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SYNTHESIS OF PEPTIDE OXAZOLONES AND RELATED COMPOUNDS[1]

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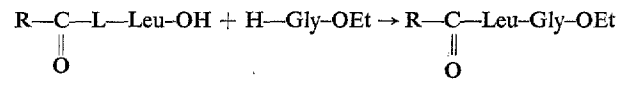
Abstract—Two optically active, crystalline, peptide oxazolones, 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4-methyl-oxazolone and 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4-benzyloxazolone, were prepared and characterized. The imidazole-accelerated *p*-nitrophenyl-active ester synthesis was found to be a good route to the dipeptides benzyloxycarbonylaminoisobutyryl-L-phenylalanine methyl ester and benzyloxycarbonylaminoisobutyryl-L-alanine methyl ester. The acids were obtained by careful hydrolysis of these esters with dilute acid, and underwent ring closure, yielding oxazolones on reacting with acetic anhydride or dicyclohexyl-carbodiimide.

The synthetic pathways leading to these compounds involved some interesting chemistry of hindered peptides. It was found that the crystalline mixed anhydride of benzyloxycarbonyl-aminoisobutyric and pivalic acids rearranges on heating to give the symmetrical anhydride of benzyloxycarbonylaminoisobutyric acid.

The problem of racemization during peptide synthesis has received much attention in recent years. Young's group carried out extensive measurement of racemization during the coupling process.[4–6] They examined the degree of racemization using the following model systems:



where R = —Me, —pH— and H.[7]

Goodman and Stueben[8] studying the alkaline hydrolysis of Z–Gly–L–Phe–ONp[7] in dioxan–water solution found that the rate of racemization of the ester was ten times greater than the rate of hydrolysis to the free acid. They postulated an oxazolone intermediate to explain these results.

Goodman and Levine[9] prepared a crystalline optically active amino acid oxazolone 2-phenyl-L-4-benzyloxazolone and compared the rates of racemization and ring-opening of this oxazolone with various nucleophiles in dioxan. They concluded on the basis of equilibrium studies that in the coupling process a small steady state concentration of oxazolone can form which racemizes much faster than it ring-opens.

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[4] N. A. Smart, G. T. Young and M. W. Williams, *J. Chem. Soc.* 3902 (1960).

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

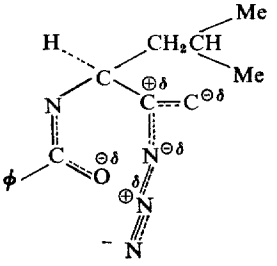
[6] A. L. Heard and G. T. Young, *J. Chem. Soc.* 5807 (1963).

[7] Abbreviations according to M. Goodman and G. W. Kenner, *Adv. in Protein Chem.* **12**, 465 (1957).

[8] M. Goodman and K. C. Stueben, *J. Org. Chem.* **27**, 3409 (1962).

[9] M. Goodman and L. Levine, *J. Am. Chem. Soc.* **86**, 2918 (1964).

Young[10] has long believed that the formation of an oxazolone intermediate is the major cause of racemization and recently[11] has provided additional evidence for the participation of oxazolone in the racemization of activated acyl dipeptide. Furthermore, he suggested that the azide method of peptide coupling has never been known to give rise to racemization because of the attraction between the negative charge developing on the amide oxygen and the positive charge on the central nitrogen atom of the azido group which inhibits the bond movements necessary to attain the conformation needed for oxazolone formation[12]



A similar intermediate structure can be visualized for 1-hydroxypiperidyl esters which are activated by protonation and couple without racemization.[12]

Anderson *et al.*[13] have shown that if Z–Gly–L–Phe–N_3 is allowed to remain in the presence of trimethylamine and then coupled in the standard manner, a yield of 1·6% of racemized product is obtained. We believe it is likely that the racemization which occurred was due to proton abstraction from the asymmetric center by the base before coupling rather than due to oxazolone formation during coupling.

In view of these results we undertook to prepare optically active peptide oxazolones and to use them as model systems for the kinetic investigation of racemization reactions. Our initial attempts were directed towards the isolation of the oxazolone from Z–Gly–L–Phe–OH. The material was a rather unstable oil which we were unable to purify. Young[11] has used this material in some racemization experiments but no experimental details were given in his preliminary communication. Kemp[14] describes the material as a syrupy liquid.

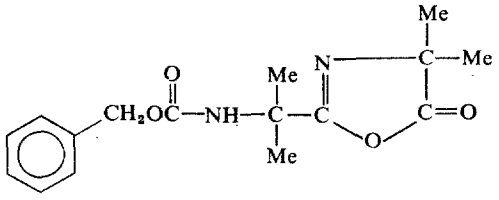
Schnabel[15] however was successful in isolating the solid oxazolone. It is difficult to say from his report how optically pure this material is.

In our efforts to prepare the oxazolone from Z–Gly–Gly–L–Phe–OH we obtained evidence of oxazolone formation from polarimetry, IR spectroscopy and TLC but again the material was an oil which partially racemized during purification.

More than thirty years ago Steiger[16] prepared a stable oxazolone from an aminoisobutyric acid derivative.

[10] G. T. Young, *Proc. Symposium on Methods of Peptide Synthesis*, Prague (1958), *Coll. Czech. Chem. Comm.* Special Issue, **24**, 39 (1959).
[11] I. Antonovics and G. T. Young, *Chem. Comm. Chem. Soc.* No. 17, 398 (1965).
[12] S. M. Beaumont, B. O. Handford and G. T. Young, *Acta Chim. Hung.* **44**, 37 (1965).
[13] G. W. Anderson, J. E. Zimmerman and F. M. Callahan, *J. Am. Chem. Soc.* **88**, 1338 (1966).
[14] D. S. Kemp, Ph.D. Dissertation, Harvard University (1964).
[15] E. Schnabel, *Liebigs Ann* **688**, 238 (1965).
[16] R. E. Steiger, *Helv. Chim. Acta* **17**, 563 (1934).

In 1960 Kenner *et al.*[17] reported the isolation of a stable peptide oxazolone 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone I

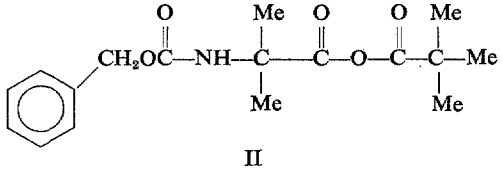


I

They obtained this oxazolone by heating Z–Aib–Aib–OH[18] with acetic anhydride at 110–120° for 15 min. Their work caused us to speculate that the material Z–Aib–L–Ala–OH might also give a stable oxazolone. The first derivative needed in the synthetic sequence which would lead to such an oxazolone was Z–Aib–Oh. Bergmann *et al.*[19] and Faust and Lange[20] found that this material melts at 78°.

The Kenner group prepared an analytical sample of the material with a melting range 72·5–74·5°. We have prepared Z–Aib–OH many times by the method described by Kenner and by the method reported by Faust and Lange and we have consistently obtained a melting range 88–89° for the pure material. It is possible that the compound exhibits polymorphism and under the conditions of our work the same crystalline form was obtained on each occasion.

Kenner[17] recommended the use of pivaloyl chloride to form the mixed anhydride II with Z–Aib–OH and more recently Zaoral[21] reported cases where the use of pivaloyl mixed anhydride is advantageous. Kenner found that II could couple smoothly with



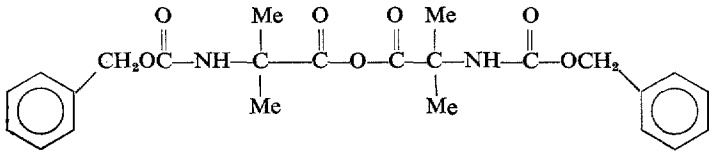
II

another aminoisobutyric ester. However, we found that when the ester used was phenylalanine methyl ester or alanine methyl ester some attack occurs at the pivaloyl carbonyl group so that pivaloyl amino acid ester is produced as an impurity in the dipeptide product. In a recent publication[22] Kenner and his group discussed the possibility of concurrent formation of some pivaloyl amino acid methyl esters in more polar solvents.

From the coupling of L-phenylalanine methyl ester with the pivaloyl mixed anhydride in toluene we recovered about 12% of Z–Aib–OH by workup of the alkaline extracts of the reaction mixture. By fractional crystallization of the neutral product we recovered a final fraction with an IR spectrum identical with that of authentic pivaloyl-L-phenylalanine methyl ester.

[17] M. T. Leplawy, D. S. Jones, G. W. Kenner and R. C. Sheppard, *Tetrahedron* **11**, 39 (1960).
[18] Aib refers to the aminoisobutyryl residue.
[19] M. Bergmann, L. Zervas, J. S. Fruton, S. Schneider and H. Schleich, *J. Biol. Chem.* **109**, 325 (1935).
[20] V. G. Faust and H. Lange, *J. Prakt. Chem.* **4**, 11 (1960).
[21] M. Zaoral, *Coll. Czech. Chem. Commun.* **27**, 1273 (1962).
[22] D. S. Jones, G. W. Kenner, J. Preston and R. C. Sheppard, *J. Chem. Soc.* 6227 (1965).

Kenner's group[22] reported that anhydride II showed no tendency to disproportionate even when heated to 100°. On the other hand, we found that prolonged heating in concentrated ethyl acetate or toluene solution causes this unsymmetrical anhydride to rearrange to the symmetrical anhydride III



III

Anhydride III can also be synthesized in fair yield by treatment of Z–Aib–OH with half the stoichiometric amount of thionyl chloride at ice temperature in the presence of a tertiary amine.

Coupling of the symmetrical anhydride III and L-phenylalanine methyl ester gave us for the first time unequivocally the dipeptide Z–Aib–L–Phe–OCH_3.

In 1963 Mazur[23] reported the acceleration of *p*-nitrophenyl ester peptide synthesis by imidazole. We made Z–Aib–ONp and in the presence of imidazole this compound coupled smoothly, in good yield and with retention of optical activity using several amino acid esters. We were not successful in obtaining Z–Aib–ONp as a crystalline compound. This ester could be purified chromatographically to the extent that on removal of solvent and storage of the resultant oil for several days, a white solid was obtained which gave satisfactory elemental analysis. We should point out at this stage, some limitations of the imidazole accelerated *p*-nitrophenyl ester method. With the hindered amino acid esters L-proline and aminoisobutyric methyl esters this method gave only 5% yields of dipeptide esters upon reaction with Z–Aib–ONp. Furthermore under certain conditions the method leads to racemization. When Z–Aib–ONp was allowed to react with D-phenylglycine methyl ester hydrochloride in the presence of triethylamine and excess imidazole in dimethylformamide for twelve hours a completely racemized dipeptide product was obtained. However, if the free ester D-phenylglycine methyl ester and Z–Aib–ONp were allowed to react in ethyl acetate in the presence of excess imidazole for one hour the optically active dipeptide ester was obtained. There is little doubt that racemization occurs by proton abstraction from the asymmetric center since there is no possibility of oxazolone formation in this reaction. Under the more drastic conditions described above the corresponding alanine and phenylalanine esters do not racemize. It may be useful at this point to refer to some references which indicate that racemization by proton abstraction does occur during peptide synthesis.

Anderson[24] demonstrated that *p*-nitrophenyl and the newer hydroxysuccinimide amino acid esters lose optical activity in the presence of tertiary amines even when such secure blocking groups as benzyloxycarbonyl and t-butyloxycarbonyl are used.

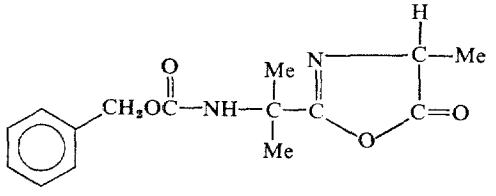
Liberek in a series of communications[25–29] has presented evidence for the base

[23] R. H. Mazur, *J. Org. Chem.* **28,** 2498 (1963).
[24] G. W. Anderson, F. M. Callahan and J. E. Zimmerman, *J. Am. Chem. Soc.* **85,** 3039 (1963).
[25] B. Liberek, *Tetrahedron Letters* No. 14, 925 (1963).
[26] B. Liberek, A. Nowicka and Z. Grzonka, *Tetrahedron Letters*, No. 22, 1479 (1963).
[27] B. Liberek, Z. Grzonka, *Tetrahedron Letters*, No. 3, 159 (1964).
[28] B. Liberek, Z. Grzonka and A. Michalik, *Bull. Acad. Polon. Sci. ser. sci. chim.* **14,** 375 (1964).
[29] B. Liberek and A. Michalik, *Acta Chim. Hung.* **44,** 71 (1965).

catalyzed proton abstraction from the asymmetric center resulting in possible resonance stabilized intermediates through which optical activity is lost.

We converted the compound Z–Aib–L–Ala–OCH_3 to the free acid by dilute acid hydrolysis in refluxing dioxan. Attempts at alkaline hydrolysis under mild conditions yielded impure products which could not be crystallized. Faust and Lange[20] encountered similar difficulties following hydrolysis of compounds of this type with sodium hydroxide.

From the pure free acid using acetic anhydride in dioxane or dicyclohexylcarbodiimide (DCCi) in ether the oxazolone IV 2-(1′-benzyloxycarbonylamino-1′-methyl)-ethyl-L-4-methyloxazolone was obtained in good yield.

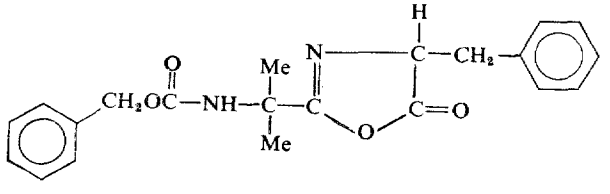


IV

Of the two methods, the DCCi procedure gave the more satisfactory results. Products obtained by the acetic anhydride method had less well-defined m.ps and lower specific rotations. Since the corresponding racemic oxazolone melts only slightly higher than the optically pure oxazolone, fractional crystallization of the optically active material is difficult.

We obtained the free acid from Z–Aib–L–Phe–OCH_3 by dilute acid hydrolysis. Attempted crystallization of the product from ether yielded a gelatinous material with a broad melting range. When this solid was added to cold benzene a gel was obtained which dissolved on warming. On cooling, beautiful, needle-like crystals were deposited which still exhibited a broad melting range. From polarimetric data it was clear that the crystals deposited from benzene contained substantial quantities of solvent.

To obtain 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-L-4-benzyloxazolone V from Z–Aib–L–Phe–OH the acetic anhydride method of ring closure proved more satisfactory than the DCCi method. The oxazolone V melts at 97–98° while the corresponding DL-oxazolone melts at 84–85°, hence the optically pure material can be obtained by fractional crystallization. We have reported preliminary data on this



V

optically active crystalline peptide oxazolone.[30] Because of its high specific rotation $[\alpha]_D$ −131·8 in dioxan, the phenylalanine oxazolone (V) is extremely useful for kinetic studies of racemization.

[30] M. Goodman and W. J. McGahren, *J. Am. Chem. Soc.* **87**, 3028 (1965).

Tripeptides. Our kinetic studies on oxazolone V were concerned with the racemization and coupling of this oxazolone with amino acid esters in various solvents. In order to interpret the results of these studies it was necessary to prepare a series of tripeptides of the type:

Z–Aib–L–Phe–amino acid ester

where the third amino acid residue included –Gly–OEt, –Ala–OCH_3, –ϕgly–OCH_3,[31] –Phe–OCH_3 and –Aib–OCH_3. The tripeptide Z–Aib–L–Phe–Gly–OEt was synthesized using the *p*-nitrophenyl ester coupling procedure to join Z–L–Phe–ONp and H–Gly–OEt. The benzyloxycarbonyl group was removed from the resultant Z–L–Phe–Gly–OEt. The free dipeptide ester was allowed to react with Z–Aib–ONp in the presence of imidazole. This approach does not involve noticeable racemization since material of identical properties was also made by the safe azide procedure. All the other tripeptides were made using the azide coupling method, eliminating the possibility of racemization by oxazolone mechanism. Table 1 lists the physical data on the tripeptides we prepared and characterized during the course of our studies.

TABLE 1. PHYSICAL DATA ON OPTICALLY ACTIVE TRIPEPTIDES

Tripeptide	M.P.	$[\alpha]_D^{25}$ in $CHCl_3$	% Yield
Z–Aib–L–Phe–Gly–OEt	93·5–95°	−47·7	55
Z–Aib–L–Phe–Aib–OCH_3	156–157·5°	−42·3	25
Z–Aib–L–Phe–L–Ala–OCH_3	151·5–152·5°	−43·2	60
Z–Aib–L–Phe–D–Ala–OCH_3	45–7°	−57·8	19
Z–Aib–L–Phe–L–ϕgly–OCH_3	145–6°	+23·2	53
Z–Aib–L–Phe–D–ϕgly–OCH_3	98·5–99·5°	−102·0	23
Z–Aib–L–Phe–L–Phe–OCH_3	137–8°	−13·8	60
Z–Aib–L–Phe–D–Phe–OCH_3–H_2O	81–3°	−87·7	15

Tripeptides of the type Z–Aib–L–Phe–L–amino acid ester are readily crystallizable compounds with fairly high m.ps and relatively low specific rotations. In contrast to this, tripeptides of the type Z–Aib–L–Phe–D–amino acid ester have low melting points and are generally very difficult to crystallize. These materials have high specific rotations. Yields were also lower in the latter case though this may be partially due to their reluctance to crystallize from solution. The crystalline stability and ease of formation is markedly different in going from the L–L series to the L–D series and most probably this is due to specific stereochemical effects. It is likely that solid state studies by X-ray diffraction might be a useful means of investigating such phenomena.

EXPERIMENTAL

All m.ps are uncorrected. They were obtained using capillary tubes and Anschutz thermometers. Analyses were carried out by Alfred Bernhardt, Mikroanalytisches Laboratorium, Max-Planck Institut, Mulheim, West Germany.

1. *Benzyloxycarbonylaminoisobutyric acid*

Method A. This compound was prepared as described by Leplawy *et al.*[17] with a minor adjustment. The method is a modification of the usual Schotten-Bauman conditions in that the alkaline soln of the

[31] ϕgly refers to the phenylglycyl residue.

amino acid is diluted one to one with acetone and the carbobenzoxy chloride is added in acetone soln. Before workup of the reaction mixture the acetone, or most of it, must be removed. We included an ether extraction step after removal of the acetone to remove any unreacted carbobenzoxy chloride, yield 60%, m.p. 88–89°, [lit.[17] m.p. 72·5–74·5[19] m.p. 78°].

Method B. We also prepared this compound by the method of Faust and Lange[20] in a yield of 60%, m.p. 88–89, [lit.,[20] m.p. 78°].

2. *Benzyloxycarbonylaminoisobutyric pivalic acid mixed anhydride*

This anhydride was prepared as described by Leplawy *et al.*[17] to get a 55% yield of beautiful crystalline needles, m.p. 87–88° [lit.[17] m.p. 81–83°].

3. *Symmetrical anhydride of benzyloxycarbonylaminoisobutyric acid*

Method A. A soln of 4·8 g (20 mmole) of Z–Aib–OH in 5 ml dry ethyl acetate was cooled in an ice-salt bath and protected from atmospheric moisture. To this mechanically stirred cold soln 2·8 ml (20 mmole) of EtN_3 and 0·9 ml (slightly less than 10 mmole) $SoCl_2$ were added by syringe through a rubber diaphragm. After 3 hr of stirring the reaction mixture was diluted with 30 ml AcOEt and the $Et_3N{\cdot}HCl$ was removed by filtration. The filtrate was extracted twice with 25 ml 1N HCl and 3 times with 25 ml 10% K_2CO_3al. After drying over $MgSO_4$ the solvent was evaporated under reduced press to give an oil which on treatment with a little ether solidified. Recrystallization from AcOEt–hexane yielded 1·3 g (34%) product, m.p. 95–96°. (Found: C, 63·33; H, 6·22; N, 6·01. Calc. for $C_{24}H_{28}N_2O_7$: C, 63·15; H, 6·10; N, 6·10%.)

Method B. A 50 ml Erlenmeyer containing 3·2 g (10 mmole) of the mixed anhydride of pivalic and benzyloxycarbonylaminoisobutyric acids and 0·5 ml toluene was kept at 50° overnight. The resultant, darkened, viscous oil was diluted with ether, Norite treated and following filtration was concentrated slowly with stirring. After 1 hr the pptd material was removed by filtration and recrystallized from AcOEt–hexane to yield 1·0 g (49%) of the symmetrical anhydride, m.p. 93–94°.

Method C. The disproportionation of the mixed anhydride could also be carried out by refluxing for 4 hr in conc AcOEt soln. A soln of 3·2 g (10 mmole) of the mixed anhydride in 2·0 ml AcOEt was refluxed for 4 hr. The reaction mixture was worked up as described under method B to get 1·2 g (57%) of product m.p. 92–94°.

4. *Tris-(p-nitrophenyl)phosphite*

This compound was prepared as outlined[32] in a yield of 68%, m.p. 165–168° [lit.[32] m.p. 167–170°].

5. p-*Nitrophenyl ester of benzyloxycarbonylaminoisobutyric acid*

Method A. This ester was prepared by a procedure outlined.[33] A suspension of 7·1 g (30 mmole) Z–Aib–OH and 5·0 g (35 mmole) *p*-nitrophenol and 6·2 g (30 mmole) dicyclohexylcarbodiimide (DCCi) in 20 ml of dry AcOEt was stirred for 2 hr. The pptd dicyclohexyl urea was removed by filtration and the filtrate was diluted to 100 ml with ether and extracted twice with 50 ml 1N HCl and many times with 10% K_2CO_3aq until the yellow color of the carbonate extract became very faint. The extracted soln was dried and concentrated to an oil which weighed 9·5 g. TLC of the oil on silica gel H using benzene:dioxan:acetic acid: :50:50:2 developing soln showed that the oil contained dicyclohexylurea. It was necessary to pass the oil over a silica gel (28–200 mesh) column to obtain chromatographically pure material which remained an oil and was used as such.

Method B. To a soln of 12 g (50 mmole) Z–Aib–OH in 25 ml pyridine 13·5 g (27 mmole) tris-(*p*-nitrophenyl) phosphite were added and the resultant suspension was stirred until a clear dark red soln was obtained (3 to 4 hr). The soln was diluted to 150 ml with ether and worked up as described in Method A. The oil obtained on removal of solvent was chromatographically pure and solidified in 3 days. Repeated attempts to get the material to crystallize failed. It could be obtained as a white solid by removing the solvent. The yield was 12 g (66%) m.p. 50–54°. (Found: C, 60·22; H, 5·21; N, 7·74. Calc. for $C_{18}H_{18}N_2O_6$: C, 60·33; H, 5·02; N, 7·82%.)

[32] F. Boardman, Ph.D. Thesis in Chemistry, Polytechnic Institute of Brooklyn, June (1962).
[33] D. F. Elliott and D. W. Russell, *Biochem. J.* **66**, 49P (1957).

6. *Pivaloyl-L-phenylalanine methyl ester*

A suspension of 0·64 g (3 mmole) L-phenylalanine methyl ester hydrochloride and 0·42 ml (3 mmole) Et_3N in 10 ml dry toluene was cooled in an ice-bath and 0·36 ml (3 mmole) pivaloyl chloride was added with stirring. After 2 hr stirring the Et_3N·HCl was removed by filtration and after normal workup isolation of the neutral product gave on recrystallization from ether–hexane 200 mg (33%) beautiful needle-like crystals, m.p. 90·5–91°, $[\alpha]_D^{25}$ +37·1 (c = 1, dioxan).

7. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanine methyl ester*

Method A. The free ester of L-phenylalanine methyl ester hydrochloride was obtained by stirring 1·08 g (5 mmole) of the ester hydrochloride with 0·70 ml (5 mmole) of Et_3N in 10 ml toluene for 45 min at room temp. Et_3N·HCl was removed by filtration and washed with toluene until the volume of the filtrate was 20 ml. This filtrate was stirred for 14 hr at room temp with 1·6 g (5 mmole) of the mixed anhydride of benzyloxycarbonylaminoisobutyric and pivalic acids. The soln was diluted with toluene to 75 ml and extracted 3 times with 20 ml 1N HCl and 4 times with the same volume of 10% K_2CO_3aq. After drying over $MgSO_4$ the solvent was removed under reduced press to get 1·45 g crude product. The crude material was dissolved in CH_2Cl_2, norite treated, passed through a pre-coated celite filter, diluted with hexane and refrigerated overnight to yield 0·8 g crystalline product m.p. 91–93°, $[\alpha]_D^{25}$ +28·0 (c = 1·5, dioxan). The filtrate was diluted further with hexane and allowed to stand in the refrigerator for 12 hr to yield a second crop 110 mg, m.p. 78–85° $[\alpha]_D$ +31·8 (c = 1·3, dioxan). The IR spectrum on this latter material matched that on authentic pivaloyl-L-phenylalanine methyl ester. The aqueous potassium carbonate extracts from the above workup were combined, acidified and extracted with AcOEt. The AcOEt extract was dried and concentrated to dryness to yield a solid which on recrystallization from benzene–hexane gave 100 mg, m.p. 82–86°. An IR spectrum showed the material to be Z–Aib–OH.

Method B. A suspension of 700 mg (3·2 mmole) L-phenylalanine methyl ester hydrochloride in 10 ml AcOEt was sitrred with 0·5 ml (3·2 mmole) Et_3N for 45 min and passed through a filter into 1·37 g (3 mmole) of the symmetrical anhydride of Z–Aib–OH. The reaction mixture was stirred at room temp for 3 hr and the neutral product was isolated as described under Method A. Recrystallization of the product from AcOEt–hexane gave 800 mg (66%) beautiful needle crystals, m.p. 95–96°. $[\alpha]_D^{25}$ +26·4 (c = 2·0, dioxan).

Method C. A suspension of 0·65 g (3 mmole) L-phenylalanine methyl ester hydrochloride was stirred in 7 ml toluene for 1 hr at room temp with 0·42 ml (3 mmole) Et_3N ethylamine. The Et_3N·HCl was removed by filtration and washed with 5 ml toluene. To the filtrate 1·07 g (3 mmole) Z–Aib–ONp and 2·0 g of imidazole were added. The reaction mixture was stirred at room temp for 4 hr and after dilution with AcOEt the neutral product was isolated in the usual manner. To discharge the yellow color of the *p*-nitrophenylate ion as many as 12 extractions with 10% K_2CO_3aq were necessary. After recrystallization of neutral product from AcOEt–hexane 0·65 g (54%) was obtained, m.p. 94·5–95·5°, $[\alpha]_D^{25}$ +26·5 (c = 2·0, dioxan).

Method D. A suspension of 2·15 g (6 mmole) Z–Aib–ONp, 1·3 g (6 mmole) L-phenylalanine methyl ester hydrochloride, 0·84 ml (6 mmole) Et_3N and 6 g imidazole was allowed to stir in 10 ml DMF at room temp overnight. Dilution of the reaction mixture with AcOEt and isolation of the neutral product yielded following recrystallization from AcOEt–hexane 1·8 g (75%) m.p. 94·2–94·8°, $[\alpha]_D^{25}$ +26·7 (c = 2·0, dioxan). (Found: C, 66·57; H, 6·60; N, 7·10. Calc. for $C_{22}H_{26}N_2O_5$: C, 66·33; H, 6·54; N, 7·04%.)

8. *Benzyloxycarbonylaminoisobutyryl-DL-phenylalanine methyl ester*

Prepared as described under No. 7, method D in a yield of 70%, m.p. 102·5–103·5°. (Found: C, 66·60; H, 6·68; N, 7·15. Calc. for $C_{22}H_{26}N_2O_5$: C, 66·33; H, 6·54; N, 7·04%.)

9. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanine*

Method A. To a soln of 0·4 g (1 mmole) Z–Aib-L–Phe–OCH_3 in 0·5 ml acetone 2 ml 0·5N NaOH were added dropwise with stirring. After 15 min the soln was diluted to 10 ml with water and a ppt appeared which was removed by filtration and shown to be 50 mg of unhydrolyzed ester. The faintly yellow filtrate (traces of *p*-nitrophenylate ion always persisted) was acidified and extracted with ether. The ether extract was dried and concentrated to an oil which was extremely difficult to

obtain as a solid. After considerable time and effort 200 mg solid product were obtained from ether soln, m.p. 55–70°. TLC indicated one major and one very minor less polar product. The $[\alpha]_D^{25}$ on the material was +33·5 (c = 1·5, dioxan).

Method B. Because alkaline hydrolysis of the Me ester was unsatisfactory the acidic hydrolysis procedure[17] was tried. A soln of 4·4 g (11 mmole) Z–Aib–L–Phe–OCH_3 in 20 ml dioxan with 5 ml 2N HCl added was refluxed for 3 hr. The solvent was then removed to obtain an oil which was treated dropwise with 75 ml 10% K_2CO_3aq. An unhydrolyzed residue of 300 mg was removed by filtration and the filtrate was acidified and extracted with ether. After drying over $MgSO_4$ the ether soln was concentrated to an oil. The oil was redissolved in ether, norite treated, passed through a filter, concentrated slightly and refrigerated overnight to yield 3·1 g (74%) product, m.p. 60–65°, $[\alpha]_D^{25}$ +34·2 (c = 2·0, dioxan). (Found: C, 65·46; H, 6·60; N, 7·11. Calc. for $C_{21}H_{24}N_2O_5$: C, 65·62; H, 6·24; N, 7·29%.)

If the product obtained from ether was added to cold benzene a gel formed in a few min which gave a colorless soln on warming. On being allowed to cool the soln deposited beautiful needle crystals, m.p. 50–55° $[\alpha]_D^{25}$ +33·2 (c = 1·5, dioxan). If these crystals, after thorough vacuum-drying were allowed to remain for a few days in a stoppered vial, they released benzene and their crystalline form became impaired and $[\alpha]_D^{25}$ was then +31·0° in dioxan.

The crystals deposited from benzene were probably solvated to some degree (estimated from polarimetric readings—one molecule of benzene for every 6 molecules of dipeptide acid).

10. *Benzyloxycarbonylaminoisobutyryl-*DL*-phenylalanine*

This crystalline material was obtained according to method B, No. 9. The yield was 70%, m.p. 144·5–145·5°. (Found: C, 65·57; H, 6·40; N, 7·29. Calc. for $C_{21}H_{24}N_2O_5$: C, 65·62; H, 6·24; N, 7·29.)

11. *Oxazolone from benzyloxycarbonylaminoisobutyryl-*L*-phenylalanine*

Method A. A soln of 2·7 g (7 mmole) Z–Aib–L–Phe–OH in 11 ml dioxan was diluted with 11 ml Ac_2O and allowed to stand at 20° ± 1°. When the negative rotation was a maximum [a typical max reading would be 10·70 after 14 hr] the solvent was removed at room temp under reduced press. The oil or solid remaining was freed from the last traces of Ac_2O by two treatments with 20 ml toluene followed by concentration to dryness. The crude solid was recrystallized from ether–hexane to give a first crop of 1·2 g (49%), m.p. 97·4–98·8°, $[\alpha]_D^{25}$ −131·2 (c = 1·0, dioxan). (Found: C, 68·85; H, 6·22; N, 7·72. Calc. for $C_{21}H_{22}N_2O_4$: C, 68·85; H, 6·01; N, 7·65%.)

A second crop of 0·56 g (20%) m.p. 88–91°, $[\alpha]_D^{25}$ −43·5° (c = 1·2, dioxan) was also recovered.

Method B. To a soln of 0·73 g (2 mmole) Z–Aib–L–Phe–OH in 10 ml ether 0·41 g (2 mmole) DCCi were added with stirring. Precipitation of solid began in about a min and after 10 min the dicyclohexylurea was filtered off and the filtrate was concentrated to dryness. The residue was stirred with 20 ml ether, the insoluble material was removed by filtration and again the filtrate was concentrated to a solid 0·5 g, $[\alpha]_D^{25}$ −111·0, (c = 1·5, dioxan). This material was recrystallized from ether to give a first crop of 350 mg (50%), m.p. 98–99·5°, $[\alpha]_D^{25}$ −124·6 (c = 1·0, dioxan). A second crop of 70 mg, m.p. 96–98° $[\alpha]_D^{25}$ −115·0 (c = 1·2, dioxan) was also recovered.

12. *Oxazolone from benzyloxycarbonylaminoisobutyryl-*DL*-phenylalanine*

This compound was prepared by method B, No. 11 in a yield of 68%, m.p. 84–85·4°. (Found: C, 69·29; H, 6·28; N, 7·52. Calc. for $C_{21}H_{22}N_2O_4$: C, 68·85; H, 6·01; N, 7·65%.)

13. *Benzyloxycarbonylaminoisobutyryl-*L*-alanine methyl ester*

This dipeptide ester was made as described under method D, No. 7 in 60% yield, m.p. 68·6–69·6°, $[\alpha]_D^{25}$ −5·5 (c = 4·2, dioxan). (Found: C, 59·69; H, 6·85; N, 8·90. Calc. for $C_{16}H_{22}N_2O_5$: C, 59·93; H, 6·80; N, 8·70%.)

14. *Benzyloxycarbonylaminoisobutyryl-*DL*-alanine methyl ester*

This material was prepared as described under method D, No. 7, in 60% yield, m.p. 101·4–102°. (Found: C, 60·06; H, 6·92; N, 8·50. Calc. for $C_{16}H_{22}N_2O_5$: C, 59·93; H, 6·80; N, 8·70%.)

15. *Benzyloxycarbonylaminoisobutyryl-L-alanine*

This crystalline product was obtained according to method B, No. 9 in 70% yield, m.p. 139·5–140·5°, $[\alpha]_D^{25}$ +5·9° (c = 5·0, dioxan) (Found: C, 58·59; H, 6·66; N, 9·16. Calc. for $C_{15}H_{20}N_2O_5$: C, 58·44; N, 9·09.

16. *Benzyloxycarbonylaminoisobutyryl-DL-alanine*

This DL-acid was prepared in the manner of method B, No. 9. The yield was 70%, m.p. 105·6–107°. (Found: C, 58·62; H, 6·61; N, 9·09. Calc. for $C_{15}H_{20}N_2O_5$: C, 58·44; H, 6·44; N, 9·09%.)

17. *Oxazolone from benzyloxycarbonylaminoisobutyryl-L-alanine*

When prepared as described by method A, No. 11, this material was obtained in 35% yield, m.p. 108–112°, $[\alpha]_D^{25}$ −48·1 (c = 1·0, dioxan). A typical maximum negative rotation of −6·50 was obtained in about 3 hr. Method B, No. 11 was the preferred method of prep of this oxazolone. A yield of 60% was obtained, m.p. 110–112°, $[\alpha]_D^{25}$ −52·1 (c = 1·0, dioxan). (Found: C, 62·58; H, 6·43; N, 9·60. Calc. for $C_{15}H_{18}N_2O_4$: C, 62·06; H, 6·21; N, 9·31%.)

18. *Oxazolone from benzyloxycarbonylaminoisobutyryl-DL-alanine*

The DL-oxazolone was obtained in 85% yield by method B, No. 11, m.p. 119–121°. (Found: C, 61·99; H, 6·58; N, 9·55. Calc. for $C_{15}N_{18}N_2O_4$: C, 62·06; H, 6·21; N, 9·31%.)

19. *Benzyloxycarbonylaminoisobutyryl-DL-phenylglycine methyl ester*

This compound was prepared by method D, No. 7 in 80% yield, m.p. 127·5–128·5°. (Found: C, 65·56; H, 6·31; N, 7·35. Calc. for $C_{21}H_{26}N_2O_5$: C, 65·62; H, 6·25; N, 7·29%.)

20. *Benzyloxycarbonylaminoisobutyryl-D-phenylglycine methyl ester*

An attempt to prepare this compound by method D, No. 7, gave a product in 75% yield, m.p. 128–129°, $[\alpha]_D^{25}$ −1·7 (c = 2, dioxan). The material had racemized by proton abstraction from the asymmetric center. The following method was then used to get optically active material.

A suspension of 2·4 g (12 mmole) D-phenylglycine methyl ester hydrochloride in 15 ml AcOEt was stirred for 45 min at room temp with 1·7 ml (12 mmole) Et_3N. The Et_3N·HCl was removed by filtration and 4·3 g (12 mmole) Z–Aib–ONp were added to the filtrate together with 6 g imidazole. This reaction mixture was stirred at room temp for 1 hr, diluted with AcOEt and the neutral product isolated in the normal manner. The product was recrystallized from AcOEt–hexane to get 1·6 g (40%) m.p. 121·5–123°. An analytical sample was recrystallized from ether, m.p. 122—123° $[\alpha]_D^{25}$ −85·9 (c = 1·2, dioxan). (Found: C, 65·72; H, 6·35; N, 7·08. Calc. for $C_{21}H_{24}N_2O_5$: C, 65·62; H, 6·25; N, 7·29%.)

It should be noted that this material was not made by another method such as the symmetrical anhydride procedure to verify optical purity.

21. *Benzyloxycarbonylaminoisobutyryl-DL-phenylglycine*

This compound was prepared using the procedure of method B, No. 9. The acid was obtained in 80% yield, m.p. 158–159·5°. (Found: C, 64·60; H, 6·02; N, 7·51. Calc. for $C_{20}H_{22}N_2O_5$: C, 64·84; H, 5·97; N, 7·56%.)

22. *Benzyloxycarbonylaminoisobutyryl-D-phenylglycine*

This compound was prepared in 70% yield according to method B, No. 9, m.p. 175·5–176·5°, $[\alpha]_D^{25}$ −96·1 (c = 1·0, dioxan). (Found: C, 65·06; H, 6·06; N, 7·49. Calc. for $C_{20}H_{22}N_2O_5$: C, 64·84; H, 5·97; N, 7·56%.)

23. *Attempted formation of oxazolone from benzyloxycarbonylaminoisobutyryl-D-phenylglycine*

We did not anticipate any difficulty in obtaining this oxazolone; however we were unsuccessful in preparing it by either the Ac_2O or DCCi methods. We have preliminary indications that the HBr salt of this oxazolone may be isolated following the use of the reagent PBr_3 for ring closure of the dipeptide acid.

24. *Benzyloxycarbonylaminoisobutyryl-L-proline methyl ester*

An attempt to prepare this material by method D, No. 7, gave the blocked dipeptide ester product in 5% yield, m.p. 95–97°.

25. *Benzyloxycarbonylaminoisobutyryl-aminoisobutyric methyl ester*

An attempt to prepare this compound by method D, No. 7, gave the product in a yield of 5%, m.p. 108–109° [lit.[17] m.p. 109–111°]. This compound was prepared using the mixed anhydride of benzyloxycarbonylaminoisobutyric and pivalic acids in 70% yield, m.p. 110–112°. Using the symmetrical anhydride procedure of method B, No. 7, the material was obtained in 80% yield, m.p. 110–112°. The work described in No. 24 and No. 25 indicates that the imidazole-accelerated *p*-nitrophenyl ester coupling technique is not effective when the incoming amino group is hindered.

26. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanyl glycine ethyl ester*

A suspension of 8·4 g (20 mmole) of Z–L–Phe–ONp (obtained from the Cyclo Corporation), 2·8 g (20 mmole) ethyl glycinate hydrochloride, 2·8 ml (20 mmole) of Et_3N and 25 g imidazole in 25 ml of DMF was stirred at room temp for 1 hr. Workup of the reaction mixture yielded 6·5 g Z–L–Phe–Gly–OEt which on recrystallization from AcOEt hexane gave 5·5 g (71%) m.p. 111–112° [lit.,[34] m.p. 111°].

A soln of 5·0 g (13 mmole) Z–L–Phe–Gly–OEt) in 25 ml glacial AcOH was saturated with HBr gas and then the temp was maintained at 50–60° until CO_2 evolution ceased. The soln was cooled and diluted with 200 ml ether to give 3·3 g ppt which was recrystallized from EtOH–ether to give 2·9 g (57%) HBr·H–L–Phe–Gly–OEt, m.p. 134–135°, $[\alpha]_D^{25}$ +40·3 (c = 1·7, water) [lit.[35] m.p. 135–136° $[\alpha]_D^{25}$ +40·3 (c = 2·0)].

A suspension of 1·3 g (4 mmole) HBr·H–L–Phe–Gly–OEt in 8 ml of AcOEt containing 0·56 ml (4 mmole) Et_3N was stirred at room temp for 1 hr and passed through a filter into a flask containing 1·07 g (3 mmole) Z–Aib–ONp and 5 g imidazole. The Et_3N·HBr was washed with 5 ml AcOEt and the wash was added to the reaction mixture which was then allowed to stir at room temp for 3 hr. On dilution of the reaction soln with AcOEt the neutral product was recovered in the normal fashion. The crude neutral product 0·9 g of a slightly yellowish oil did not solidify readily. It was passed over a silica gel (28–200 mesh) column and eluted with chf–hexane 80:20. The solid white product from the column was recrystallized from ether to get 0·75 g (50%) crystalline Z–Aib–L–Phe–Gly–OEt, m.p. 93·5–95°, $[\alpha]_D^{25}$ −47·2 (c = 4·0, chf). (Found: C, 63·91; H, 6·61; N, 8·95. Calc. for $C_{25}H_{31}N_3O_6$: C, 63·96; H, 6·61; N, 8·95%.)

This tripeptide was also prepared by the azide coupling procedure of No. 34 in a yield of 35%, m.p. 93–95°, $[\alpha]_D^{25}$ −47·7 (c = 2·5. chf).

27. *Benzyloxycarbonylaminoisobutyryl-DL-phenylalanylglycine ethyl ester*

This material was prepared as described in No. 26 starting with Z–DL–Phe–ONp. The product Z–Aib–DL–Phe–Gly–OEt was recrystallized from AcOEt, m.p. 161·5–162·5°. (Found: C, 64·27; H, 6·62; N, 8·94. Calc. for $C_{25}H_{31}N_3O_6$: C, 63·96; H, 6·61; N, 8·95%.)

28. *Attempted preparation of benzyloxycarbonyl-L-phenylalanyl-DL-phenylglycine methyl ester by p-nitrophenyl ester technique*

A mixture of 8·4 g (20 mmole) of Z–L–Phe–ONp, 4·0 g (20 mmole) of DL-phenylglycine methyl ester hydrochloride, 2·8 ml (20 mmole) of Et_3N and 20 g imidazole in 15 ml of DMF was stirred at room temp. In 3 min the suspension became a soln and in 10–15 min a heavy yellow ppt appeared, making further magnetic stirring difficult. After 30 min the heavy suspension was diluted with 100 ml AcOEt and filtered to get 5 g of crude, yellow residue which was very insoluble in the usual organic solvents. It was recrystallized from boiling dioxan to get 2·0 g white crystals, m.p. 210–212°, $[\alpha]_D^{25}$ −67·6 (c = 0·6, dioxan). It was later shown that this material was partially racemized Z–L–Phe–D–ϕgiy–OCH_3 (see No. 29). The filtrate from which the crude yellowish ppt had been obtained was worked up in the usual manner to get the neutral product which was recrystallized from chf to get

[34] M. Bergmann and J. S. Fruton, *J. Biol. Chem.* **118,** 414 (1937).
[35] G. W. Anderson, J. Blodinger and A. D. Welcher, *J. Am. Chem. Soc.* **74,** 5309 (1952).

3·5 (m.p. 162–165°, $[\alpha]_D^{25}$ +18·8 (c = 1·1, dioxan). This material was impure Z–L–Phe–L–ϕgly–OCH_3 (see No. 30).

29. *Benzyloxycarbonyl-L-phenylalanyl-D-phenylglycine methly ester*

A suspension of 4·2 g (10 mmole) Z–L–Phe–ONp, 2·0 g (10 mmole) D-phenylglycine methyl ester hydrochloride, 1·4 ml (10 mmole) Et_3N and 15 g imidazole in 15 ml DMF was stirred at room temp. Soln was effected in 3 to 4 mins and very shortly thereafter the reaction became a solid suspension which was diluted with AcOEt and the ppt was recovered by filtration to get 3·0 g crude yellowish product. Re-crystallization from AcOEt gave 2·2 g (50%) m.p. 213–214° $[\alpha]_D^{25}$ −75·0 (c = 1·0, dioxan). (Found: C, 69·76; H, 5·79; N, 6·34. Calc. for $C_{26}H_{26}N_2O_5$: C, 69·90; H, 5·83; N, 6·27%.)

30. *Benzyloxycarbonyl-L-phenylalanyl-L-phenylglycine methyl ester*

The material was made by procedure No. 29. However, this time no precipitation occurred and the soln was stirred for 1·5 hr and worked up to get the neutral product in a yield of 65% m.p. 166–167·5°, $[\alpha]_D^{25}$ +31·2° (c = 1·0, dioxan). (Found: C, 69·89; H, 5·80; N, 6·52. Calc. for $C_{26}H_{26}N_2O_5$: C, 69·90; H, 5·83; N, 6·27%.)

31. *Resolution of DL-phenylglycine*

Betti and Mayer[36] resolved DL-phenylglycine making use of the reagent D-10 camphorsulfonic acid. They obtained the D-form readily $[\alpha]_D^{25}$ −157·8° (c = 3·4, 1N HCl) but they had some difficulty in getting a very small amount of the L-form. The D-form is commerically available and the material which we used had $[\alpha]_D^{25}$ −153·8 (c = 2·0 1N HCl). Our best effort at this resolution involved a slight modification of a procedure outlined.[37]

A blend of 13·0 g (82 mmole) of DL-phenylglycine and 20 g (86 mmole) D-10 camphorsulfonic acid was added to boiling water in a 250 ml Erlenmeyer so that the total volume of the soln did not exceed 50 ml. The soln was filtered through a warm, pre-coated filter bed. On cooling and standing for 2 hr the filtrate deposited a heavy ppt which was removed by filtration to give 15·5 g of the DD-complex. The filtrate was stirred with an added 20 ml of 2·2N NaOH. After ½ hr of stirring the ppt was recovered by filtration and re-slurried in 30 ml of cold de-ionized water and stirred for 15 min. Upon recovery by filtration the solid was re-slurried in acetone and finally in ether. The dried crude product 4·0 g (61%) had $[\alpha]_D^{25}$ +140° (c = 1·0, 1N HCl). By re-crystallization from dilute, de-ionized-water soln white flaky crystals were obtained $[\alpha]_D^{25}$ +151·0 (c = 0·7, 1N HCl). Marvel[37] reported products with $[\alpha]_D$'s varying from +127° to +146° in dil HClaq.

32. *Benzyloxycarbonylaminoisobutyryl-DL-phenylalanine hydrazide*

This material was prepared by a slight modification of the procedure described by Hofmann *et al.*[38] A soln consisting of 2·6 g (6·5 mmole) Z–Aib–DL–Phe–OCH_3 in 8 ml MeOH was heated to boiling. Then an excess, 0·6 ml, of an 85% aqueous soln of hydrazine hydrate was added and the soln was left standing overnight. All dipeptide esters prepared by the *p*-nitrophenyl ester technique became faintly yellow on addition of hydrazine hydrate due to traces of *p*-nitrophenylate ion. The solvent was removed to give 2·6 g product, m.p. 159·9–161·5° which was recrystallized from chf to give 2·5 g (92%) m.p. 162·5–163·5. (Found: C, 63·49; H, 6·67; N, 14·13. Calc. for $C_{21}H_{26}N_4O_4$: C, 63·31; H, 6·53; N, 14·07%.)

33. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanine hydrazide*

To a soln of 4·4 g (11 mmole) Z–Aib–L–Phe–OCH_3 in 15 ml of boiling MeOH 1·1 ml (excess) 85% hydrazine hydrate soln were added and the reaction soln was allowed to stand at room temp overnight. The solvent was removed under reduced press to give a yellowish oil which was dissolved in chf and extracted with Na_2CO_3aq. The dried chf soln was concentrated to a cotton candy solid which could be broken to a fine powder, 3·8 g (83%), and was easily manipulated in a room at 20°. If allowed to reach a temp of 25° or above it became tacky. Despite considerable effort we were unable to get the material crystalline. The bulk of the material melted between 48–55° but a trace

[36] M. Betti and M. Mayer, *Ber. Dtsch. Chem. Ges.* **41,** 2071 (1908).
[37] C. S. Marvel and W. A. Noyes *J. Am. Chem. Soc.* **42,** 2264 (1920).
[38] K. Hofmann, W. Haas, M. J. Smithers and Z. Guido, *J. Am. Chem. Soc.* **87,** 631 (1965).

persisted beyond 120°. The material was prepared 4 times, always with the same yields and physical characteristics. The rotations and melting ranges were as follows:

(i) $[\alpha]_D^{25}$ −30·9 (c = 1·5, chf), m.p. 48–55°.
(ii) $[\alpha]_D^{25}$ −34·4 (c = 1·9, chf), m.p. 51–56°
(iii) $[\alpha]_D^{25}$ −32·2 (c = 1·3, chf), m.p. 48–55°
(iv) $[\alpha]_D^{25}$ −30·5 (c = 0·7, chf), m.p. 46–56°

Some of the material was passed over a silica gel (60–200 mesh) column but the product from the column was identical with the material before chromatography. Despite the difficulties with purity and crystallinity of this compound it was used successfully in subsequent azide synthesis of tripeptides.

34. *Benzyloxycarbonylaminoisobutyryl-*L*-phenylalanyl-*L*-phenylglycine methyl ester*

To make this compound the azide coupling technique as described by Honzyl and Rudinger[39] was generally followed. To a magnetically stirred soln of 1·0 g (2·5 mmole) Z–Aib–L–Phe–$NHNH_2$ in 2 ml dry THF which had previously been made 2·2N in HCl by passing dry HCl gas into the dry solvent, in a rubber capped 50 ml Erlenmeyer cooled in dry ice/acetone an excess, 0·35 ml, of butyl nitrate was added through the cap from a syringe. Stirring for 10–15 min produced a dark brown oil which was dissolved in 40 ml precooled AcOEt and extracted with an ice-cold sat bicarbonate–brine soln. The aqueous layer was quickly removed and the AcOEt soln of azide was dried over $MgSO_4$ while at the same time being cooled in a dry-ice/acetone bath.

A suspension of 0·5 g (2·5 mmole) L-phenylglycine methyl ester hydrochloride which had been stirred for 1 hr at room temp with 0·35 ml (2·5 mmole) Et_3N was passed through a filter into a 50 ml Erlenmeyer. The Erlenmeyer was capped and cooled in dry ice/acetone. Into this cold free ester solution was poured the cold AcOEt soln of the azide which had been separated from $MgSO_4$ by filtration. The reaction soln was placed in an ice-packed Dewar in the refrigerator for 24 hr. The reaction mixture was then extracted several times with ice-cold 1N HCl and ice-cold 10% K_2CO_3aq. Recovery of the neutral product by solvent removal gave 0·8 g crude material which was recrystallized from AcOEt–hexane to give 0·7 g (53%) m.p. 145–146°, $[\alpha]_D^{25}$ +23·2 (c = 1·0, chf). (Found: C, 67·96; H, 6·18; N, 8·06. Calc. for $C_{30}H_{33}N_3O_6$: C, 67·79; H, 6·21; N, 7·91%.)

35. *Benzyloxycarbonylaminoisobutyryl-*L*-phenylalanyl-*D*-phenylglycine methyl ester*

This material was prepared by the method of No. 34. In this case it was extremely difficult to obtain crystalline material from the final oil product. When 0·66 g solid material was finally obtained it had a broad melting range 55–80° with decomposition. Once the solid material was dissolved in a solvent, it was difficult to get it back to the solid form again. A soln of the material in ether after one week at dry ice/acetone temp deposited 0·35 g (23%) of crystals, m.p. 98·5–99·5°, $[\alpha]_D^{25}$ −102·0 (c = 1·0, chf). (Found: C, 67·89; H, 6·32; N, 8·02. Calc. for $C_{30}H_{33}N_3O_6$: C, 67·79; H, 6·21; N, 7·91%.)

36. *Benzyloxycarbonylaminoisobutyryl-*L*-phenylalanyl-*L*-alanine methyl ester*

This tripeptide was obtained in the crystalline form in 60% yield by the method of No. 34, m.p. 151·5–152°, $[\alpha]_D^{25}$ −43·2 (c = 1·2, chf). (Found: C, 64·14; H, 6·76; N, 8·92. Calc. for $C_{25}H_{31}N_3O_6$: C, 63·96; H, 6·61; N, 8·95%.)

37. *Benzyloxycarbonylaminoisobutyryl-*L*-phenylalanyl-*D*-alanine methyl ester*

This material obtained by the procedure of No. 34 was extremely difficult to crystallize. Finally after one week it crystallized from toluene soln as scaly crystals, m.p. 45·5–47·5°, $[\alpha]_D^{25}$ −57·8 (c = 1·0, chf). (Found: C, 64·12; H, 6·84; N, 8·94. Calc. for $C_{25}H_{31}N_3O_6$: C, 63·96; H, 6·61; N, 8·95%.)

38. *Benzyloxycarbonylaminoisobutyryl-*L*-phenylalanyl-*L*-phenylalanine methyl ester*

This tripeptide was obtained as a readily crystallizable product by procedure No. 34 in 60% m.p. 137–138° $[\alpha]_D^{25}$ −13·8 (c = 2·6, chf). (Found: C, 68·34; H, 6·52; N, 7·72. Calc. for $C_{31}H_{35}N_3O_6$: C, 68·25; H, 6·42; N, 7·70%.)

[39] J. Honzyl and J. Rudinger, *Coll. Czech. Chem. Commun.* **26,** 2333 (1961).

39. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanyl-D-phenylalanine methyl ester*

This material obtained as an oily product by the method of No. 34 was after much time and effort obtained crystalline from toluene in 15% yield, m.p. 81–83°, $[\alpha]_D^{25}$ −87·7 (c = 1·0, chf). (Found: C, 66·23; H, 6·47; N, 7·63. Calc. for $C_{31}H_5N_3O_6$: C, 68·25; H, 6·42; N, 7·70%.) Because of the discrepancy in the carbon analysis the tripeptide was prepared again by the azide method of No. 34 to obtain a material m.p. 81–83° which gave the following analysis: (Found: C, 65·60; H, 6·61; N, 7·61%.)

On TLC both preparations had the same R_f as Z–Aib–L–Phe–L–Phe–OCH_3 and the IR spectra of these materials were very similar. If we assume that Z–Aib–L–Phe–D–Phe–OCH_3 crystallizes as a monohydrate the calculated values for $C_{31}H_{35}N_3O_6 \cdot H_2O$ are as follows: (C, 66·08; H, 6·57; N, 7·46%) which agree satisfactorily with the values found for both preparations. Furthermore the material did not crystallize from dry toluene. If the soln in dry toluene were exposed to atmospheric humidity, the tripeptide crystallized out in 4 to 5 days.

40. *Benzyloxycarbonylaminoisobutyryl-L-phenylalanyl-aminoisobutyric methyl ester*

This material was made according to No. 34 procedure in 25% yield, m.p. 156–157·5° $[\alpha]_D^{25}$ −42·3 (c = 1·2, chf). (Found: C, 64·43; H, 6·74; N, 8·73. Calc. for $C_{26}H_{31}N_3O_6$: C, 64·59; H, 6·83; N, 8·69%.)

41. *Benzyloxycarbonylaminoisobutyryl-DL-phenylalanyl aminoisobutyric methyl ester*

This optically inactive material was also made by the method of No. 34 in 45% yield, m.p. 169·5–170.5°. (Found: C, 64·43; H, 7·00; N, 8·82. Calc for $C_{21}H_{31}N_3O_6$: C, 64·59; H, 6·83; N, 8·69%)