0.18 ml (1.3 mmol) of Et_3N in 10 ml of MeCN containing 1.8 ml (90 mmol) of D_2O was added 0.305 g (1.3 mmol) of the perchlorate salt of **1a**. After evaporation of the solvent, stirring the residue with water and recrystallization from benzene–petroleum ether, the nmr spectrum of the product **4a** (R_3 = $ZNHCH_2$) was in accord with only 0.3–0.4 vinyl hydrogens at δ 5.4, in comparison with the integration for the methylene signal at δ 4.08. In a control experiment, the enol ester was subjected to the work-up conditions, and no diminution of the δ 5.4 signal was observed.

***N-tert*-Butyl-β-trimethylacetoxy-α-methylcinnamamide (4f, R_3 = CMe_3).**—The usual test procedure adding pivalic acid to excess **2f** gave, after 3 hr, a product mixture that had nmr signals for unreacted **2f**, the expected product **4f** (R_3 = CMe_3), and no by-products. Precipitation with petroleum ether gave colorless needles, mp 102–105.3°. Recrystallization from heptane gave material of mp 104.5–106.5°: uv max (MeCN) 244 nm (ϵ 12,600); ir (CH_2Cl_2) 5.78 μ; nmr ($CDCl_3$) δ 1.22 (s, 9), 1.32 (s, 9), 1.87 (s, 3), 5.93 (br, 1), 7.23 (s, 5).

Anal. Calcd for $C_{19}H_{27}NO_3$: C, 71.88; H, 8.59; N, 4.41. Found: C, 71.79; H, 8.39; 6, 4.51.

(25) D. F. DeTar, R. Silverstein, and J. Rodgers, Jr., *J. Amer. Chem Soc.*, **88**, 1024 (1966).

(26) M. Brenner, J. P. Zimmerman, J. Wehrmuller, P. Quitt, A. Hartmann W. Schneider, and J. Beglinger, *Helv. Chim. Acta*, **40**, 1497 (1957).

(27) J. Baddiley, J. G. Buchanan, R. Hodges, and J. F. Prescott, *J. Chem. Soc.*, 4769 (1957).

(28) A second, smaller signal at lower field was also observed to build in several cases where the disappearance of the transient was monitored, but the structure of the minor decomposition product has not been established.

(29) E. Hope and W. H. Perkin, Jr., *J. Chem. Soc.*, **95**, 2045 (1909).

Preparation of Enol Esters (4a).—The general procedure reported previously[4] was used for the acids below with the modifications noted.

tert-**Butyloxycarbonyl-L-leucine.**—The residue from the procedure with 2.07 g (8.0 mmol) of BOC-Leu-OH·H_2O (prepared according to the procedure of Schwyzer[30]), mp 78–82.5° (lit.[30] mp 78–81°), was taken up in 150 ml of EtOAc, and the solution was washed with aqueous NaCl and 5% aqueous $NaHCO_3$. After drying (Na_2SO_4), the solution was evaporated to a small volume and flooded with 80 ml of petroleum ether (bp 40–60°). Seeding, chilling overnight, filtration and drying gave 1.8 g (76%) of tan solid: mp 98–101° (mp 103–104° after recrystallization from CCl_4–heptane and EtOAc–petroleum ether); nmr ($CDCl_3$) δ 5.64 (br, 1), 5.42 (unresolved m, 1), 5.16 (unresolved m, 1), 4.41 (unresolved m, 1), 1.95 (unresolved m, 3), 1.70 (br, 3), 1.42 and 1.31 (both s, total 18), and 0.97 (d, 6).

Anal. Calcd for $C_{19}H_{34}N_2O_5$: C, 61.58; H, 9.27; N, 7.56. Found: C, 61.72; H, 9.21; N, 7.62.

Carbobenzoxy-L-serine.—The general procedure directly gave 87% of the ester: mp 77–81° (mp 81–83° after recrystallization from CCl_4 or C_6H_6 and petroleum ether); nmr ($CDCl_3$) δ 7.38 (s, 5), 5.86 (br, 2), 5.49 (unresolved m, 1), 5.18 (s, 2), 4.42 (br, 2), 3.89 (br, 1), 1.93 (s, 3), and 1.40 (s, 9); $[\alpha]^{19}D$ −92.3° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.29; H, 6.94; N, 7.40. Found: C, 60.16; H, 7.06; N, 7.28.

Carbobenzoxy-L-glutamine.—The general procedure (extraction work-up) followed by precipitation with $CHCl_3$–petroleum ether gave 73% of the ester: mp 96.5–98.5° after drying several days *in vacuo* (mp 103–105° after recrystallization from $CHCl_3$–petroleum ether); nmr (MeCN) δ 7.37 (s, 5), 5.53 (unresolved m, 1), 5.12 (s, 2), and 1.25 (s, 9); $[\alpha]^{24}D$ −42.2° (*c* 1, MeCN).

Anal. Calcd for $C_{21}H_{29}N_3O_6$: C, 59.97; H, 7.20; N, 9.99. Found: C, 59.81; H, 7.01; N, 9.87.

Carbobenzoxy-L-alanine, Carbobenzoxy-L-methionine, and Carbobenzoxy-L-tyrosine.—The general procedure gave oils showing the expected enol ester ir absorption near 5.65 μ but which resisted a variety of attempts at initiating crystallization.

Carbobenzoxy-L-asparagine.—To a solution of 0.266 g (1.0 mmol) of carbobenzoxy-L-asparagine and 0.14 ml (1.0 mmol) of Et_3N in 10 ml of MeCN was added 0.240 g (1.0 mmol) of *N-tert*-butylacetylketenimine After evaporation of the solvent, the residue was dissolved in EtOAc and washed with aqueous NaCl and 5% $NaHCO_3$. The nmr spectrum of the neutral residue showed keto amide to be a major constituent. Acidification of the $NaHCO_3$ phase, extraction with CH_2Cl_2, evaporation, and several recrystallizations of the residue gave 0.01 g of white powder, mp 125–126°, identical with authentic carbobenzoxy-β-cyanoalanine prepared by the method of Liberek.[31]

Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.05; H, 4.88; N, 11.29. Found: C, 57.95; H, 4.78; N, 11.09.

Benzoyl-L-leucine.—To 0.169 g (1.21 mmol) of *N-tert*-butylacetylketenimine in 6 ml of 2-picoline was added with stirring 0.285 g (1.21 mmol) of benzoyl-L-leucine (prepared by the method of Williams and Young)[32] along with 6 ml more of solvent. After 20 hr, the solvent was evaporated. The ir spectrum (MeCN) of the residue contained weak azlactone absorption at 5.52 μ as well as a strong enol ester peak at 5.69 μ.

In a duplicate experiment, isolation of the enol ester possessing optical activity established, at least, that complete racemization *via* azlactone does not result under these conditions. Treatment of the reaction residue with petroleum ether gave 0.217 g (48%) of tan solid: mp 115–118.5° (needles of mp 118–119° after two recrystallizations from $CHCl_3$–heptane); nmr ($CDCl_3$) δ 8.30–7.17 (m, 6), 6.15 (s, 1), 5.49 (s, 1), 4.93 (unresolved m, 1), 1.88 (unresolved m, 6), 1.33 (s, 9), and 1.0 (m, 6); $[\alpha]^{23.1}D$ −84.5° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{21}H_{30}N_2O_4$: C, 67.34; H, 8.09; N, 7.48. Found: C, 67.16; H, 7.87; N, 7.46.

Coupling Tests.—As a general procedure for the preparation of the compounds below, exact equivalents each of the appropriate enol ester, the amino acid ester hydrochloride, and Et_3N were combined in MeCN (~0.2 *M* in each reactant). The solution was stirred overnight or for 24 hr and evaporated under reduced pressure. The product was isolated by thorough stirring with hot water, filtration, and drying over P_2O_5 *in vacuo*.

(30) R. Schwyzer, P. Sieber, and H. Kappeler, *Helv. Chim. Acta*, **42**, 2622 (1959).

(31) B. Liberek, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, **10**, 407 (1962).

(32) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).

Carbobenzoxytriglycine Ethyl Ester.—The procedure gave 90% of the protected tripeptide, mp 168.5–169.5° with softening (mp 168–169° after recrystallization from EtOH).

Carbobenzoxyglycyl-L-serine Methyl Ester.—The residue from the general reaction procedure (EtOAc solvent) was partitioned between aqueous NaCl and EtOAc. The dried (Na_2SO_4) organic layer was evaporated and the residue was crystallized from CCl_4 at −20°. Recrystallization from benzene gave 80% of solid, mp 96–97°.

Phthaloyldiglycine Ethyl Ester.—The procedure gave 92% of white powder, mp 195–196.5° (fine needles, mp 197–199° after recrystallization from EtOH–H_2O).

Anal. Calcd for $C_{14}H_{14}N_2O_5$: C, 57.91; H, 4.87; N, 9.65. Found: C, 58.04; H, 4.84; N, 9.61.

Carbobenzoxy-L-glutaminyl-L-tyrosine Methyl Ester.—The procedure ($MeNO_2$ solvent) gave 82% of solid, mp 197–200° dec after recrystallization from DMF–H_2O.

Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.35; H, 5.96; N, 9.19. Found: C, 60.21; H, 5.87; N, 9.16.

β-Benzylamino-*N-tert*-butylcrotonamide (11).—A solution of 0.314 g (2.0 mmol) of *N-tert*-butylacetoacetamide (**5a**) and 0.22 ml (2.0 mmol) of $PhCH_2NH_2$ in 20 ml of MeCN showed carbonyl absorption for **5a** after standing overnight at room temperature. To force the reaction to completion, the solvent was evaporated under reduced pressure, leaving 0.49 g (100%) of white solid: mp 96–101° (mp 103–105° after recrystallization from CCl_4–petroleum ether); nmr ($CDCl_3$) τ 0.65 (br, 1), 2.72 (s, 5), 5.20 (br, 1), 5.65 (unresolved m, 3), 8.20 (s, 3), and 8.65 (s, 9).

Anal. Calcd for $C_{15}H_{22}N_2O$: C, 73.12; H, 9.01; N, 11.37. Found: C, 73.05; H, 8.75; N, 11.30.

Rate of Condensation of Benzylamine with *N-tert*-Butylacetoacetamide 5a.—A solution of 0.157 g (1.0 mmol) of **5a** and 0.107 g (1.0 mmol) of $PhCH_2NH_2$ in 10 ml of MeCN at ambient temperature (22°) was assayed by ir to estimate the concentration of **5a** from the corrected absorbance at 5.88 μ. A plot of 1/[**5a**] was linear over two half-lives, giving an apparent second-order rate constant of approximately 5×10^{-3} l./mol min.

Reaction of β-Pivaloxy-*N-tert*-butylcrotonamide[4] and Benzylamine.—A solution of 0.085 g (0.35 mmol) of the enol ester **4a** (R_3 = CMe_3) of pivalic acid[5] and 0.038 ml (0.35 mmol) of $PhCH_2NH_2$ in 0.5 ml of $CDCl_3$ was assayed by nmr. After 20 hr, the ratio of enamine **11** to desired amide was 3:4 with considerable unreacted enol ester remaining. On long standing, loss of **11** and enol ester accompanied by an increase in **5a** and amide (1:3 ratio after 3 weeks) was observed.

β-Pivaloxy-*N-tert*-butylcinnamamide (4b, R_3 = CMe_3).—To a solution of 0.21 ml (1.5 mmol) of Et_3N in 12 ml of MeCN was added 0.453 g (1.5 mmol) of *N-tert*-butyl-5-phenylisoxazolium perchlorate[33] with magnetic stirring. After addition of 1 equiv of pivalic acid, the solvent was immediately evaporated at room temperature, and the residual pale yellow oil was taken up in 30 ml of EtOAc and washed three times with 5 ml each of aqueous NaCl, 5% $NaHCO_3$, and aqueous NaCl. Evaporation of the dried (Na_2SO_4) organic solution and recrystallization of the residue from heptane gave 0.361 g (78%) of off-white crystals: mp 115–116.5° (colorless needles, mp 116–117° after recrystallization from CCl_4–heptane); nmr ($CDCl_3$) δ 7.47 (unresolved m, 5), 6.07 (s, 1), 5.88 (br, 1), and 1.42 (s, 18).

Anal. Calcd for $C_{18}H_{25}NO_3$: C, 71.28; H, 8.59; N, 4.41. Found: C, 71.79; H, 8.39; N, 4.51.

Spectral Tests of the Condensation Reaction with 5b.—After 48 hr, only trace peaks at δ 4.47 (s) and 4.18 (d, J = 6 Hz) assignable to the condensation product could be detected in the nmr spectrum of the product mixture from the reaction of 0.107 g (0.35 mmol) of **5b** (R_3 = CMe_3) and 0.038 ml (0.35 mmol) of $PhCH_2NH_2$ in 0.5 ml of $CDCl_3$.

After 15 hr, the nmr spectrum of a solution originally of 0.067 g (0.4 mmol) of **5b** and 0.044 ml (0.4 mmol) of $PhCH_2NH_2$ contained the above enamine signals corresponding to 15% conversion. In a comparison test with **5a** in place of **5b**, 80% transformation to **11** resulted in the same time.

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Registry No.—**1a** perchlorate, 10513-45-8; **1b**, 10514-54-2; **2a**, 10513-47-0; **2b**, 10513-46-9; **2e**, 42221-95-4; **2f**, 21555-07-7; **2f** perchlorate, 42221-97-6; **4a** (R_3 = H), 42221-98-7; **4a** (R_3 =

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ZNHCH$_2$), 19625-78-6; **4a** (R$_3$ = CMe$_3$), 21995-77-7; **4a** (R$_3$ = *tert*-butyloxycarbonyl-L-leucyl), 42222-15-1; **4a** (R$_3$ = carbobenzoxy-L-seryl), 42222-16-2; **4a** (R$_3$ = carbobenzoxy-L-glutamyl), 42222-17-3; **4a** (R$_3$ = benzoyl-L-leucyl), 42222-19-5; **4a** (R$_3$ = carbobenzoxyglycylglycyl), 42222-24-2; **4a** (R$_3$ = phthaloylglycyl), 21995-78-8; **4a** (R$_3$ = carbobenzoxy-L-glutaminyl), 42222-17-3; **4b** (R$_3$ = ZNHCH$_2$), 42222-01-5; **4b** (R$_3$ = CMe$_3$), 42222-02-6; **4e** (R$_3$ = ZNHCH$_2$), 42222-03-7; **4f** (R$_3$ = H), 42222-04-8; **4f** (R$_3$ = CMe$_3$), 42222-05-9; **4f** (R$_3$ = Me), 42222-14-0; **5a**, 42222-06-0; **5b**, 42222-07-1; **5f**, 42222-08-2; **6a** (R$_3$ = H), 42222-09-3; **6f** (R$_3$ = H), 42222-10-6; **7** (R$_3$ = ZNHCH$_2$), 21995-76-6; **8f**, 4767-01-5; **11**, 42222-13-9; formic acid, 64-18-6; acetic acid, 64-19-7; carbobenzoxy-β-cyanoalanine, 3309-41-9; carbobenzoxytriglycine ethyl ester, 2503-35-7; carbobenzoxyglycyl-L-serine methyl ester, 10239-27-7; phthaloyldiglycine ethyl ester, 2641-02-3; carbobenzoxy-L-glutaminyl-L-tyrosine methyl ester, 42222-23-1; benzylamine, 100-46-9; pivalic acid, 75-98-9; carbobenzoxyglycine, 1138-80-3; carbobenzoxy-L-asparagine, 2304-96-3; benzoyl-L-leucine, 1466-83-7; glycine ethyl ester hydrochloride, 623-33-6; L-serine methyl ester hydrochloride, 5680-80-8; L-tyrosine methyl ester hydrochloride, 3417-91-2.

Synthesis of 2-Nor-2-formylpyridoxal 5′-Phosphate, a Bifunctional Reagent Specific for the Cofactor Site in Proteins

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A facile and general procedure was developed for the preparation of vitamin B$_6$ amine oxides and their corresponding 2-carbinol rearrangement products. These compounds served as intermediates in the synthesis of the dicarboxaldehyde 2-nor-2-formylpyridoxal 5′-phosphate and of 2-nor-2-formyl-4-deoxypyridoxol 5′-phosphate, an isomer of pyridoxal 5′-phosphate. Both analogs were prepared for the purpose of exploring the spacial configuration of polypeptide chains at the pyridoxal 5′-phosphate site in glycogen phosphorylase (EC 2.4.1.1) and other enzymes.

The introduction of stable covalent bridges between amino acid residues has been recently utilized as a direct chemical tool to provide information about the spacial orientation of polypeptide chains in proteins.[1] It occurred to us that this approach, coupled with a systematic application of affinity labeling techniques, may reveal the architecture of important sites on enzymes and perhaps contribute to the elucidation of the catalytic mechanism. 2-Nor-2-formylpyridoxal 5′-phosphate (**9**) and 2-nor-2-formyl-4-deoxypyridoxol 5′-phosphate (**4**)[2] were synthesized as part of a project designed to probe the three-dimensional structure around the pyridoxal 5′-phosphate pocket in glycogen phosphorylase (EC 2.4.1.1)[3] and a number of other enzymes. Earlier investigations indicated that the absence of the 2-methyl group has little effect on the activity of the cofactor.[3,4] Consequently, an additional functionality was anchored at this position in the expectation that the enzyme will conserve a high degree of specificity for this analog and thus promote the formation of a bisazomethine cross-linkage at the PLP site.

The synthetic pathway, as depicted in Scheme I, is based on an improved two-step synthesis of 2-pyridine carbinol analogs serving as intermediates for the final products described herein. When *m*-chloroperbenzoic acid[5] is employed in either protic or aprotic solvents in the cold, a smooth N-oxidation of vitamin B$_6$ compounds proceeds. Although several syntheses of pyridoxol *N*-oxide had been published,[6,7] none would seem to equal the general applicability or good yields obtained with this reagent. Ethanol was frequently used as solvent from which the amine oxides crystallized out during the course of oxygenation. Protection of a formyl group was effected by hemiacetal formation; otherwise extensive oxidation to the corresponding carboxylic acid is the preferred pathway as noticed in the quantitative conversion of pyridoxal 5′-phosphate to 4-pyridoxic acid 5′-phosphate. On the other hand, carbinol groups are attacked only on a limited scale and the relatively small improvements in yield do not warrant the work involved in protection and deprotection. Thus several compounds in the vitamin B$_6$ group were readily converted to the amine oxides with the exception of the PLP diethyl acetal, which under a variety of conditions gave rise to low yields and a number of by-products. In a second step, an intramolecular rearrangement[8] of the amine oxides to the corresponding 2-pyridine carbinols was effected by trifluoroacetic anhydride [(TFA)$_2$O], a reagent which was not previously utilized for such conversions on a preparative scale.[9] We found this anhydride to be superior to acetic anhydride in that it required mild conditions for acylation and gave rise to fewer by-products.

With ω-hydroxypyridoxol readily available, attempts were made to oxidize **7c** to **8** by activated manganese dioxide. The complex mixture of products thus obtained was fractionated on an ion-exchange column and was found to include ω-hydroxypyridoxal (**7a**) and 2-nor-2-formylpyridoxol as well as other acidic components, but only small amounts of the desired dicarbox-

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(2) Abbreviations used are as follows: ω-hydroxy indicates the introduction of a 2′-hydroxyl group and 2-nor-2-formyl, the replacement of a 2-methyl with a 2-formyl; PLP, pyridoxal 5′-phosphate; *m*-CPBA, *m*-chloroperbenzoic acid; PPA, polyphosphoric acid; THF, tetrahydrofuran; (TFA)$_2$O, trifluoroacetic anhydride.

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(7) G. R. Bedford, A. R. Katritzky, and H. M. Wuest, *J. Chem. Soc.*, 4601 (1963).

(8) S. Oae, *Tetrahedron*, **20**, 2677 (1966).

(9) Trifluoroacetic anhydride was previously employed on a preparative scale in a Beckmann rearrangement [see W. D. Emmons, *J. Amer. Chem. Soc.*, **79**, 6522 (1957)] and in mechanistic studies of N–O bond cleavage of amine oxides [T. Koenig, *ibid.*, **88**, 4045 (1966)].