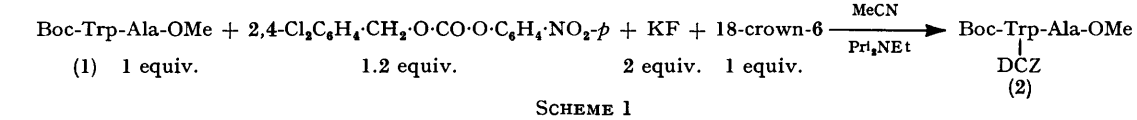
Crown Ethers as Catalysts of Fluoride-anion-mediated Reactions in Peptide Synthesis. Part 1. Protection of Tryptophan by Benzyloxycarbonyl and 2,4-Dichlorobenzyloxycarbonyl Groups

By **Yakir S. Klausner** * and **Michael Chorev**, Department of Organic Chemistry, The Hebrew University, Jerusalem, Israel

Protection of the indole ring of tryptophan by benzyloxycarbonyl and 2,4-dichlorobenzyloxycarbonyl groups is described. Unsolvated fluoride anion, generated by the solubilization of potassium fluoride with 18-crown-6, acts as a powerful base which abstracts the proton from the indole nitrogen atom, and the indolide ion can then be acylated with suitable carbonates. No racemization occurs during acylation. The protecting groups can be removed by catalytic hydrogenation or with liquid hydrogen fluoride or hydrazine.

THE sensitivity of the indole ring of tryptophan toward oxidation, especially in acidic media, is an obstacle in the synthesis of tryptophan-containing peptides. This is especially pronounced in the solid-phase method,[1] where extensive destruction occurs during the repetitive removal of the N^{α}-protecting group. The protection of tryptophan may be achieved in two ways: either by the addition of scavengers such as 2-mercaptoethanol,[2] dithiothreitol,[3] or 1,2-ethanedithiol;[4] or by substitution at the indole nitrogen atom. The formyl group has been successfully employed for *N*-protection.[5,6] It is removable by hydrazine or 1M-ammonium hydrogen carbonate buffer at pH 9, and is stable to liquid hydrogen fluoride.[7] In order to increase the scope of protection methods, we investigated the applicability in conventional synthesis of the benzyloxycarbonyl [8] and the more acid-resistant 2,4-dichlorobenzyloxycarbonyl [9] groups.

For acylation of the indole nitrogen atom a strong base, *e.g.* sodium hydride, has to be applied in order to produce the indolide ion. Experiments with this base did not meet with success. We therefore investigated acylation reactions in the presence of unsolvated (' naked ' [10]) fluoride ion, generated by solubilization of potassium fluoride in organic solvents. This solid–liquid transfer is brought upon by complex formation between crown ethers such as 18-crown-6 [11] or dicyclohexyl-18-crown-6 [11] and the potassium ion. The unsolvated fluoride ion becomes a powerful base, as well as a strong nucleophile.



SCHEME 1

A typical acylation reaction is shown in Scheme 1. Various reaction conditions were examined, from which several conclusions can be drawn. The carboxy-group

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[2] G. R. Marshall in ' Pharmacology of Hormonal Polypeptides and Proteins,' eds. N. Back, R. Paoletti, and L. Martini, Plenum Press, New York, 1968, p. 48.
[3] C. H. Li and D. Yamashiro, *J. Amer. Chem. Soc.*, 1970, **92**, 7608.
[4] J. J. Sharp, A. B. Robinson, and M. D. Kamen, *J. Amer. Chem. Soc.*, 1973, **95**, 6097.
[5] M. Ohno, S. Tsukamoto, and N. Izumiya, *J.C.S. Chem. Comm.*, 1972, **663**; M. Ohno, S. Tsukamoto, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Japan*, 1972, **45**, 2852.
[6] D. Yamashiro and C. H. Li, *J. Org. Chem.*, 1973, **38**, 2594.
[7] S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, 1967, **40**, 2164.
[8] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[9] B. W. Erickson and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1973, **95**, 3757.
[10] C. L. Liotta and H. P. Harris, *J. Amer. Chem. Soc.*, 1974, **96**, 2250.
[11] C. J. Pedersen, *J. Amer. Chem. Soc.*, 1967, **89**, 7017.

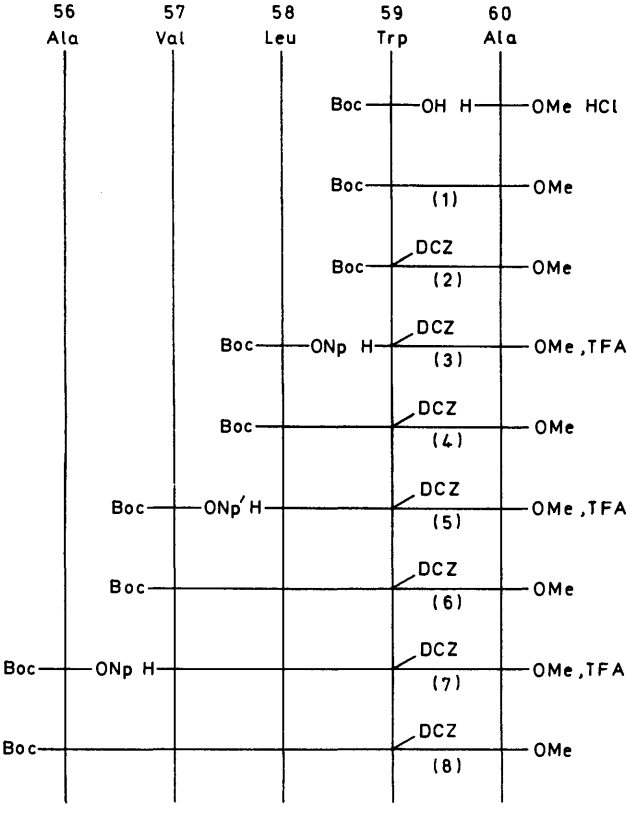
of the N^{α}-protected tryptophan has to be protected, by amide or ester formation. With a free carboxy-group the acylation failed, presumably because it is more difficult to obtain the indolide anion in the presence of carboxylate anion.

The solvent of choice is acetonitrile. In this solvent yields were in the range 60—65%. Use of tetrahydrofuran gave a lower yield (45%). The molar ratio of crown ether to peptide was 1 : 1. Reduction to 0.8 equiv. crown ether still resulted in reasonable, although longer reaction times. With 0.4 equiv. of crown ether the acylation became very slow. Reaction times were usually 24 h, although in most reactions after 4—6 h the starting material was largely acylated, as judged by t.l.c. Acylations with benzyloxycarbonyl chloride resulted in poor yields. Those with benzyloxycarbonyl *p*-nitrophenyl carbonate [12] and 2,4-dichlorobenzyloxycarbonyl *p*-nitrophenyl carbonate were successful. Azides, such as t-butyloxycarbonyl azide,[13] are also applicable, as demonstrated in the synthesis of 1-t-butoxycarbonylindole-3-carbaldehyde.[14] Significantly, because of the greater acidity of this indole derivative, only 0.1 equiv. of crown ether was needed. Acylation with azides has the advantage of readier isolation of products.

In order to try to increase the yields, a reaction was carried out at 37 °C for 24 h. The yield was 55%. A reaction with 2 equiv. of crown ether resulted in 50% yield. Increasing the excess of acylating agent did have beneficial results: the yield was raised to 75%. In most acylations the bulky base ethyldi-isopropylamine [15] was employed. A reaction without tertiary base gave 54% yield. Although this finding indicates that the base is not essential, we prefer to keep the reaction mixture slightly basic.

As fluoride ion is a powerful base the danger of racemisation exists, in our case of either the tryptophan or the alanine residue. In order to test whether the tryptophan was racemised, the 2,4-dichlorobenzyloxycarbonyl group was removed from the dipeptide (2) (Scheme 1) by catalytic hydrogenation and the product was digested with α-chymotrypsin. Complete cleavage was observed, indicating that optical purity was preserved. To find out whether the alanine residue was racemised, the n.m.r. spectra of the dipeptide (1) and of Boc-Trp-Ala-OMe, obtained after catalytic hydrogenation of the dipeptide (2), were compared. According to Weinstein and Pritchard,[16] racemisation of the alanine residue should result in a different chemical shift for the methyl protons (of the D-isomer). However, the spectra of the two dipeptides were identical, indicating that little or no racemisation had occurred.

In addition to its basic properties, the unsolvated fluoride ion is a potent nucleophile. The possibility exists that it attacks the acylating reagent with formation of the corresponding fluoride, which in turn may acylate the indole ring. Thus a reaction between the active carbonate and fluoride ion should liberate *p*-nitrophenolate ion, easily estimated by its strong u.v. absorption at 410 nm. We treated benzyloxycarbonyl *p*-nitrophenyl carbonate with the crown ether and potassium fluoride, without the tryptophan-containing peptide. After correction for hydrolysis, we found that no *p*-nitrophenolate ion had been formed. This evidence, together with the finding that acylation of the more acidic 3-formylindole requires only 0.1 equiv. of crown ether, leads us to infer that in the acylation reaction the fluoride ion acts predominantly as a base.



SCHEME 2

We have reported briefly [17] the protection of tryptophan with the benzyloxycarbonyl group. The 2,4-dichlorobenzyloxycarbonyl group [9] is *ca.* 80 times more resistant to acids and has been recommended for use in combination with the t-butoxycarbonyl group. In the present work we employed it for the synthesis of a simple model peptide which constitutes positions 56—60 in

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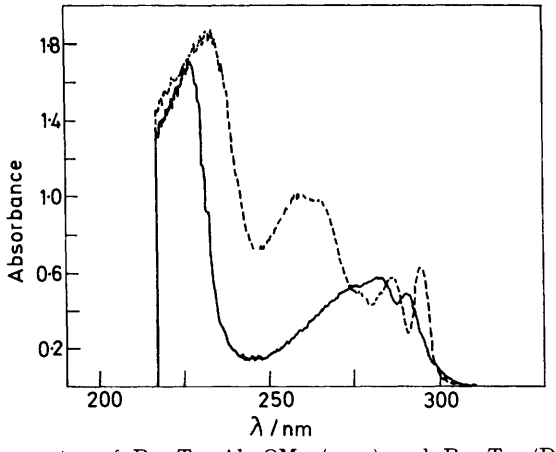
[13] L. A. Carpino, *J. Amer. Chem. Soc.*, 1957, **79**, 4427.
[14] I. Ugi, A. Arora, H. Burghard, G. Eberle, H. Eckert, G. George, G. Gokel, H. Herlinger, E. v. Hinrichs, P. Hoffmann, H. Kleinmann, H. Klusacek, H. L. Lam, D. Marquarding, H. S. Nah, I. Offermann, D. Rehn, S. Stuber, M. Tamasi, R. Urban, L. Wackerle, S. Zahr, and H. V. Zychlinski, in 'Peptides 1974,' Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, Israel Universities Press, Jerusalem, 1975, p. 71.
[15] M. Bodanszky and A. Bodanszky, *Chem. Comm.*, 1967, 591.
[16] B. Weinstein and A. E. Pritchard, *J.C.S. Perkin I*, 1972, 1015.
[17] M. Chorev and Y. S. Klausner, *J.C.S. Chem. Comm.*, 1976, 596.

parsnip (*Plastinaca sativa L.*) cytochrome C.[18] t-Butoxycarbonyltryptophan was condensed with alanine methyl ester by the dicyclohexylcarbodi-imide–1-hydroxybenzotriazole method [19] to yield t-butoxycarbonyltryptophylalanine methyl ester (1) in high yield. The ester (1) was acylated (Scheme 2) and the chain was then lengthened in stepwise manner,[20] by reactions with *p*-nitrophenyl (Np) [21] and *o*-nitrophenyl (Np') esters,[22] catalysed by 1-hydroxybenzotriazole.[23] The coupling of the leucine residue is noteworthy: this required a longer time and larger excess of both active ester and catalyst to achieve complete acylation, probably owing to steric interaction between the 2,4-dichlorobenzyloxycarbonyl group and the indole ring. All the peptides with indole-protected tryptophan were isolated in crystalline state, without the pink–violet colour which results from oxidation products. None responded to the Ehrlich test. Indole-protected peptides exhibit characteristic u.v. spectra (see Figure). The i.r. spectra lack the sharp



U.v. spectra of Boc-Trp-Ala-OMe (——) and Boc-Trp-(DCZ)-Ala-OMe (– – – –) (10^{-4}M in ethanol)

indole NH band at 3 400 cm^{-1}. The 2,4-dichlorobenzyloxycarbonyl group withstands treatment with trifluoroacetic acid, as well as with 0.12N-hydrogen chloride in formic acid [5] and with 4N-hydrochloric acid in ethyl acetate.

Protecting groups were removed by catalytic hydrogenation or treatment with liquid hydrogen fluoride or liquid hydrazine.

EXPERIMENTAL

Thin-layer chromatograms were run on Kieselgel $60F_{254}$ plates (Merck). R_F Values refer to the following systems: (A) methylene chloride–ethyl acetate (3 : 1); (B) chloroform–methanol–acetic acid (18 : 2 : 1); (C) chloroform–methanol (9 : 1); (D) chloroform–light petroleum (1 : 1). The solvents were of reagent grade; dimethylformamide was distilled *in vacuo* from ninhydrin and stored over Linde 4 Å molecular sieves. Spots were detected by u.v. illumination, and by ninhydrin and Ehrlich tests. M.p.s were determined with a Thomas Hoover apparatus. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. U.v. spectra were recorded for solutions in ethanol. Abbreviations follow the I.U.P.A.C.–I.U.B. rules; [24] in addition DCZ = 2,4-dichlorobenzyloxycarbonyl; DCC = dicyclohexylcarbodi-imide; DCU = dicyclohexylurea; DMF = dimethylformamide; HOBt = 1-hydroxybenzotriazole; TFA = trifluoroacetic acid. Amino-acids are of the L-configuration.

N-*t-Butoxycarbonyltryptophylalanine Methyl Ester* (1).—To a cold solution of alanine methyl ester hydrochloride (3.07 g, 22 mmol) in DMF (15 ml) and ethyl acetate (25 ml), t-butoxycarbonyltryptophan (6.08 g, 20 mmol), HOBt (3.38 g, 25 mmol), *N*-methylmorpholine (2.44 ml, 22 mmol), and DCC (4.24 g, 21 mmol) were added. After 1 h stirring at 0 °C and 20 h at room temperature the DCU was filtered off and the organic solvents were removed *in vacuo*. The residue was dissolved in ethyl acetate (200 ml) and the organic phase was washed with water (50 ml), aqueous 5% $KHSO_4$–K_2SO_4 (1 : 1; 2 × 50 ml), saturated aqueous $NaHCO_3$ (2 × 50 ml), and finally saturated aqueous NaCl (3 × 50 ml). Drying (Na_2SO_4), filtration, and evaporation gave an oil. Light petroleum (b.p. 40—60 °C) was added, and the *dipeptide* solidified when kept overnight at 0 °C. It was filtered off, washed, and dried under high vacuum; yield 6.98 g (90%); m.p. 61—62°; R_{F_A} 0.29 (positive Ehrlich test); $[\alpha]_D^{24}$ −17° (*c* 1 in DMF) (Found: C, 61.5; H, 7.1; N, 10.75. $C_{20}H_{27}N_3O_5$ requires C, 61.7: H, 7.0; N, 10.7); $\lambda_{max.}$ 275 (ε 5 200), 282 (5 630), and 290.5 nm (4 900); $\nu_{max.}$ (KBr) 3 400, 3 325, 1 735, 1 680, 1 650, and 1 520 cm^{-1}; δ($CDCl_3$) 7.63 and 7.19 (1 H and 4 H, respectively, m, ArH), 6.41 (1 H, m, amide NH), 5.19 (1 H, m, urethane NH), 4.50 (2 H, m, α-CH), 4.13 (1 H, m, indole NH), 3.63 (3 H, s, OCH_3), 3.26 (2 H, m, CH_2), 1.41 (9 H, s, Bu^t), and 1.26 (3 H, d, CH_3·CH).

N-*t-Butoxycarbonyl*-in-2,4-*dichlorobenzyloxycarbonyltryptophylalanine Methyl Ester* (2).—Into a weighed flask (50 ml), 18-crown-6 (1.056 g, 4 mmol) was placed, followed by acetonitrile (12 ml), in which the polyether dissolved. The dipeptide (1) (1.56 g, 4 mmol), 2.4-dichlorobenzyloxycarbonyl *p*-nitrophenyl carbonate (1.71 g, 5 mmol) and ethyldi-isopropylamine (0.8 ml, 5 mmol) were then added, followed by dry potassium fluoride (464 mg, 8 mmol; predried for 12—16 h at 120 °C and added as quickly as possible). The suspension was stirred with protection from light. After 10—15 min dissolution had occurred (except for a small amount of potassium fluoride). After 24 h the solvent was removed *in vacuo*, and methylene chloride (a few ml) was added and evaporated off. The residue was suspended in a small amount of methylene chloride and chromatographed on a two-layer column [60 × 3 cm silica gel (lower) and 30 × 3 cm neutral alumina (upper)]. The column was equilibrated with the same solvent. After elution with *ca.* 200 ml, 20% ethyl acetate were added to the eluant. Monitoring was performed by u.v. spectroscopy (282 nm) and t.l.c. [system (A)]. The fractions which contained the product were pooled, the solvents were evaporated off *in vacuo*, and the *dipeptide* was precipitated with light petroleum ether, filtered off, and dried in high

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[19] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
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[21] M. Bodanszky, *Nature*, 1955, **175**, 685.
[22] M. Bodanszky, K. W. Funk, and M. L. Fink, *J. Org. Chem.*, 1973, **38**, 3565.
[23] W. König and R. Geiger, *Chem. Ber.*, 1973, **106**, 3626.
[24] Reprinted in the Chemical Society Specialist Periodical Report, ' Amino-acids, Peptides, and Proteins,' ed. G. T. Young, 1972, vol. 4, p. 441.

vacuum (P_2O_5); yield 1.43 g (60%); m.p. 105—106°; R_{F_A} 0.58 (negative Ehrlich test); $[\alpha]_D^{24}$ 0° (*c* 1 in DMF) (Found: C, 56.5; H, 5.6; Cl, 12.25; N, 7.25. $C_{28}H_{31}Cl_2N_3O_7$ requires C, 56.75; H, 5.25; Cl, 11.95; N, 7.1%); $\lambda_{max.}$ 258 (ε 10 300), 264 (10 100), 285.5 (5 900), and 294 nm (6 500); $\nu_{max.}$ (KBr) 3 320, 1 735, 1 680, 1 652, and 1 520 cm^{-1}; δ($CDCl_3$) 8.14 and 7.5 (1 H and 7 H, respectively, m, ArH), 6.60 (1 H, d, amide NH), 5.48 (2 H, s, OCH_2), 5.30 (1 H, d, urethane NH), 4.50 (2 H, m, α-CH), 3.66 (3 H, s, OCH_3), 3.17 (2 H, d, β-CH_2), 1.41 (9 H, s, Bu^t), and 1.30 (3 H, d, CH_3·CH).

N-*t-Butoxycarbonyl*-in-*benzyloxycarbonyltryptophylalanine Methyl Ester* (2a).—The dipeptide (1) (0.78 g, 2 mmol) was acylated with benzyloxycarbonyl *p*-nitrophenyl carbonate as in the preparation of the dipeptide (2). After 24 h the reaction was worked up as described for compound (2), to give the *product* (2a) (0.68 g, 65%); m.p. 147—148°; R_{F_A} 0.55 (negative Ehrlich test); $[\alpha]_D^{24}$ 0°, (*c* 1 in DMF) (Found: C, 64.5; H, 6.05; N, 8.2. $C_{28}H_{33}N_3O_7$ requires C, 64.25; H, 6.35; N, 8.05%); $\lambda_{max.}$ 258.5 (ε 10 000), 264 (9 750), 285.5 (5 600), and 294 nm (6 200); $\nu_{max.}$ (KBr) 3 400, 1 728, 1 690, 1 654, and 1 520 cm^{-1}; δ($CDCl_3$) 8.13 and 7.43 (1 H and 9 H, respectively, m, ArH), 6.43 (1 H, d, amide NH), 5.40 (2 H, s, OCH_2), 5.18 (1 H, d, urethane NH), 4.44 (2 H, m, α-CH), 3.62 (3 H, s, OCH_3), 3.12 (2 H, d, β-CH_2), 1.41 (9 H, s, Bu^t), and 1.26 (3 H, d, CH_3·CH).

t-Butoxycarbonyl-leucyl-in-2,4-*dichlorobenzyloxycarbonyltryptophylalanine Methyl Ester* (4).—The dipeptide (2) (475 mg, 0.8 mmol) was dissolved in TFA (3 ml). After 10 min the acid was removed *in vacuo* and dry ether (50 ml) was added. The clear solution was stored at 0 °C for 1 h, and the precipitate was then filtered off, washed with dry ether and dried for 1 h *in vacuo* (P_2O_5–KOH); yield 454 mg (80%); m.p. 131—132°; R_{F_B} 0.58 (u.v.- and ninhydrin-positive, Ehrlich test negative). All the dipeptide trifluoroacetate (3) (0.75 mmol) was dissolved in DMF (2 ml). t-Butoxycarbonyl-leucine *p*-nitrophenyl ester [25] (358 mg, 1.02 mmol), HOBt (200 mg, 1.5 mmol), and *N*-methylmorpholine (83 μl, 0.75 mmol) were added. After 24 h the reaction was practically complete, as indicated by the negative ninhydrin test. The solvent was removed *in vacuo* and the residue was worked up exactly as described for the dipeptide (2). The *product* was isolated by precipitation with hexane, after cooling for several h at 0 °C; yield 446 mg (94%); m.p. 89—90°; R_{F_A} 0.36; $[\alpha]_D^{24}$ −25° (*c* 1 in DMF) (Found: C, 57.95; H, 6.45; Cl, 10.55; N, 7.75. $C_{34}H_{43}Cl_2N_4O_8$ requires C, 57.75; H, 6.45; Cl, 10.05; N, 7.95%); $\lambda_{max.}$ 258.5 (ε 10 100), 263.5 (9 900), 285.5 (5 750), and 294 nm (6 270); $\nu_{max.}$ (KBr) 3 420, 1 738, 1 643, and 1 523 cm^{-1}.

t-Butoxycarbonylvalyl-leucyl-in-2,4-*dichlorobenzyloxycarbonyltryptophylalanine Methyl Ester* (6).—The tripeptide (4) (300 mg, 0.424 mmol) was dissolved in TFA (2 ml). After 10 min the acid was removed *in vacuo* and dry ether (50 ml) was added. The precipitate was filtered off and dried *in vacuo* for 1 h (P_2O_5–KOH); yield 300 mg; m.p. 216°; R_{F_B} 0.52 (u.v.- and ninhydrin-positive, Ehrlich test negative). All the tripeptide trifluoroacetate (5) was dissolved in DMF (1.5 ml). t-Butoxycarbonylvaline *o*-nitrophenyl ester [22] (186 mg, 0.55 mmol) was added, followed by HOBt (67 mg, 0.5 mmol) and ethyldi-isopropylamine (64 μl). The mixture was kept slightly basic by addition of the same base.. Next day the DMF was decanted and the remaining *crystals* were digested with ether, filtered off, washed with ether, and dried in air; yield 241 mg (70%); m.p. 214—215; R_{F_A} 0.30; $[\alpha]_D^{24}$ −27° (*c* 1 in DMF). To the decanted DMF, ether (40 ml) was added. After cooling for several h the crystals were filtered off, washed with ether and air-dried; yield 65 mg (19%). This fraction had the same physical characteristics as the first; total yield 89% (Found: C, 58.15; H, 6.2; Cl, 8.85; N, 8.45. $C_{39}H_{52}Cl_2N_5O_9$ requires C, 58.1; H, 6.5; Cl, 8.8; N, 8.7%); $\lambda_{max.}$ 258.5 (ε 10 100), 264 (10 000), 285 (5 700), and 294 nm (6 400); $\nu_{max.}$ (KBr) 3 280, 1 740, 1 640, and 1 530 cm^{-1}.

t-Butoxycarbonylalanylvalyl-leucyl-in-2,4-*dichlorobenzyloxycarbonyltryptophylalanine Methyl Ester* (8).—The tetrapeptide (6) (200 mg, 0.25 mmol) was deblocked as described for the tripeptide (4); yield 205 mg (quantitative); m.p. 218°; R_{F_C} 0.23 (u.v.- and ninhydrin-positive, Ehrlich test negative). All the tetrapeptide trifluoroacetate (7) was dissolved in DMF (1.5 ml). t-Butoxycarbonylalanine *p*-nitrophenyl ester [26] (96 mg, 0.31 mmol), HOBt (40 mg, 0.3 mmol), and ethyldi-isopropylamine (40 μl) were added and the mixture was kept basic. Next day ether (50 ml) was added and after several hours at 0 °C the *crystals* were filtered off, washed with ether, and dried in air; yield 190 mg (87%), m.p. 192°, R_{F_A} 0.17; R_{F_C} 0.66; $[\alpha]_D^{24}$ −30° (*c* 1 in DMF) (Found: C, 57.2; H, 6.45; Cl, 8.45; N, 9.2. $C_{42}H_{57}Cl_2N_6O_{10}$ requires C, 57.5; H, 6.55; Cl, 8.1; N, 9.6%); $\lambda_{max.}$ 258.5 (ε 9 500), 263 (9 300), 285.5 (5 400), and 294 nm (5 800); $\nu_{max.}$ (KBr) 3 280, 1 735, 1 660, 1 630, and 1 520 cm^{-1}.

Removal of the in-2,4-*Dichlorobenzyloxycarbonyl Group by Liquid Hydrogen Fluoride*.—The dipeptide (2) (50 mg) was dissolved at 0 °C in liquid hydrogen fluoride (1 ml), in the presence of anisole (0.1 ml) and mercaptoacetic acid (70 mg). After 45 min the hydrogen fluoride was removed *in vacuo*. Dry ether was added and the crystals were filtered off, washed with ether, and dried *in vacuo* (P_2O_5–NaOH); yield 25 mg. T.l.c. showed a single spot (ninhydrin-, u.v.- and Ehrlich-positive), moving similarly (R_{F_B} 0.13) to the deprotected dipeptide (1).

Removal of the in-2,4-*Dichlorobenzyloxycarbonyl Group by Catalytic Hydrogenation*.—The dipeptide (2) (165 mg, 0.28 mmol) was suspended in ethanol (50 ml). Catalytic hydrogenation at atmospheric pressure was performed for 4 h in the presence of 10% Pd–C (45 mg). The catalyst was filtered off, the solution was evaporated *in vacuo*, and light petroleum (30 ml) was added. After several h at 0 °C the product was filtered off, washed with light petroleum, and dried in air and under high vacuum; yield 85 mg (78%); m.p. 61—62°; R_{F_A} 0.29 (positive Ehrlich test); $[\alpha]_D^{24}$ −17° (*c* 1 in DMF).

Removal of the in-*Benzyloxycarbonyl Group with Hydrazine*.—The dipeptide (2a) (150 mg) was suspended in methanol (5 ml). The mixture was flushed with nitrogen and hydrazine hydrate (100%; 0.7 ml) was added. After 7 h the suspension was filtered and the precipitate was washed with dry ether. The filtrate was evaporated *in vacuo* and the residue dried *in vacuo* (conc. H_2SO_4). Next day the residue was triturated with methanol and ether and the resulting *crystals* were filtered off, washed with ether and dried *in vacuo* (conc. H_2SO_4); yield 80 mg; m.p. 177—178° R_{F_C} 0.28; $[\alpha]_D^{24}$ −6° (*c* 1 in DMF) (Found: C, 53.8; H, 7.35; N, 22.75. $C_{19}H_{27}N_5O_4,NH_2NH_2$ requires C, 54.1; H,

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[26] K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *J. Amer. Chem. Soc.*, 1965, **87**, 611.

7.45; N, 23.25%). A sample dried *in vacuo* at 65 °C for 24 h (P_2O_5) gave essentially the same analytical figures.

2,4-Dichlorobenzyloxycarbonyl p-Nitrophenyl Carbonate.—To a cold solution of 2,4-dichlorobenzyl alcohol (1.77 g, 10 mmol) in pyridine (10 ml) *p*-nitrophenyl chloroformate (2.01 g, 10 mmol) was added. After stirring for 1 h in the cold and 4 h at room temperature the solvent was removed *in vacuo*. Ethyl acetate (100 ml) was added to the residue and the organic phase was washed three times with 1.5N-hydrochloric acid and twice with saturated brine, dried (Na_2SO_4), filtered and evaporated *in vacuo*. Propan-2-ol (20 ml) was added, and after cooling the *product* was filtered off and dried in air and then *in vacuo* (P_2O_5); yield 1.52 g (44.5%); m.p 97—98°. A recrystallized sample (propan-2-ol) had the same m.p. (Found: C, 49.3; H, 2.7; Cl, 20.25; N, 3.9. $C_{14}H_9Cl_2NO_5$ requires C, 49.1; H, 2.65; Cl, 20.75; N, 4.1%).

t-Butoxycarbonylindole-3-carbaldehyde.—Indole-3-carbaldehyde (145 mg, 1 mmol) was dissolved in acetonitrile (2 ml). Dicyclohexyl-18-crown-6 (37 mg, 0.1 mmol), triethylamine (0.154 ml, 1.1 mmol), t-butyl azidoformate[13] (0.17 ml, 1.2 mmol), and dry potassium fluoride (116 mg, 2 mmol) were added and the mixture was stirred for 3 h. T.l.c. [system (D)] revealed that the reaction was practically complete. The solvent was removed *in vacuo* and the residue was dissolved in methylene chloride and applied to a column of silica gel (10 × 1 cm), equilibrated with the same solvent. The fractions which contained the product were pooled and evaporated *in vacuo*. Light petroleum was added and the crystals were filtered off, washed with petroleum and air-dried; yield 145 mg. A second crop was isolated from the filtrate (64 mg; total yield 209 mg, 85%); m.p. 127° (lit.,[27,28] 121—122°) (Found: C, 68.7; H, 6.1; N, 5.7. Calc. for $C_{14}H_{15}NO_2$: C, 68.55; H, 6.1; N, 5.7%).

Enzymic Cleavage of Boc-Trp-Ala-OMe with α-Chymotrypsin.—t-Butoxycarbonyltryptophylalanine methyl ester [obtained by catalytic hydrogenation of the dipeptide (2a) (3.9 mg)] was suspended in 0.04N-Tris–HCl buffer, 0.1N in KCl, and 0.01M in $CaCl_2$ (pH 8.1) (5 ml). α-Chymotrypsin (2 mg) was added and the mixture was stirred at 37 °C. After 16 h the starting material had completely disappeared as judged by t.l.c. [system (C)].

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