β-alanine, 7436-73-9; Cbz-L-γ-cyano-γ-aminobutyric acid, 31883-94-0.

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2,4-Dimethoxybenzyl as a Protecting Group for Glutamine and Asparagine in Peptide Synthesis

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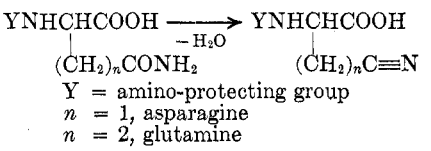
The properties of 2,4-dimethoxybenzyl (Dmb) as a protecting group for the amide side chain of glutamine and asparagine during peptide synthesis are described. 2,4-Dimethoxybenzylamine was prepared by the reduction of 2,4-dimethoxybenzyaldoxime with sodium bis(2-methoxyethoxy)aluminum hydride. The Dmb derivatives obtained by reaction of 2,4-dimethoxybenzylamine and either *N,N'*-dicyclohexylcarbodiimide or *N*-diethylamino-1-propyne with the appropriate amine acid derivatives are crystalline and the Dmb group can be removed by trifluoroacetic acid or anhydrous hydrogen fluoride to give the free amide. No formation of pyroglutamyl peptides or of other side reactions was detected with Dmb-protected glutamyl derivatives, even during saponification. On the contrary, use of alkali with either 2,4-dimethoxybenzyl- or bis(2,4-dimethoxybenzyl)-protected asparaginyl peptides resulted in a mixture of products and is not recommended.

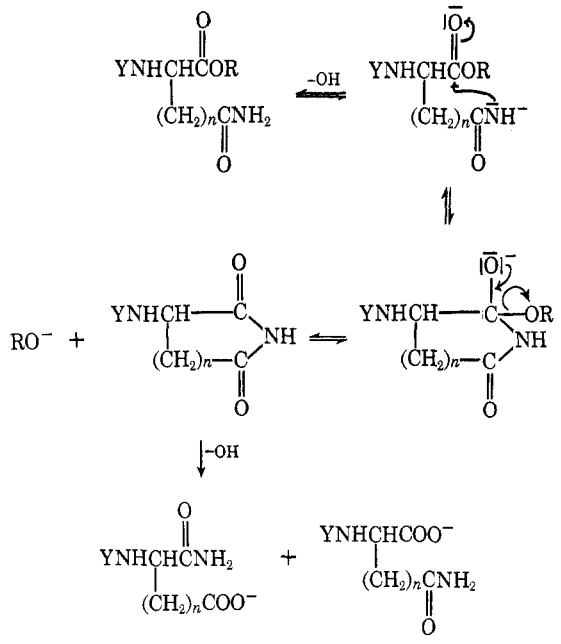
The amide groups of asparagine and glutamine undergo the following side reactions (eq 1–3) during peptide synthesis: (1) dehydration to the corresponding



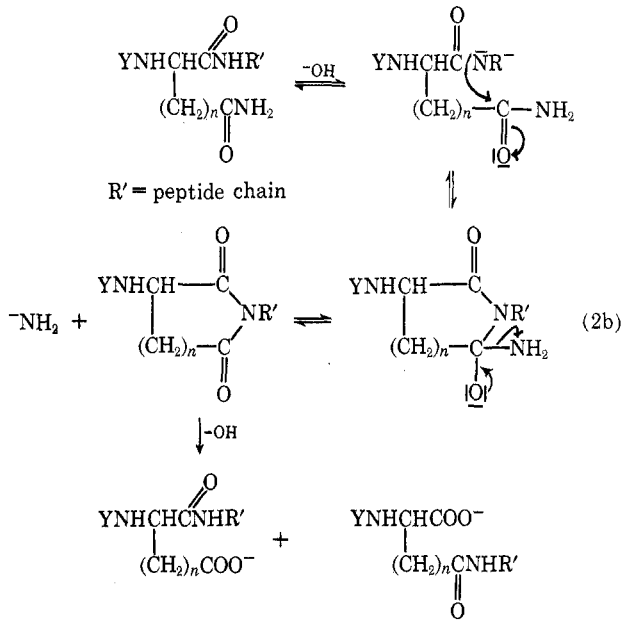
(1)

Y = amino-protecting group
n = 1, asparagine
n = 2, glutamine

cyano derivatives;[2–6] (2) formation of imides and subsequent hydrolysis[7–10] [N-protected asparagine or glutamine esters (a), asparaginyl or glutaminyl peptides (b). In this case, the loss of a proton by the action of alkali occurs in both position α and ω. The α site is more reactive because of the greater electrophilic strength of the α carbon atom as compared with that of the ω carbon atom. The subsequent release of the NH_2 group leads to formation of α and ω isomeric peptides, though the latter is obtained in greater amount. Reaction at the ω site causes cleavage of the peptide



(2a)



(2b)

(1) To whom any correspondence should be sent.

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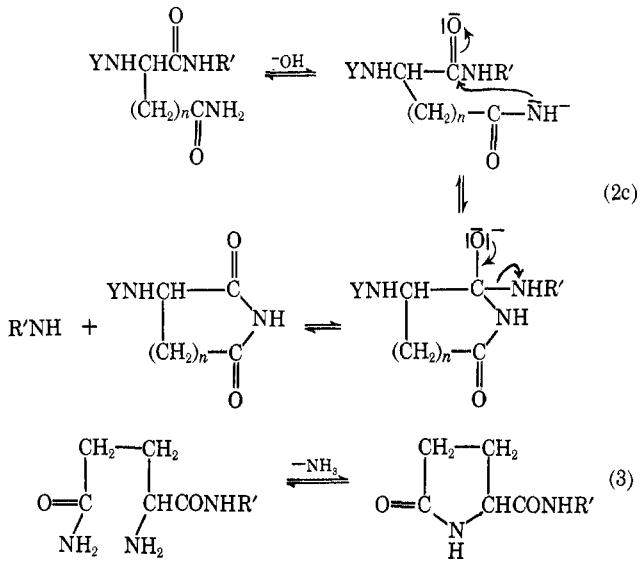
(c)]; and (3) formation of pyroglutamyl derivatives from glutaminyl peptides.[11,12]



(2c)

(3)

Weygand and his colleagues[13,14] have introduced the protecting group bis(2,4-dimethoxybenzyl), $(Dmb)_2$, in order to prevent the previously mentioned side reactions. The preparation of $(Dmb)_2NH$ is laborious and the $(Dmb)_2$-protected derivatives are usually amorphous; therefore, their characterization is difficult. The possibility of using just one 2,4-dimethoxybenzyl group has been further investigated. 2,4-Dimethoxybenzylamine has been synthesized by a new and easier method, *i.e.*, reduction with sodium bis(2-methoxyethoxy)aluminum hydride of the 2,4-dimethoxybenzaldoxide. The Dmb derivatives are crystalline products which can be easily obtained by reacting 2,4-dimethoxybenzylamine with the corresponding esters of *N*-benzyloxycarbonyl or *N*-*tert*-butyloxycarbonylaspartic and glutamic acids, *via* *N*-diethylamino-1-propyne or *N*,*N*′-dicyclohexylcarbodiimide.[15]

On removal of the ester group or the amino-protective group, the carboxyl or amino components are obtained, respectively. The synthesis of the Dmb-protected asparaginyl or glutaminyl peptides has been carried out from these compounds. The Dmb group was removed using trifluoroacetic acid or anhydrous hydrofluoric acid.

With regard to the action of base, it has been found that Dmb-protected glutaminyl derivatives are stable. In fact, alkaline hydrolysis of *N*-benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine methyl ester, *N*-benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester, and *N*-*tert*-butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester gave the corresponding free acids in high yield.

(11) D. Theodoropoulos and I. Souchleris, *Acta Chim. Acad. Sci. Hung.*, **44**, 183 (1965).

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(15) Attempts to prepare these intermediates directly from the symmetrical anhydrides and 2,4-dimethoxybenzylamine were not successful because of the difficulties in separating the mixture of the α and ω amide derivatives which resulted.

On the contrary, alkaline hydrolysis of *N*-benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparaginyl-L-alanine methyl ester and *N*-benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparaginyl-L-alanyl-L-leucyl-L-alanine methyl ester resulted in a mixture of products, according to the side reactions described in 2b. In the case of *N*-benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine methyl ester, however, the desired product is easily obtained from the mixture by crystallization.

Even the $(Dmb)_2$ protective group, which makes reaction at the ω site impossible, is not able to prevent the more preferred reaction at the α site, however, in contrast to previously reported observations.[13] In fact, alkaline hydrolysis with 3 equiv of 1 *N* NaOH of *N*-benzyloxycarbonyl-N^{β}-bis(2,4-dimethoxybenzyl)-L-asparaginyl-L-leucine methyl ester[13] and *N*-benzyloxycarbonyl-N^{β}-bis(2,4-dimethoxybenzyl)-L-asparaginyl-L-alanyl-L-leucyl-L-alanine methyl ester yielded, as ascertained by thin layer chromatography, three different products. Therefore, use of alkali with asparaginyl peptides protected either by Dmb or $(Dmb)_2$ is not recommended. No formation of pyroglutamyl derivatives was observed with Dmb-protected carboxamide groups. Thus, on the reaction of the dipeptide ester, prepared by hydrogenolysis of *N*-benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester in 80% acetic acid, with *N*-*tert*-butyloxycarbonyl-L-alanine *p*-nitrophenyl ester, the tripeptide *N*-*tert*-butyloxycarbonyl-L-alanyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester was obtained in high yield. In addition, formation of pyroglutamyl derivatives was not observed during removal of the protective groups of *N*-*tert*-butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine, *N*-*tert*-butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine, and *N*-*tert*-butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanyl-L-valyl-L-valine *tert*-butyl ester with trifluoroacetic acid.

Experimental Section

Ascending thin layer chromatograms were run on silica gel G with butan-1-ol–acetic acid–water (4:1:1 v/v) (R_{FA}), butan-1-ol–acetic acid–water–pyridine (15:10:2:3) (R_{FB}), and benzene–ethyl acetate–acetic acid–water (10:10:2:1) (R_{FC}). Descending chromatograms were run on Whatman No. 3 MM paper with butan-1-ol–acetic acid–water (4:1:1) (R_{FD}) or liquified phenol saturated with water and in the presence of a beaker of 0.3% NH_4OH in the tank during each run (R_{FE}). Spots were revealed with ninhydrin solution, and sodium hypochlorite followed by potassium iodide (1%)–starch (1%).[16] Acid hydrolysates of peptides were prepared using 6 *N* hydrochloric acid (110°, 16 hr) and the amino acid composition was determined with a Technicon Auto-Analyzer. Optical rotation were determined with a Perkin-Elmer polarimeter, Model 141. Organic extracts were dried with anhydrous sodium sulfate and evaporations were carried out under reduced pressure in a rotary evaporator. Melting points (uncorrected) were determined in capillary tubes in a Tottoli melting point apparatus (manufactured by W. Büchi).

2,4-Dimethoxybenzaldoxime.—2,4-Dimethoxybenzaldehyde (16.6 g, 0.1 mol) and hydroxylamine hydrochloride (6.9 g, 0.1 mol) in 12% sodium hydroxide (50 ml) and ethanol (12 ml) were gently refluxed for 10 min. After cooling overnight at 0°, the precipitate was filtered and crystallized from ethanol–water, yielding the product (14.8 g, 82%), mp 104–105°.

Anal. Calcd for $C_9H_{11}NO_3$: C, 59.64; H, 6.12; N, 7.73. Found: C, 59.61; H, 6.12; N, 7.77.

(16) H. N. Rydon and P. Smith, *Nature*, **169**, 922 (1952).

2,4-Dimethoxybenzylamine (Dmb-NH$_2$).—A solution of 2,4-dimethoxybenzaldoxime (13.6 g, 75 mmol) in benzene (100 ml) was added under stirring to a solution of sodium bis(2-methoxyethoxy)aluminum hydride (60 g, 0.3 mol) in benzene (45 ml). The mixture was boiled for 1 hr, cooled, and decomposed with 20% sulfuric acid at 0°. After washing with ether, the solution was made alkaline with 10% sodium hydroxide, filtered, and extracted with ether. The ether extracts were dried, concentrated, and treated with HCl–ether. The salt which precipitated was filtered and crystallized from ethanol–ether to give the product hydrochloride (13 g, 86%), mp 185–186°.[13]

A. N^β-(2,4-Dimethoxybenzyl)-L-asparagine Derivatives and Peptides. N-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine α-Methyl Ester [Z-Asn(Dmb)-OMe].—Freshly distilled 1-diethylamino-1-propyne[17,18] (2.6 g, 23.5 mmol) in methylene chloride (25 ml) was added dropwise at 5–10° during 30 min to a stirred solution of α-methyl-N-benzyloxycarbonyl-L-aspartic acid (6.6 g, 23.5 mmol) and 2,4-dimethoxybenzylamine (3.92 g, 23.5 mmol) in methylene chloride (100 ml). Stirring was continued at 20–25° for 30 min and then the mixture was evaporated. Crystallization of the residue from ethyl acetate gave the product (7.4 g, 74%): mp 162–163°; R_{FA} 0.80, R_{FC} 0.86; $[\alpha]^{27}D$ +60.1° (c 1.03, dimethylformamide).

Anal. Calcd for $C_{22}H_{26}N_2O_7$: C, 61.38; H, 6.08; N, 6.50. Found: C, 61.37; H, 5.94; N, 6.50.

***N*-*tert*-Butyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine α-Benzyl Ester [Boc-Asn(Dmb)-OBzl].**—This product (mp 102–103°), which crystallized from ethyl acetate–petroleum ether (bp 60–80°), was obtained similarly in 38% yield: R_{FA} 0.62, R_{FC} 0.70; $[\alpha]^{25}D$ −6.4° (c 1.0, methanol).

Anal. Calcd for $C_{25}H_{32}N_2O_7$: C, 63.54; H, 6.83; N, 5.93. Found: C, 63.30; H, 6.94; N, 6.06.

***N*-*tert*-Butyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-isoasparagine β-Benzyl Ester [Boc-Asp(Bzl)-NH-Dmb].**—This product (mp 99–100°), which crystallized from ethyl acetate–petroleum ether, was obtained similarly in 78% yield: R_{FA} 0.9, R_{FA} 0.86; $[\alpha]^{25}D$ −10.4° (c 1.0, methanol).

Anal. Calcd for $C_{25}H_{32}N_2O_7$: C, 63.54; H, 6.83; N, 5.93. Found: C, 63.20; H, 6.83; N, 6.01.

***N*-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine [Z-Asn(Dmb)-OH].**—N-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine α-methyl ester (4.3 g, 10 mmol) in dioxane (50 ml) and 1 N NaOH (11 mmol) were kept at 23–25° for 90 min. After evaporation, the resulting residue was dissolved in water, and the solution was acidified with 1 N HCl and then extracted with ethyl acetate. The extracts were washed with water, dried, and evaporated. Crystallization from ethyl acetate gave the product (3.4 g, 81%): mp 152–154°; R_{FA} 0.88, R_{FC} 0.77; $[\alpha]^{27}D$ +3.2° (c 1.0, methanol).

Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.56; H, 5.80; N, 6.72. Found: C, 60.34; H, 5.70; N, 6.71.

The dicyclohexylammonium salt had mp 161–162°.

Anal. Calcd for $C_{33}H_{47}N_3O_7$: C, 66.31; H, 7.92; N, 7.03. Found: C, 66.45; H, 7.70; N, 7.05.

***N*-*tert*-Butyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine Dicyclohexylammonium Salt [Boc-Asn(Dmb)·DCHA].**—N-*tert*-Butyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine α-benzyl ester (0.94 g, 2 mmol) was hydrogenated in ethanol (15 ml) over 10% palladium on charcoal (0.2 g) for 10 hr. The catalyst was then filtered off and the solution was concentrated. Addition of dicyclohexylamine (0.36 g, 2 mmol) in ether (5 ml) precipitated the corresponding salt, which was filtered and crystallized from ethyl acetate, yielding the product (0.77 g, 68%), mp 124–125°, R_{FC} 0.80.

Anal. Calcd for $C_{30}H_{49}N_3O_7$: C, 63.93; H, 8.50; N, 7.45. Found: C, 63.98; H, 8.47; N, 7.51.

***N*-*tert*-Butyloxycarbonyl-N^α-(2,4-dimethoxybenzyl)-L-isoasparagine Dicyclohexylammonium Salt [Boc-Asp(·DCHA)-NH-Dmb].**—This compound (mp 166–167°, R_{FB} 0.70, R_{FC} 0.75) was prepared similarly in 85% yield.

Anal. Calcd for $C_{30}H_{49}N_3O_7$: C, 63.93; H, 8.50; N, 7.45. Found: C, 64.02; H, 8.45; N, 7.58.

***N*-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine p-Nitrophenyl Ester [Z-Asn(Dmb)-ONp].**—N,N'-Dicyclohexylcarbodiimide (1.7 g, 8.2 mmol) was added at 0° to a stirred solution of N-benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine (3.4 g, 8.2 mmol) and p-nitrophenol (1.36 g, 10 mmol) in dimethylformamide (40 ml). The mixture was kept at 0° for 3 hr and then filtered. After evaporation under reduced pressure, the residue was crystallized from ethanol, yielding the product (3.1 g, 70%), mp 148–149°, R_{FC} 0.9.

Anal. Calcd for $C_{27}H_{27}N_3O_9$: C, 60.33; H, 5.06; N, 7.81. Found: C, 60.31; H, 5.06; N, 7.86.

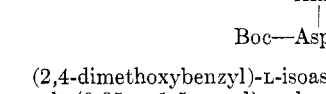
***N*-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparaginyl-L-alanine Methyl Ester [Z-Asn(Dmb)-Ala-OMe].**—N-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine p-nitrophenyl ester (5.90 g, 11 mmol) was added to a solution of L-alanine methyl ester hydrochloride (1.39 g, 10 mmol) and triethylamine (1.4 ml, 10 mmol) in pyridine (40 ml). The mixture was kept overnight at room temperature and then the solvent was removed under reduced pressure. The residue was washed with ether and crystallized from methanol–ethyl acetate. The product had mp 181–182° (3.7 g, 74%), R_{FA} 0.77, R_{FC} 0.70.

Anal. Calcd for $C_{25}H_{31}N_3O_8$: C, 59.88; H, 6.23; N, 8.38. Found: C, 59.77; H, 6.18; N, 8.23.

***N*-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparaginyl-L-alanyl-L-leucyl-L-alanine Methyl Ester [Z-Asn(Dmb)-Ala-Leu-Ala-OMe].**—This compound was prepared similarly in 70% yield starting from N-benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine p-nitrophenyl ester and L-alanyl-L-leucyl-L-alanine methyl ester (obtained by hydrogenolysis of N-benzyloxycarbonyl-L-alanyl-L-leucyl-L-alanine methyl ester): mp 235–236° (from methanol); R_{FA} 0.82, R_{FC} 0.65; $[\alpha]^{27}D$ −9.4° (c 1.0, dimethylformamide); amino acid ratios, Asp 1.00, Ala 1.94, Leu 1.02.

Anal. Calcd for $C_{34}H_{47}N_5O_{10}$: C, 59.55; H, 6.90; N, 10.21. Found: C, 59.47; H, 6.90; N, 10.29.

***N*-*tert*-Butyloxycarbonyl-N^α-(2,4-dimethoxybenzyl)-L-isoasparaginyl-L-alanine Benzyl Ester.**—N-*tert*-Butyloxycarbonyl-N^α-



(2,4-dimethoxybenzyl)-L-isoasparagine dicyclohexylammonium salt (0.85 g, 1.5 mmol) and L-alanine benzyl ester hydrochloride (0.33 g, 1.5 mmol) were dissolved in methylene chloride (20 ml). After 15-min stirring, N,N'-dicyclohexylcarbodiimide (0.315 g, 1.5 mmol) was added at 0°, and the mixture was kept at 0° for 12 hr and then filtered. After evaporation under reduced pressure, the residue was crystallized from ethyl acetate, yielding the product (0.57 g, 70%): mp 140–141°; R_{FA} 0.80, R_{FC} 0.70; $[\alpha]^{27}D$ −15.8° (c 1.0, dimethylformamide).

Anal. Calcd for $C_{28}H_{37}N_3O_8$: C, 61.86; H, 6.86; N, 7.73. Found: C, 61.86; H, 6.78; N, 7.76.

***N*-*tert*-Butyloxycarbonyl-N^α-(2,4-dimethoxybenzyl)-L-isoasparaginyl-L-alanine.**—N-*tert*-Butyloxycarbonyl-N^α-(2,4-dimeth-

oxybenzyl)-L-isoasparagine-L-alanine benzyl ester was hydrogenated similarly to the N-*tert*-butyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine benzyl ester in 88% yield, mp 194–195° crystallized from ethanol–ether, R_{FA} 0.70, R_{FC} 0.64.

Anal. Calcd for $C_{21}H_{31}N_3O_8$: C, 55.63; H, 6.89; N, 9.26. Found: C, 55.49; H, 6.87; N, 9.29.

***N*$^\beta$-(2,4-Dimethoxybenzyl)-L-asparagine [H-Asn(Dmb)-OH].**—N-Benzyloxycarbonyl-N^β-(2,4-dimethoxybenzyl)-L-asparagine (0.83 g, 2 mmol) in acetic acid (20 ml) was hydrogenated over 10% palladium on charcoal (0.25 g) for 3 hr. The catalyst was filtered off and the solution was evaporated. Crystallization from methanol gave the product (0.64 g, 82%), mp 230–231°, R_{FA} 0.61, R_{FB} 9.72.

Anal. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.50; H, 6.07; N, 9.94.

***N*$^\alpha$-(2,4-Dimethoxybenzyl)-L-isoasparagine (H-Asp-NH-Dmb).**—N-*tert*-Butyloxycarbonyl-N^α-(2,4-dimethoxybenzyl)-L-isoasparagine dicyclohexylammonium salt (0.84 g, 1.5 mmol) was treated with 1 N HCl–acetic acid (10 ml) for 30 min. After evaporation, the residue was crystallized from ethanol–ether, yielding the product (0.28 g, 69%), mp 160–162°, R_{FA} 0.65, R_{FB} 0.76.

Anal. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.48; H, 6.09; N, 9.92.

B. N-(2,4-Dimethoxybenzyl)-L-glutamine Derivatives and Peptides. N-Benzyloxycarbonyl-N^γ-(2,4-dimethoxybenzyl)-L-

(17) H. G. Viehe, *Angew. Chem.*, **76**, 571 (1964).

(18) A. S. vanMourik, E. Harryvan, and J. F. Arens, *Recl. Trav. Chim. Pays-Bas*, **84**, 1344 (1965).

glutamine α-Methyl Ester [Z-Gln(Dmb)-OMe].—Freshly distilled 1-diethylamino-1-propyne[17] (2.2 g, 20 mmol) in 20 ml of methylene chloride was added dropwise at 5–10°, during 30 min to a stirred solution of α-methyl-*N*-benzyloxycarbonyl-L-glutamate (5.9 g, 20 mmol) and 2,4-dimethoxybenzylamine (3.34 g, 20 mmol) in 80 ml of methylene chloride. The mixture was kept under stirring at 20–25° for 30 min; then the solvent was evaporated and the residue was extracted with ethyl acetate. The extracts were washed with aqueous sodium bicarbonate, 10% aqueous citric acid, and water, then dried and evaporated. Crystallization of the residue from ethyl acetate–petroleum ether (bp 60–80°) gave the product (6.2 g, 70%): mp 125°; R_{FA} 0.79, R_{FC} 0.7; $[\alpha]^{27}D$ −11.9° (*c* 1.0, methanol).

Anal. Calcd for $C_{28}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 61.95; H, 6.45; N, 6.23.

α-Benzyl-*N*-*tert*-butyloxycarbonyl-L-glutamate Dicyclohexylammonium Salt [Boc-Glu(·DCHA)-OBzl].—Freshly distilled 1-diethylamino-1-propyne[17] (3.78 g, 34 mmol) in 20 ml of anhydrous tetrahydrofuran was added dropwise at 5–10° over 30 min to a stirred solution of *N*-*tert*-butyloxycarbonyl-L-glutamic acid (8.4 g, 34.5 mmol) in anhydrous tetrahydrofuran (20 ml). Stirring was continued for 15 min and then benzyl alcohol (10.8 g, 100 mmol) was added; the mixture was treated dropwise with dicyclohexylamine (6.24 g, 34.5 mmol) in ether (100 ml) and was kept at room temperature overnight. The solid precipitate was collected, washed with ether, and crystallized from ethanol, yielding α-benzyl-*N*-*tert*-butyloxycarbonyl-L-glutamate dicyclohexylammonium salt (10.35 g, 58%), mp 174–175° (lit.[19] mp 172°), R_{FA} 0.80, R_{FC} 0.65.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine α-Benzyl Ester [Boc-Gln(Dmb)-OBzl].**—α-Benzyl-*N*-*tert*-butyloxycarbonyl-L-glutamate (obtained as an oil by acidification of the salt) (9.9 g, 29.4 mmol) and 2,4-dimethoxybenzylamine (4.9 g, 29.4 mmol) in methylene chloride (250 ml) were treated with *N*,*N*′-dicyclohexylcarbodiimide (6.2 g, 30 mmol) at 0°. The mixture was stirred at 0° for 3 hr and then at room temperature for 2 hr. After filtration and evaporation, the resulting residue was dissolved in ethyl acetate, and the solution was washed with aqueous sodium bicarbonate, 10% aqueous citric acid, and water, then dried and evaporated. Crystallization of the residue from ethyl acetate–petroleum ether (bp 30–60°) yielded *N*-*tert*-butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine α-benzyl ester (10 g, 70%): mp 111–112°; R_{FA} 0.90, R_{FC} 0.87; $[\alpha]^{25}D$ −6.5° (*c* 1.0, methanol).

Anal. Calcd for $C_{26}H_{34}N_2O_7$: C, 64.18; H, 7.04; N, 5.75. Found: C, 64.33; H, 7.12; N, 5.78.

***N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine [Z-Gln(Dmb)-OH].**—Sodium hydroxide hydrate (0.64 g, 16 mmol) in water (16 ml) was added dropwise over 10 min at 20–25° to a stirred solution of *N*-benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine α-methyl ester (6.2 g, 14 mmol) in dioxane (90 ml). The mixture was stirred at 20–25° for 60 min and then, after removal of the dioxane, acidified to pH 3 with 1 *N* HCl and extracted with ethyl acetate. The extracts were washed with water, dried, and evaporated, and the residue was treated with dicyclohexylamine (2.54 g, 14 mmol) in ethyl acetate (35 ml), giving the salt (7.5 g, 88%), mp 110°, $[\alpha]^{25}D$ +5.5° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{34}H_{49}N_3O_7$: C, 66.75; H, 7.97; N, 6.86. Found: C, 66.74; H, 8.07; N, 6.86.

The acid, prepared by acidification of the salt with 0.5 *M* citric acid, was crystallized from ethyl acetate, mp 136–137°, R_{FA} 0.85, R_{FC} 0.48.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine [Boc-Gln(Dmb)-OH].**—*N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine α-benzyl ester (6.5 g, 13.4 mmol) in ethanol (100 ml) was hydrogenated at room temperature and pressure over 10% palladium on charcoal (1.2 g) for 8 hr. After removal of the catalyst, the solution was evaporated and the residue was dissolved in ethyl acetate. The solution was extracted with aqueous sodium bicarbonate. The extracts were acidified with HCl and extracted with ethyl acetate. The extracts were dried and evaporated and the resulting residue was crystallized from ethyl acetate–petroleum ether, yielding the product (4.65 g, 88%), mp 110–111°, R_{FA} 0.75, R_{FC} 0.68.

Anal. Calcd for $C_{19}H_{28}N_2O_7$: C, 57.56; H, 7.12; N, 7.07. Found: C, 57.43; H, 7.22; N, 7.18.

(19) E. Schröder and E. Klieger, *Justus Liebigs Ann. Chem.*, **673**, 196 (1964).

***N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine *p*-Nitrophenyl Ester [Z-Gln(Dmb)-ONp].**—This compound was prepared similarly to the asparagine analog in 72% yield: mp 149–150° (from ethanol); R_{FC} 0.90; $[\alpha]^{25}D$ −16.0° (*c* 1.0, methanol).

Anal. Calcd for $C_{28}H_{29}N_3O_9$: C, 60.97; H, 5.30; N, 7.62. Found: C, 60.88; H, 5.43; N, 7.52.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine *p*-Nitrophenyl Ester [Boc-Gln(Dmb)-ONp].**—This compound was prepared similarly in 90% yield: mp 134–135°; R_{FC} 0.90; $[\alpha]^{25}D$ −18.1° (*c* 1.0, methanol).

Anal. Calcd for $C_{25}H_{31}N_3O_9$: C, 58.01; H, 6.04; N, 8.12. Found: C, 57.86; H, 6.31; N, 8.24.

***N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine Methyl Ester [Z-Gln(Dmb)-Ala-OMe].**—This was prepared similarly to the asparagine analog in 70% yield: mp 179–180° (from ethyl acetate), R_{FA} 0.75, R_{FC} 0.65; $[\alpha]^{25}D$ −6.3° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{36}H_{33}N_3O_8$: C, 60.57; H, 6.45; N, 8.15. Found: C, 60.47; H, 6.47; N, 8.16.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine Methyl Ester [Boc-Gln(Dmb)-Ala-OMe].**—This peptide was prepared similarly in 86% yield: mp 133–134° (from ethyl acetate–petroleum ether); R_{FA} 0.82, R_{FC} 0.71; $[\alpha]^{25}D$ 7.0° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{23}H_{35}N_3O_8$: C, 57.37; H, 7.33; N, 8.73. Found: C, 57.37; H, 7.35; N, 8.57.

***N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine [Z-Gln(Dmb)-Ala-OH].**—*N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester (1.0 g, 2 mmol) in dioxane (30 ml) and 0.5 *N* NaOH (5 mmol) were kept at 23–25° for 90 min. The mixture was acidified with 1 *N* HCl, evaporated, and then extracted with ethyl acetate. The extracts were washed with water, dried, and evaporated. Crystallization from ethyl acetate gave the product (0.80 g, 80%): mp 193–194°; R_{FA} 0.71, R_{FC} 0.55; $[\alpha]^{25}D$ −1.9° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{25}H_{31}N_3O_8$: C, 59.87; H, 6.23; N, 8.38. Found: C, 59.51; H, 6.29; N, 8.30.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine [Boc-Gln(Dmb)-Ala-OH].**—This compound was prepared similarly in 93% yield: mp 159–160° (from ethanol–ether); R_{FA} 0.68, R_{FC} 0.54; $[\alpha]^{25}D$ 1.2° (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{22}H_{33}N_3O_8$: C, 56.52; H, 7.12; N, 8.99. Found: C, 56.87; H, 6.91; N, 8.84.

***N*-*tert*-Butyloxycarbonyl-L-alanyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine Methyl Ester [Boc-Ala-Gln(Dmb)-Ala-OMe].**—*N*-Benzyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanine methyl ester (0.21 g, 0.41 mmol) in 90% aqueous acetic acid (25 ml) was hydrogenated at room temperature and pressure over 10% palladium on charcoal (0.05 g) for 4–5 hr. After removal of the catalyst, the solution was evaporated and the residue was dissolved in pyridine (20 ml). To this solution *N*-*tert*-butyloxycarbonyl-L-alanine *p*-nitrophenyl ester (0.155 g, 0.5 mmol) was added and the mixture was kept at room temperature for 40 hr. Then the solvent was removed under reduced pressure and the residue was extracted with ethyl acetate. The extracts were washed with aqueous sodium bicarbonate, 10% aqueous citric acid, and water, then dried and evaporated. Crystallization of the residue from ethyl acetate–ether gave the product (0.17 g, 75%), mp 126–127°, R_{FA} 0.64, R_{FC} 0.57.

Anal. Calcd for $C_{26}H_{40}N_4O_9$: C, 56.51; H, 7.28; N, 10.14. Found: C, 56.87; H, 7.23; N, 10.18.

L-Valyl-L-valine *tert*-Butyl Ester (H-Val-Val-OtBu).—*N*,*N*′-Dicyclohexylcarbodiimide (3.7 g, 18 mmol) in methylene chloride (10 ml) was added at 0° to a stirred solution of L-valine *tert*-butyl ester (3.1 g, 18 mmol) and *N*-benzyloxycarbonyl-L-valine (4.5 g, 18 mmol) in methylene chloride (50 ml). The mixture was kept at 20–22° for 20 hr and then filtered. The filtrate was washed with aqueous sodium bicarbonate, 10% aqueous citric acid, and water, then dried and evaporated. The residue in ethanol (60 ml) was hydrogenated at room temperature and pressure over 10% palladium on charcoal (1.5 g) until evolution of CO_2 ceased (5 hr). After removal of the catalyst, the solution was evaporated to give an oil (4.28 g), R_{FA} 0.47, R_{FB} 0.55.

***N*-Benzyloxycarbonyl-L-alanyl-L-valyl-L-valine *tert*-Butyl Ester (Z-Ala-Val-Val-OtBu).**—*N*-Benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester (5.5 g, 16 mmol) was added to a solution of

L-valyl-L-valine *tert*-butyl ester (4.28 g, 15.8 mmol) in pyridine (40 ml). The mixture was kept at room temperature for 40 hr and then the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed with aqueous sodium bicarbonate, 10% aqueous citric acid, and water, then dried and evaporated. Crystallization from ethyl acetate–petroleum ether gave the product (4.47 g, 60%), mp 150–151°, R_{FA} 0.70, R_{FC} 0.87.

Anal. Calcd for $C_{25}H_{39}N_3O_6$: C, 62.87; H, 8.23; N 8.8 0. Found: C, 62.86; H, 8.06; N, 8.58.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanyl-L-valyl-L-valine *tert*-Butyl Ester [Boc-Gln(Dmb)-Ala-Val-Val-OtBu].**—*N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutamine *p*-nitrophenyl ester (0.7 g, 1.35 mmol) was added to a solution of L-alanyl-L-valyl-L-valine *tert*-butyl ester (0.47 g, 1.35 mmol, obtained by hydrogenolysis of the corresponding *N*-benzyloxycarbonyl derivative) in pyridine (15 ml). The mixture was kept at room temperature for 40 hr and then the solvent was evaporated. After ether trituration, the residue was crystallized from ethyl acetate to give the product (0.65 g, 68%), mp 151–152°, R_{FC} 0.84.

Anal. Calcd for $C_{36}H_{59}N_5O_{10}$: C, 59.89; H, 8.24; N, 9.70. Found: C, 59.83; H, 8.08; N, 9.82.

N^{γ}-(2,4-Dimethoxybenzoyl)-L-glutamine [H-Gln(Dmb)-OH].—This product was prepared similarly to the asparagine analog, mp 240–241° (from methanol), R_{FA} 0.56, R_{FB} 0.64.

Anal. Calcd for $C_{14}H_{20}N_2O_5$: C, 56.75; H, 6.80; N, 9.46. Found: C, 56.77; H, 6.83; N, 9.44.

C. Cleavage of the 2,4-Dimethoxybenzyl Protecting Group. L-Asparagine. a.—*N*-Benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine (0.134 g, 0.32 mmol) in 90% aqueous acetic acid (10 ml) was hydrogenated at room temperature and pressure over 10% palladium on charcoal (0.025 g) until evolution of CO_2 ceased. After removal of the catalyst, the solution was evaporated and the residue was dissolved in trifluoroacetic acid (1 ml). The solution was kept at room temperature for 18 hr and then evaporated. The trifluoroacetic salt obtained after ether titration was crystallized from 50% aqueous ethanol (in the presence of a few crystals of sodium acetate) to give L-asparagine (25 mg, 60%): R_{FA} 0.17, R_{FD} 00.8; $[\alpha]^{25}D$ −12° (*c* 2.0, 1 *N* NaOH).

b.—*N*-Benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine or *N*-*tert*-butyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine were added to trifluoroacetic acid (5 ml) and the resulting solution was refluxed for 1 hr. Then the solution was evaporated and the residue was crystallized from 50% ethanol (in the presence of a few crystals of sodium acetate) to give L-asparagine in 75–80% yield.

c.—*N*-Benzyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine or *N*-*tert*-butyloxycarbonyl-N^{β}-(2,4-dimethoxybenzyl)-L-asparagine were treated with anhydrous hydrofluoric acid[20] for 3 hr. Crystallization of the residue from 50% ethanol (in the presence of a few crystals of sodium acetate) gave L-asparagine in 80% yield.

Isoasparagine.—This compound (R_{FA} 0.27, R_{FD} 0.15) was prepared similarly in 70% yield.

L-Glutamine.—This compound (R_{FA} 0.15, R_{FE} 0.55) was prepared similarly in 70% yield. It appeared identical with an authentic sample of L-glutamine.

(20) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.

L-Glutaminyl-L-alanine (H-Gln-Ala-OH).—This peptide was prepared similarly in 75% yield, mp 174–175° (lit.[21] 174–178°) (from acetic acid–water), R_{FA} 0.41, $[\alpha]D$ +8.30° (*c* 1.0, 1 *N* hydrochloric acid).

L-Glutaminyl-L-alanyl-L-valyl-L-valine (H-Gln-Ala-Val-Val-OH).—*N*-*tert*-Butyloxycarbonyl-N^{γ}-(2,4-dimethoxybenzyl)-L-glutaminyl-L-alanyl-L-valyl-L-valine *tert*-butyl ester (0.65 g, 0.9 mmol) was added at 0° to trifluoroacetic acid (10 ml) and the resulting solution was kept at room temperature for 48 hr. Dry ether (50 ml) was added and the peptide trifluoroacetate was collected, dissolved in water (10 ml), and chromatographed on Amberlite IRA-400 in the OH^- form. Crystallization of the residue obtained by evaporation from ethanol yielded the product (30 mg, 80%): R_{FA} 0.45, R_{FB} 0.50; $[\alpha]^{20}D$ −33.88° (*c* 1.0, acetic acid).

Anal. Calcd for $C_{18}H_{33}N_5O_6 \cdot H_2O$: C, 49.88; H, 8.18; N, 16.10. Found: C, 50.0; H, 8.16; N, 16.01.

Registry No.—2,4-Dimethoxybenzaldoxime, 31874-34-7; Dmb-NH_2 HCl, 20781-21-9; Z-Asn(Dmb)-OMe, 31874-64-3; Boc-Asn(Dmb)-OBzl, 31874-36-9; Boc-Asp(Bzl)-NH-Dmb, 31874-37-0; Z-Asn(Dmb)-OH, 31874-38-1; Z-Asn(Dmb)-OH dicyclohexylammonium salt, 31874-39-2; Boc-Asn(Dmb)·DCHA, 32017-42-8; Boc-Asp(·DCHA)-NH-Dmb, 31874-40-5; Z-Asn(Dmb)-ONp, 31874-41-6; Z-Asn(Dmb)-Ala-OMe, 31874-42-7; Z-Asn(Dmb)-Ala-Leu-Ala-OMe, 31874-43-8; H-Asn(Dmb)-OH, 31874-46-1; H-Asp-NH-Dmb, 31874-47-2; Z-Gln(Dmb)-OMe, 31874-48-3; Boc-Glu(·DCHA)-OBzl, 30924-91-5; Boc-Gl(Dmb)-OBzl, 31874-50-7; Z-Gln(Dmb)-OH, 31874-51-8; Boc-Gln(Dmb)-OH, 31874-52-9; Z-Gln(Dmb)-ONp, 31874-53-0; Boc-Gln(Dmb)-ONp, 31874-54-1; Z-Gln(Dmb)-Ala-OMe, 31874-56-3; Boc-Gln(Dmb)-Ala-OMe, 31874-57-4; Z-Gln(Dmb)-Ala-OH, 31874-58-5; Boc-Gln(Dmb)-Ala-OH, 31874-59-6; Boc-Ala-Gln(Dmb)-Ala-OMe, 31874-60-9; H-Val-Val-OtBu, 31874-61-0; Z-Ala-Val-Val-OtBu, 31874-62-1; Boc-Gln(Dmb)-Ala-Val-Val-OtBu, 31874-63-2; H-Gln(Dmb)-OH, 31874-55-2; Z-Gln(Dmb)-OH·DCHA, 31874-65-4; H-Gln-Ala-Val-Val-OH, 31874-66-5; glutamine, 56-85-9; asparagine, 70-47-3; *N*-*tert*-butyloxycarbonyl-N^{α}-(2,4-dimethoxybenzyl)-L-isoasparaginyl-L-alanine benzyl ester, 31874-44-9; *N*-*tert*-butyloxycarbonyl-N^{α}-(2,4-dimethoxybenzyl-L-isoasparaginyl-L-alanine, 31874-45-0.

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(21) E. Sondheimer and R. W. Holley, *J. Amer. Chem. Soc.*, **76**, 2816 (1954).