1.22 (s, CH_3), 1.00 (s, 2 CH_3); MS m/e 303, 305, and 307 (M^+ - CHO), 267, 269, 187, 145 (base peak).

Epoxide 8 to Olefin Epoxide 10. Compound 8 (5 mg) was added to freshly prepared t-BuONa/t-BuOH (2 mL). This mixture was warmed to 60 °C for 18 h, diluted with 10 mL of benzene, and dried over $MgSO_4$. The solvent was removed and the residue passed through a pad of silica gel with benzene to give 3-chloro-4-bromo-Δ^9-chamigrene 7,8-epoxide (**10**; 3.5 mg, 83% yield): ^1H NMR (100 MHz, $CDCl_3$) δ 5.64 (br s, 2 H, $H_{9,10}$), 4.59 (dd, J = 12, 4 Hz, H_4), 2.92 (br s, H_8), 1.66 (s, Me_{12}), 1.57 (s, Me_{13}), 1.07 (s, CH_3), 1.00 (s, CH_3); MS m/e 332, 334, and 336 (M^+), 289, 291, 293, 253, 255, 173, 98 (base peak).

Acid Treatment of Deoxyprepacifenol 7. Deoxyprepacifenol (1.0 mg) was dissolved in 5 mL of dry benzene with a single crystal of p-toluenesulfonic acid and the mixture stirred for 18 h at room temperature. After neutralization with saturated $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$, and the solvent was removed. The residue was passed through a silica gel pad with benzene. The product was analyzed by GC/MS and ^1H NMR, and no kylinone or deoxyprepacifenol was detected.

Silica Treatment of Deoxyprepacifenol 7. Deoxyprepacifenol (1.0 mg) was dissolved in 5 mL of chloroform with activated silica (0.5 g) and the mixture stirred at room temperature for 24 h. The silica was removed by filtration to give deoxyprepacifenol, identical with the starting material.

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Active Esters of 9-Fluorenylmethyloxycarbonyl Amino Acids and Their Application in the Stepwise Lengthening of a Peptide Chain

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Preparation and properties of p-nitrophenyl esters of several 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids are described. The Fmoc derivatives of the hindered amino acids valine and isoleucine were converted to the more reactive o-nitrophenyl esters while N-Fmoc-O-benzyl-L-tyrosine was esterified with pentachlorophenol. In the selection of experimental conditions for coupling reactions an effort was made to keep premature cleavage of the Fmoc group at a minimum. As an example for chain lengthening with Fmoc amino acid active esters the preparation of the C-terminal 7-peptide segment of chicken VIP is described.

The 9-fluorenylmethyloxycarbonyl (Fmoc) group was proposed by Carpino and Han[1] for the protection of the α-amino function in peptide synthesis. A particular advantage of the new protecting group is that it can be removed by basic reagents under mild conditions and yet is quite resistant to acidolysis.[2] These properties of the Fmoc group allow the use of acid-labile protecting groups for the blocking of the C-terminal carboxyl and various side-chain functions. Thus, final deprotection can be carried out with relatively weak acids, again under mild conditions. The Fmoc protection has already been applied, and with considerable success, in solid-phase syntheses of biologically active peptides.[3,4] We report here our experiments on stepwise chain lengthening[5] *in solution*, with nitrophenyl esters[6] of Fmoc amino acids[7] as acylating agents. In this process premature deprotection could be caused by the action of the amino component. Indeed, a moderate loss of Fmoc groups during coupling was experienced[8] in our laboratory, but we expected that under carefully chosen reaction conditions this side reaction can be kept at an acceptable minimum. Therefore, the acylations with active esters of Fmoc amino acids were catalyzed with 1-hydroxybenzotriazole (HOBt).[9] This acidic additive diminished the basicity of the reaction mixture and considerably reduced the time during which the Fmoc derivatives were exposed to the action of the nucleophilic component.

For removal of the Fmoc group the use of diethylamine[1] was also explored. The reagent is more easily removed from the reaction mixtures and less readily forms a tertiary amine by addition to dibenzofulvene (the byproduct of deprotection) than the recommended[1] and somewhat more efficient piperidine. The polymeric derivative of piperazine proposed[10] for the removal of the Fmoc group was not

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(2) The originally observed[1] resistance of the Fmoc group to catalytic hydrogenation could not be confirmed in our laboratory: J. Martinez, J. C. Tolle, and M. Bodanszky, *J. Org. Chem.*, **44**, 3596 (1979).

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(7) The active esters Fmoc-glycine N-hydroxysuccinimide ester and Fmoc-glycine 8-hydroxyquinoline ester were described in ref 1. The p-nitrophenyl ester of Fmoc-L-asparagine was used in solid-phase syntheses[3,4] but its properties were not reported.

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adopted because its elimination by filtration from reaction mixtures, which often have a tendency to become viscous and even gelatinous, could become too cumbersome.

For the preparation of *p*-nitrophenyl esters of Fmoc amino acids a general procedure[11] with dicyclohexylcarbodiimide (DCC) as condensing agent[12] was used. The Fmoc derivatives of the hindered amino acids valine and isoleucine were activated in the form of their *o*-nitrophenyl esters[13] because these are somewhat more reactive.[14] The *o*-nitrophenyl esters were prepared in pyridine; the Fmoc group is quite resistant to this solvent. In the case of *N*-Fmoc-*O*-benzyl-L-tyrosine the pentachlorophenyl ester[15] was prepared, to extend the area of active esters of Fmoc amino acids. Further details of the preparation of active esters are described in the Experimental Section, and their physical constants are summarized in Table I.

Preparation of the C-terminal seven-peptide segment of the vasoactive intestinal polypeptide (VIP)[16] from chicken[17] was used to test the applicability of Fmoc amino acid active esters in syntheses carried out in solution. To avoid diketopiperazine formation during deprotection of the dipeptide derivative, we secured the C-terminal three-peptide derivative [(benzyloxy)carbonyl]-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threonine methyl ester via the DCC-mediated[18] coupling of *Z*-L-Val-L-Leu to L-Thr(*t*-Bu)OCH_3. Racemization was kept at a minimum by the addition of HOBt.[19] The protected three-peptide ester was purified by chromatography and ammonolyzed in methanol. The three-peptide amide was purified by recrystallization and hydrogenated to afford Val-Leu-Thr-(*t*-Bu)-NH_2 which in turn was acylated with Fmoc-Ser(*t*-Bu)-ONp. In a parallel experiment the same protected amino acid was incorporated by the DCC–HOBt procedure, but with slightly less satisfactory results. Some consideration was given also to the adaptation of symmetrical anhydrides[20,21] for coupling, but the large excess used in the reported syntheses[3,4] combined with the inherent loss of part of their molecules and the relatively high cost of Fmoc amino acids were discouraging.

The active ester approach gave satisfactory results also in the subsequent lengthening of the chain with the derivatives of Fmoc-L-asparagine, Fmoc-L-leucine and finally *N*-Fmoc-*O*-*tert*-butyl-L-tyrosine *p*-nitrophenyl esters. The protected seven-peptide amide Fmoc-Tyr(*t*-Bu)-Leu-Asn-Ser(*t*-Bu)-Val-Leu-Thr(*t*-Bu)-NH_2[22] was obtained in good yield and homogeneous form. The experience gained in the application of active esters of Fmoc amino acids for the synthesis of the C-terminal seven-peptide segment of chicken VIP (Chart I) encourages us to carry out the

Table I. Active Esters of 9-Fluorenylmethyloxycarbonyl Amino Acids

active ester (all L config)	yield, %	mp, °C	$[\alpha]^{22}_D$[e]	R_f A	R_f B	formula	calcd C	calcd H	calcd N	found C	found H	found N
Fmoc-Ala-ONp	64	166–167	−41.5	0.86	0.20	$C_{24}H_{20}N_2O_6$	66.7	4.7	6.5	66.4	4.7	6.5
Fmoc-Asp(O-*t*-Bu)-ONp	65	45–50[a]	−34.2	0.82	0.31	$C_{29}H_{28}N_2O_8$				*d*		
Fmoc-Asn-ONp	78	170–172	−37.8	0.45	0.02	$C_{25}H_{21}N_3O_7$	63.2	4.5	8.8	63.4	4.6	9.0
Fmoc-Glu(O-*t*-Bu)-Onp	72	oil	−14.0	0.60	0.45	$C_{30}H_{30}N_2O_8$				*d*		
Fmoc-Gln-ONp	38	182–185	−40.3	0.71	0.03	$C_{26}H_{23}N_3O_7$	63.7	4.9	8.6	63.8	5.2	8.3
Fmoc-Gly-ONp	77	133–136		0.73	0.17	$C_{23}H_{18}N_2O_6 \cdot 0.5H_2O$	64.6	4.5	6.6	64.3	4.7	6.5
Fmoc-Ile-ONo	50	101–103	−45.4	0.76	0.31	$C_{27}H_{26}N_2O_6$	68.3	5.5	5.9	68.6	5.3	5.9
Fmoc-Leu-ONp	85	115–116	−50.0	0.86	0.35	$C_{27}H_{26}N_2O_6$	68.3	5.5	5.9	68.5	5.8	5.9
Fmoc-Lys(Boc)-ONp	71	105–107	−35.3	0.81	0.13	$C_{32}H_{35}N_3O_8$	65.2	6.0	7.1	65.4	6.2	7.3
Fmoc-Met-ONp	75	125–127	−51.6	0.85	0.26	$C_{26}H_{24}N_2O_6S$	63.4	4.9	5.7	63.7	5.1	5.6
Fmoc-Phe-ONp	76	207–209	−43.0	0.85	0.33	$C_{30}H_{24}N_2O_6$	70.9	4.8	5.5	70.6	5.0	5.7
Fmoc-Pro-ONp	62	112–113	−62.7	0.72	0.25	$C_{26}H_{22}N_2O_6$	68.1	4.8	6.1	68.5	5.2	6.2
Fmoc-Ser(*t*-Bu)-ONp	89	54–58[a]	−29.7	0.82	0.24	$C_{28}H_{28}N_2O_7$	66.7	5.6	5.6	66.3	5.8	5.8
Fmoc-Thr(*t*-Bu)-ONp	94	49–53[a]	−23.9	0.72	0.37	$C_{29}H_{30}N_2O_7 \cdot H_2O$[c]	66.9	6.2	5.4	66.2	6.4	5.8
Fmoc-Trp-ONp	84	213–215	−19.6	0.69	0.24	$C_{32}H_{25}N_3O_6$	70.2	4.6	7.7	69.5	4.6	7.6
Fmoc-Tyr(*t*-Bu)-ONp[b]	72	90–91	21.1	0.85	0.36	$C_{34}H_{32}N_2O_7$				*d*		
Fmoc-Tyr(Bzl)-OPcp	61	182–184	−49.6	0.84	0.66	$C_{37}H_{26}NO_5Cl_5$	59.9	3.5	1.9	60.2	3.8	2.1
Fmoc-Val-ONo	69	111–112	−58.4	0.74	0.43	$C_{26}H_{24}N_2O_6$	67.8	5.3	6.1	67.5	5.5	6.0

[a] Amorphous solid. [b] This ester contained slight amounts of impurities (TLC), but no system was found for further purification. [c] The analytical values found suggest the presence of 0.5 mol of EtOH. [d] Characterized by the NMR spectrum. [e] *c* = 1–2, DMF with 1% AcOH.

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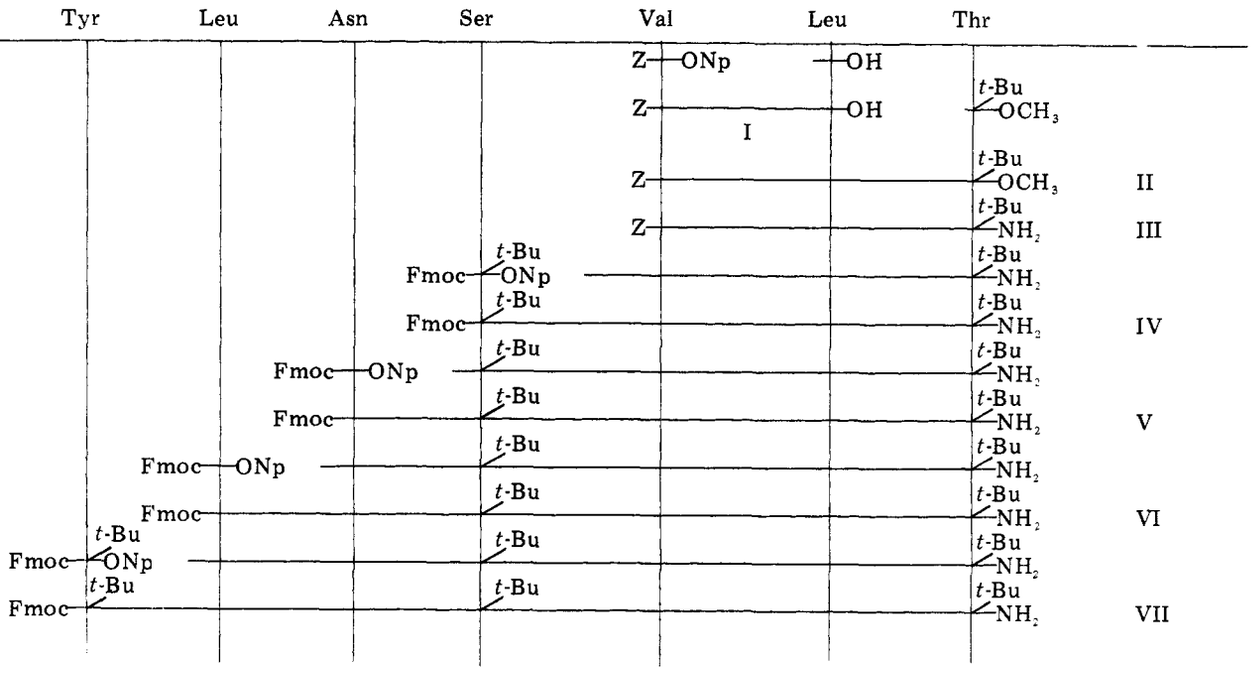
Chart I[a]



[a] Scheme of the synthesis of a protected seven-peptide amide corresponding to the C-terminal sequence of chicken VIP. In the preparation of compound IV and in the subsequent steps the "in situ" technique (cf. ref 13) was applied.

synthesis of longer chains by this procedure.

Experimental Section

Capillary melting points are reported uncorrected. Thin-layer chromatograms (TLC) were run on precoated plates of silica gel (Merck) in the following solvent systems: A, $CHCl_3$–CH_3OH (9:1); B, hexane–EtOAc (7:3); C, $CHCl_3$–CH_3OH (19:1); D, $CHCl_3$–CH_3OH–AcOH (39:3:1). Spots were revealed by their UV absorption and by charring; nitrophenyl esters were detected by holding the plates above a concentrated solution of NH_4OH. On TLC, Fmoc peptides were distinguished from dibenzofulvene or from its addition products (such as 9-fluorenyldiethylamine or *N*-9-fluorenylpiperidine) by exposure of the plates to vapors of piperidine or diethylamine followed by spraying with ninhydrin.

For amino acid analysis, samples were hydrolyzed with constant-boiling HCl in evacuated, sealed ampules at 110 °C for 16 h, evaporated, dissolved in a buffer of pH 2.2, and analyzed by the Spackman–Stein–Moore method[23] on a Beckman-Spinco instrument.

Preparation of Aryl Esters of Fmoc Amino Acids. The protected amino acids were prepared by the procedure of Carpino and Han[1] or purchased from Chemical Dynamics Corp. To a 0.3 M solution of the Fmoc amino acid in EtOAc was added *p*-nitrophenol in 20% excess. The solution was stirred and cooled in an ice–water bath while the calculated amount of DCC was added. Stirring at 0 °C was continued for 0.5 h and for about 2 h at room temperature. The disappearance of DCC was monitored by its IR band at 2100 cm^{-1}, and when necessary more time was allowed for the completion of the reaction. The precipitated *N,N'*-dicyclohexylurea was removed by filtration and the solvent by evaporation. The residue was recrystallized from 95% EtOH containing 1% AcOH. Some Fmoc acids which were not sufficiently soluble in EtOAc were esterified in different solvents, e.g., Fmoc-L-Pro in a 1:1 mixture of EtOAc and tetrahydrofuran. The pentachlorophenyl ester of Fmoc-L-Tyr(Bzl) and the *o*-nitrophenyl esters of Fmoc-L-Val and Fmoc-L-Ile were prepared in pyridine. The *o*-nitrophenyl esters of Fmoc-L-Val and Fmoc-L-Ile and the *p*-nitrophenyl esters of Fmoc-L-Ser(*t*-Bu), Fmoc-L-Asp(*O*-*t*-Bu) and Fmoc-L-Thr(*t*-Bu) were purified by chromatography on a column of silica gel with $CHCl_3$ containing 0.25% AcOH as eluent. The identity of the active esters was confirmed by their NMR spectra, the presence of an active ester CO band was ascertained in the IR spectra.

No effort was made to prepare active esters of the Fmoc derivatives of cysteine and histidine, because of the many possible variations with respect to side-chain protecting groups. In the preparation of the *p*-nitrophenyl ester of Fmoc-L-Gln the formation of several byproducts was observed; one of them was probably the glutarimide derivative. The active ester was soluble in acetone and could thus be separated from the byproduct. The acetone extract was evaporated and the residue recrystallized from 95% EtOH containing 1% AcOH. The *p*-nitrophenyl ester of Fmoc-L-Trp separated with the *N,N'*-dicyclohexylurea. The precipitate was extracted with THF, the extract evaporated, and the residue recrystallized from a 1:1 mixture of THF and EtOH.

The yields, physical properties, and analytical values of the individual active esters are summarized in Table I.

[(Benzyloxy)carbonyl]-L-valyl-L-leucine (I). Finely powdered [(benzyloxy)carbonyl]-L-valine *p*-nitrophenyl ester[24] (5.58 g, 15 mmol) was added to a suspension of L-leucine (3.94 g, 30 mmol) in H_2O (18 mL), THF (44 mL), and 2 N NaOH (13.5 mL). The mixture was stirred, and its pH was kept between 9.6 and 10.4 by the dropwise addition of 2 N NaOH. A total of 6.5 mL of 2 N NaOH was needed to reach constant pH. The mixture was acidified to pH 6.15 with 2 N HCl (9 mL) and extracted (three times) with ether, and the ether extracts were washed (twice) with 0.5 N $KHCO_3$. The aqueous solutions were acidified to pH 2.2; the oily precipitates gradually solidifed. They were disintegrated, filtered, and washed with H_2O. A total of 4.36 g of I was obtained (77%): R_fA 0.30; mp 138–139 °C, $[\alpha]^{22}_D$ −0.9 (*c* 1.6, EtOAc). In the NMR spectrum ($CDCl_3$) the aromatic protons and the methyl protons showed the expected ratios. Anal. Calcd for $C_{19}H_{28}N_2O_5$ (364.4): C, 62.6; H, 7.7; N, 7.7. Found: C, 62.6; H, 7.6; N, 7.4.

[(Benzyloxy)carbonyl]-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threonine Methyl Ester (II). To a stirred solution of I (1.2 g, 3.3 mmol) in CH_2Cl_2 (10 mL) were added *O*-*tert*-butyl-L-threonine methyl ester hydrochloride (Chemalog, 0.68 g, 3 mmol), diisopropylethylamine (0.50 mL, 3 mmol), 1-hydroxybenzotriazole monohydrate (0.46 g, 3 mmol), and DCC (0.62 g, 3 mmol) at 0 °C. After 0.5 h the ice–water bath was removed and the mixture stirred at room temperature for 1.5 h. At that time the IR spectrum of a sample showed the absence of DCC. The precip-

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itated *N,N'*-dicyclohexylurea was removed by filtration and the solution washed with 0.5 N $KHCO_3$, H_2O, 1 N HCl, and H_2O and dried over $MgSO_4$. Evaporation in vacuo left a residue which was chromatographed on a column of silica gel (60 g), starting with $CHCl_3$ as eluent. The product (II) was eluted with 1% CH_3OH in $CHCl_3$: 1.2 g (74%); R_fC 0.64; mp 123–5 °C. Anal. Calcd for $C_{28}H_{45}N_3O_7$ (535.7): C, 62.8; H, 8.5; N, 7.8. Found: C, 62.9; H, 8.7; N, 8.1.

[(Benzyloxy)carbonyl]-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threoninamide (III). A sample of compound II (1.1 g, 2 mmol) was dissolved in MeOH (30 mL), and the solution was saturated with NH_3 at 0 °C. After 5 days at room temperature the precipitated III was collected (0.36 g) and the solution concentrated to a small volume and once more saturated with NH_3. A second crop (0.12 g) of III was obtained. The combined filtrate and washings were evaporated to dryness, and the residue recrystallized from boiling MeOH. The combined product (0.485 g, 46%) melted at 229–231 °C and had R_fC 0.31. Anal. Calcd for $C_{27}H_{44}N_4O_6$ (520.7): C, 62.3; H, 8.5; N, 10.8. Found: C, 62.4; H, 8.7; N, 10.5. The combined mother liquors were chromatographed on a column of silica gel; this yielded 0.3 g of the ester II.

***N*-[[(9-Fluorenylmethyl)oxy]carbonyl]-*O*-*tert*-butyl-L-seryl-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threoninamide (IV). (A).** A sample (100 mg) of compound III was dissolved in boiling 95% EtOH. When the solution cooled, a fluffy precipitate separated. A Pd-black catalyst (10 mg) was added to the suspension which was then stirred in a H_2 atmosphere for 3 h. The white precipitate gradually dissolved during hydrogenation. The catalyst and the solvent were removed, leaving a residue (74 mg, 99%) with R_fA, 0.23.

A preparation of this compound (0.52 g, 1.35 mmol) in DMF (13 mL) was treated, at 0 °C, with HOBt (monohydrate, 0.20 g, 1.35 mmol), Fmoc-L-Ser(*t*-Bu) (0.68 g, 1.8 mmol), and DCC (0.30 g, 1.45 mmol). After 3 h at room temperature the precipitate was removed, the solvent evaporated in vacuo, and the residue triturated with ether. The product (1.1 g) was chromatographed on a column of silica gel (Baker, 2 × 60 cm). The desired material was eluted with a 1:25 mixture of CH_3OH–$CHCl_3$: 0.89 g (88%); mp 191–192 °C, R_fA 0.65. Anal. Calcd for $C_{41}H_{61}N_5O_8$ (752.0): C, 65.5; H, 8.2; N, 9.3. Found: C, 65.6; H, 8.0; N, 9.5.

(B). A sample of the free amine obtained by dehydrogenation (0.58 g, 1.5 mmol) in DMF (12 mL) was treated with HOBt (0.25 g, 1.6 mmol) and Fmoc-Ser (*t*-Bu)-ONp (1.06 g, 2.1 mmol), and the solution was kept at room temperature overnight. The solvent was removed in vacuo and the residue triturated with ether, filtered, and washed with ether. The product was homogeneous (without chromatography) on TLC (R_fA 0.65): 0.98 g (87%); mp 204–205 °C.

[[(9-Fluorenylmethyl)oxy]carbonyl]-L-asparaginyl-*O*-*tert*-butyl-L-seryl-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threoninamide (V). (A). A sample of the protected four-peptide amide (0.54 g, 0.70 mmol, prepared by procedure A in the previous section) was dissolved in a 9:1 mixture of DMF and piperidine (7 mL). After 1 h at room temperature the solvents were thoroughly removed in vacuo, and the residue was dissolved in DMF (7 mL) and treated in the presence of HOBt (0.11 g, 0.72 mmol) with Fmoc-L-Asn-ONp (0.44 g, 0.93 mmol). After 2.5 h at room temperature the solvent was removed and the product precipitated with ether and washed with ether, EtOAc, 95% EtOH, and once more with ether: 0.45 g (77%); R_fA, 0.35; mp 201 °C (no clear melt); Asp, 1.1; Ser, 1.0; Val, 1.0; Leu, 1.00. Anal. Calcd for $C_{45}H_{67}N_7O_{10}$ (866.1): C, 62.4; H, 7.8; N, 11.3. Found: C, 60.9; H, 7.8; N, 12.2

(B). A sample of the protected four-peptide amide (0.85 g, 1.13 mmol) prepared by procedure B of the previous section was deprotected with a 10% solution of diethylamine in DMF (10 mL). After 2 h the solution was concentrated in vacuo to remove the solvent and the secondary amine. The residue was treated with HOBt (0.17 g, 1 13 mmol) and Fmoc-Asn-ONp (0.69 g, 1.45 mmol). After 3 h the acylation was complete (TLC). The solvent was removed and the residue triturated with ether. The residue was collected and washed with ether, EtOAc, and a 1:1 mixture of EtOH and ether to give the product: mp, 211–212 °C; R_fA 0.35; 0.89 g (85%). Anal. Found: C, 61.3; H, 7.8; N, 11.4. Since no solvent system was found for the further purification of this material, it was used as such in the next step.

[[(9-Fluorenylmethyl)oxy]carbonyl]-L-leucyl-L-asparaginyl-*O*-*tert*-butyl-L-seryl-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threoninamide (VI). (A). A sample of the protected five-peptide amide (V; 0.44 g, 0.51 mmol, prepared by procedure A of the previous section) was dissolved in DMF (8 mL) containing 10% diethylamine. After 2 h at room temperature the solvent was removed in vacuo and residue triturated and washed with ether and dried. To the solution of this material in DMF (5 mL) were added Fmoc-L-Leu (0.23 g, 0.66 mmol, Chemalog), HOBt (0.10 g, 0.66 mmol), and DCC (0.11 g, 0.53 mmol). After 2 h at room temperature ether was added, and the precipitate was washed with ether, EtOAc, and ether, and dried in vacuo: 0.52 g (>100%, contains *N,N'*-dicyclohexylurea); mp 196–200 °C, R_fA 0.17, R_fD 0.30 with a spot (probably Fmoc-Leu) at the starting line.

(B). The protected five-peptide amide (V; 0.80 g, 0.93 mmol, prepared by active ester couplings) was deprotected as described above and acylated in DMF (9 mL) in the presence of HOBt (0.143, g, 0.93 mmol) with Fmoc-L-Leu-ONp (0.42 g, 1.3 mmol). After 2 h at room temperature no more amino component could be detected on TLC. The solvent was removed in vacuo and the product precipitated with ether: 0.88 g (97%); mp 218–222 °C (sinters at 216 °C); R_fA 0.17, R_fD 0.30. Amino acid analysis: Asp, 1.0; Thr, 0.90; Ser, 0.90; Val, 0.90; Leu, 2.0. Anal. Calcd for $C_{51}H_{78}N_8O_{11}$ (979.2): C, 62.5; H, 8.0; N, 11.5. Found: C, 62.4; H, 7.9; N, 11.7.

***N*-[[(9-Fluorenylmethyl)oxy]carbonyl]-*O*-*tert*-butyl-L-tyrosyl-L-leucyl-L-asparaginyl-*O*-*tert*-butyl-L-seryl-L-valyl-L-leucyl-*O*-*tert*-butyl-L-threoninamide (VII).** The protected six-peptide amide (VI; 0.44 g, 0.45 mmol, by procedure B of the previous section) was deprotected with a 10% solution of diethylamine in DMF (10 mL). After 2 h at room temperature the solvent was removed in vacuo, the residue triturated with ether and washed with EtOAc and ether. The dried material was dissolved in DMF (5 mL) and HOBt (0.07 g, 0.45 mmol) and Fmoc-L-Tyr(*t*-Bu)-ONp (0.35 g, 0.60 mmol) were added. After 2 h at room temperature no free amino component could be detected with fluorescamine. The next morning ether was added and the precipitate washed with ether and EtOAc and dried to give VII: 0.485 g (90%); mp 240–243 °C; R_fA 0.28. Anal. Calcd for $C_{64}H_{95}N_9O_{13}$ (1198.5): C, 64.1; H, 8.0; N, 10.5. Found: C, 63.9; H, 7.8; N, 10.3.

For further characterization the protecting groups were removed from a sample of compound VII. To a suspension of the protected peptide (105 mg) in DMF (1.5 mL) was added piperidine (0.075 mL). Soon a clear solution was obtained. After 1 h at room temperature the solution was evaporated in vacuo and the residue triturated and washed with ether and finally with EtOAc; R_f (in *n*-BuOH–AcOH–H_2O, 4:1:1) 0.61. The partially deprotected material (96 mg) was dissolved in trifluoroacetic acid (1 mL). After 1 h at room temperature the solvent was evaporated in vacuo and the residue triturated and washed with ether and dried to give 83 mg of product (calcd 81 mg). On TLC in the above mentioned system the major component moved with an R_f value of 0.37, while a minor spot had R_f 0.45. The latter gradually disappeared on further exposure (of a small sample) to trifluoroacetic acid; it is, presumably, an incompletely deprotected peptide, perhaps with an *O*-*tert*-butylthreonine residue.

The crude deprotected material was chromatographed on a column of silica gel (Baker, 1 × 31 cm) in EtOAc–pyridine–AcOH–H_2O (45:20:6:11). The elution was monitored by TLC with the same system. Fractions of 10 mL were collected. The first fraction left only a small residue which was neglected, and the second fraction yielded 36 mg of residue, mostly of the free peptide (R_f 0.40) but also containing some of the faster moving component (R_f 0.52). The third and fourth fractions produced 42 and 12 mg of the product, respectively; R_f 0.40. Amino acid analysis: Asp, 1.0; Thr, 0.9; Ser, 0.8; Val, 1.0; Leu, 2.00; Tyr, 0.8. On LC [Varian 8500; μ-Bondapak C 18 column, 30 × 4 mm, eluted at 2 mL/min with a system of CH_3CN (23%) and 0.01 M ammonium acetate (75%); monitored at 215 nm] the peptide emerged in a single peak with a retention time of 3.6 min.

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Registry No. I, 17708-79-1; II, 71989-07-6; III, 71989-08-7; IV, 71989-09-8; V, 71989-10-1; VI, 71989-11-2; VII, 71989-12-3; F-Ala-OH, 35661-39-3; F-Ala-ONp, 71989-13-4; F-Asp(O-*t*-Bu)-OH, 71989-14-5; F-Asp(O-*t*-Bu)-ONp, 71989-15-6; F-Asn-OH, 71989-16-7; F-Asn-ONp, 71989-17-8; F-Glu(O-*t*-Bu)-OH, 71989-18-9; F-Glu(O-*t*-Bu)-ONp, 71989-19-0; F-Gln-OH, 71989-20-3; F-Gln-ONp, 71989-21-4; F-Gly-OH, 29022-11-5; F-Gly-ONp, 71989-22-5; F-Ile-OH, 71989-23-6; F-Ile-ONo, 71989-24-7; F-Leu-OH, 35661-60-0; F-Leu-ONp, 71989-25-8; F-Lys(BOC)-OH, 71989-26-9; F-Lys(BOC)-ONp, 71989-27-0; F-Met-OH, 71989-28-1; F-Met-ONp, 71989-29-2; F-Phe-OH, 35661-40-6; F-Phe-ONp, 71989-30-5; F-Pro-OH, 71989-31-6; F-Pro-ONp, 71989-32-7; F-Ser(*t*-Bu)-OH, 71989-33-8; F-Ser(*t*-Bu)-ONp, 71989-34-9; F-Thr(*t*-Bu)-OH, 71989-35-0; F-Thr(*t*-Bu)-ONp, 71989-36-1; F-Trp-OH, 35737-15-6; F-Trp-ONp, 71989-37-2; F-Tyr(*t*-Bu)-OH, 71989-38-3; F-Tyr(*t*-Bu)-ONp, 71989-39-4; F-Tyr(Bzl)-OH, 71989-40-7; F-Tyr(Bzl)-OPcp, 71989-41-8; F-Val-OH, 68858-20-8; F-Val-ONo, 71989-42-9; [(benzyloxy)carbonyl]-L-valine *p*-nitrophenyl ester, 10512-93-3; L-leucine, 61-90-5; *o*-*tert*-butyl-L-threonine methyl ester HCl, 71989-43-0; pentachlorophenol, 87-86-5; *p*-nitrophenol, 100-02-7; *o*-nitrophenol, 88-75-5.

Halogenation of N-Oxygenated Pyrazoles. Preparation of N-Oxygenated 4-Halopyrazole and 4,4-Dihalo-4*H*-pyrazole Derivatives

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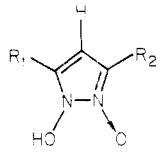
Reaction of some 1-hydroxypyrazoles and 1-hydroxypyrazole 2-oxides with iodine or with 1 equiv of *N*-chloro- or *N*-bromosuccinimide (NCS or NBS) gives high yields of the 4-halo derivatives. With 2 equiv of NBS or NCS or with *tert*-butyl hypochlorite the products are 4,4-dihalo-4*H*-pyrazole 1-oxides or 1,2-dioxides. Reaction of 3,5-diphenylpyrazole with 2 equiv of *tert*-butyl hypochlorite gives 1,4-dichloro-3,5-diphenylpyrazole, which rearranges to 4,4-dichloro-3,5-diphenyl-4*H*-pyrazole. Silver ion assisted solvolysis of the *gem*-dihalides to form 4-chloro-3*H*-pyrazole derivatives is described.

Freeman and co-workers have recently described the chlorination of 3,4,5-trisubstituted 1-hydroxypyrazoles and 1-hydroxypyrazole 2-oxides to give a series of novel 4-chloro-4*H*-pyrazole derivatives.[2] We wish to report the halogenation of some compounds of types **1** and **2**.[3] These



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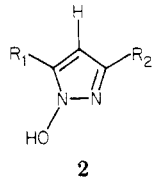

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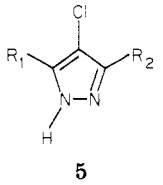
compounds undergo attack at C-4 as anticipated, but the absence of a group at that position permits aromatic substitution to occur, leading to the novel 4-halo-1-hydroxypyrazole 2-oxides and 4-halo-1-hydroxypyrazoles. Further halogenation may occur to give 4,4-dihalo-4*H*-pyrazole derivatives analogous to the 4-chloro-4*H*-pyrazoles reported by Freeman.

Results

Solutions of the 1-hydroxypyrazole 2-oxides (**1**) in aqueous ethanolic potassium carbonate reacted rapidly with 1 molar equiv of iodine–potassium iodide at room temperature, and the 4-iodo-1-hydroxypyrazole 2-oxides (**3**, X = I) precipitated upon acidification. Halogenation using *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) under similar conditions gave the corresponding **3** (X = Cl or Br). The 1-hydroxypyrazoles (**2**) could be halogenated in the same fashion to give 4-halo-1-hydroxypyrazoles (**4**). The results are summarized in Table I.

The properties of **3** are similar to those of other 1-hydroxypyrazole 2-oxides.[3,4] They exhibit limited solubility in most common solvents, give water-soluble alkali metal salts, and form complexes of their conjugate bases with transition-metal ions. The infrared and ^{1}H NMR spectra of **3** resemble those of **1**, except for the absence of the signal for the C-4 hydrogen in the NMR. The compounds **4** give broad absorptions in the infrared at 2400–2700 cm^{-1} and a highly variable signal between δ 8 and 12 in the ^{1}H NMR for the hydroxyl group.

Reduction of **3a** and **3d** with zinc in acetic acid gave the known 4-chloropyrazoles (**5**),[5] while reduction with sodium



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dithionite gave the 4-chloro-1-hydroxypyrazoles **4a** and **4d**. Reduction of **3** (X = Br or I) with either zinc or sodium dithionite resulted in reductive dehalogenation as well as deoxygenation. Dithionite reduction of **3h** gave a low yield of the previously unreported **2** (R_1 = R_2 = Me), but the major product of the reaction was 3,5-dimethylisoxazole, and small quantities of isoxazoles were also observed in some of the other dithionite reductions. Isoxazole formation by rearrangement of N-oxygenated azoles has been

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