Preparation and Applications of Pentafluorophenyl Esters of 9-Fluorenylmethyloxycarbonyl Amino Acids for Peptide Synthesis[a]

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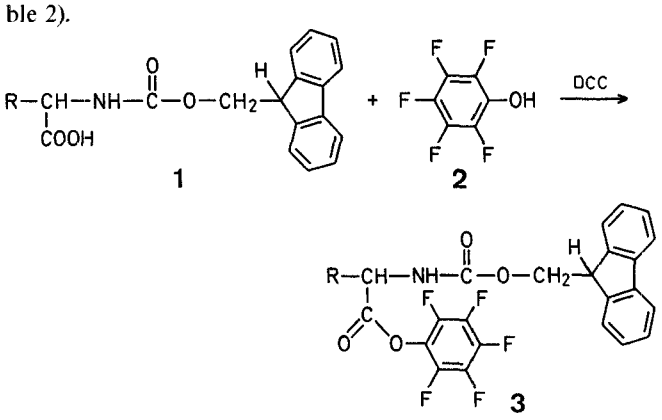
In the recent years, the application of the base-labile 9-fluorenylmethyloxycarbonyl group (Fmoc) for the protection of the amino functions has become a well established method in peptide synthesis[1-5]. Methodological experiments showed that the undesired cleavage[6] of the 9-fluorenylmethyloxycarbonyl group by the free amino component to be acylated resulted in double acylations[7] in coupling reactions carried out in solution. Theoretically this side-reaction can be prevented either by the addition of acidic additives such as 1-hydroxybenzotriazole[8], or by using highly reactive pentafluorophenyl esters[9-13] to significantly increase the rate of the coupling compared to that of the undesired 9-fluorenylmethyloxycarbonyl cleavage.

For this purpose we prepared the pentafluorophenyl esters **3** of several 9-fluorenylmethyloxycarbonyl amino acids **1** (Table 1). Some of the starting 9-fluorenylmethyloxycarbonyl amino acid derivatives **1** are reported here for the first time (Table 2).



The applicability of these active esters avoiding the side-reaction mentioned above has been proved in the step-wise synthesis of 9-fluorenylmethyloxycarbonyl-glycyl-L-tryptophyl-L-leucyl-L-aspartyl-L-phenylalaninamide (Fmoc-Gly-Trp-Leu-Asp-Phe-NH_2) with isolation of all intermediates; overall yield: 45%. The product, tested by conductometric bioassay[14,15], possesses full biological activity as compared to that of the control pentagastrin (Boc-β-Ala-Trp-Met-Asp-Phe-NH_2, Acignost®).

Melting points were determined with a Tottoli (Büchi) apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 141 automatic polarimeter. Thin-layer chromatography was performed

[a] Abbreviations used: Acm = acetylaminomethyl; Boc = *t*-butoxycarbonyl; Bzl = benzyl; Fmoc = 9-fluorenylmethyloxycarbonyl; Pip = L-piperidine-2-carboxylic acid (L-pipecolic acid); Z = benzyloxycarbonyl; and the standard amino acid abbreviations.

on precoated silica gel 60 sheets (Merck). Solvent systems were made by mixing ethyl acetate and a stock solution of pyridine/acetic acid/water = 20:6:11 in the following proportions: S1 ethyl acetate/stock = 9:1, S2 ethyl acetate/stock = 4:1 unless otherwise stated. Spots were detected under a U.V. lamp at 254 nm, followed by spraying with ninhydrin, then with toluidine/KJ after chlorination. I.R. spectra were recorded on a Perkin-Elmer 700 I.R. spectrophotometer. ^{1}H-N.M.R. spectra were obtained on a Varian EM 360 apparatus, using TMS as internal standard.

Pentafluorophenyl Esters of Fmoc-Amino Acids; General Procedure:
To a stirred, ice-cooled solution of the Fmoc-amino acid **1** (2 mmol) and pentafluorophenol (**2**; 2 mmol) in dry dioxan, ethyl acetate, or a mixture of one of these solvents with dimethylformamide (5 to 10 ml), dicyclohexylcarbodiimide (2 mmol) is added and stirring is continued for 1 h at 0 °C and for 1 h at room temperature. Dicyclohexylurea is filtered off and the solvent is evaporated in vacuo at 40 °C using a rotary evaporator. The residue is triturated with *n*-hexane, the solid filtered, and recrystallized (Table 1).

All active esters **3** show a characteristic I.R. absorption at $\nu \approx 1780$ cm^{-1} (KBr) and the expected ^{1}H-N.M.R. spectra ($CDCl_3$; $CDCl_3$ + DMSO-d_6).

Fmoc-Asn-OC$_6$F$_5$:
To a stirred, ice-cooled solution of Fmoc-Asn-OH (780 mg, 2 mmol) and pentafluorophenol (**2**; 1.1 g, 6.6 mmol) in dry dioxan (24 ml) and dimethylformamide (1 ml), dicyclohexylcarbodiimide (230 mg, 2.2

Table 1. Preparation of Pentafluorophenyl Esters of 9-Fluorenylmethyloxycarbonyl (Fmoc)-Amino Acids[a]

Starting Fmoc-Amino Acid[b] **1**	Pentafluorophenyl Ester **3**					
	m.p. [°C]	Recrystallization solvent	Yield [%]	R_f[c]	$[\alpha]_D^{25}$[d]	Molecular Formula[e]
Gly	160–161°	ethyl acetate	99	0.79	—	$C_{23}H_{14}F_5NO_4$ (463.37)
Ala	171–173°	ethyl acetate/*n*-hexane	93	0.79	−22.7°	$C_{24}H_{16}F_5NO_4$ (477.39)
Val	122–123°	ethyl acetate/*n*-hexane	85	0.81	−21.9°	$C_{26}H_{20}F_5NO_4$ (505.45)
Leu	114–116°	ethyl acetate/*n*-hexane	96	0.82	−25.7°	$C_{27}H_{22}F_5NO_4$ (519.47)
Ile	96–98°	*n*-hexane	78	0.77	−13.4°	$C_{27}H_{22}F_5NO_4$ (519.47)
Pro	127–129°	ethyl acetate	82	0.81	−59.2°	$C_{26}H_{18}F_5NO_4$ (503.43)
Pip	95–96°	ethyl acetate/*n*-hexane	55	0.77	−45.8°	$C_{27}H_{20}F_5NO_4$ (517.45)
Phe	154–157°	ethyl acetate	93	0.81	−20.3°	$C_{30}H_{20}F_5NO_4$ (553.49)
Trp	185–186°	ethyl acetate/*n*-hexane	85	0.73	−42.1°	$C_{32}H_{21}F_5NO_4$ (592.53)
Tyr(C_4H_9-*t*)	76–78°	ethyl acetate/*n*-hexane	61	0.83	−12.7°	$C_{34}H_{28}F_5NO_5$ (625.60)
Met	102–104°	ethyl acetate/*n*-hexane	93	0.83	−12.6°	$C_{26}H_{20}F_5NO_4S$ (537.51)
Cys(Bzl)	132–134°	ethyl acetate/*n*-hexane	89	0.78	−31.0°	$C_{31}H_{22}F_5NO_4S$ (599.58)
Cys(Acm)	157–158°	ethyl acetate	78	0.62	−32.6°	$C_{27}H_{21}F_5NO_5S$ (580.54)
Lys(Z)	106–108°	ethyl acetate/*n*-hexane	93	0.76	−10.5°	$C_{35}H_{29}F_5N_2O_6$ (668.62)
Lys(Boc)	89–93°	ethyl acetate/*n*-hexane	85	0.81	−14.2°	$C_{32}H_{31}F_5N_2O_6$ (634.61)
Asn	164–165°	ethyl acetate/*n*-hexane	96	—[f]	−13.1°[g]	$C_{25}H_{17}F_5N_2O_5$ (520.42)
Gln	151–153°	ethyl acetate	97	0.55	−19.8°[g]	$C_{26}H_{19}F_5N_2O_5$ (534.44)
Asp(OC_4H_9-*t*)	98–100°	*n*-hexane	89	0.78	−2.5°	$C_{29}H_{24}F_5NO_6$ (577.51)
Asp-OBzl	124–125°	ether/petroleum ether	97	0.82	−5.1°[h]	$C_{32}H_{22}F_5NO_6$ (611.53)
Asp(OBzl)	128–131°	ethyl acetate/diisopropyl ether	75	0.78	−14.0°[h]	$C_{32}H_{22}F_5NO_6$ (611.53)
Glu(OC_4H_9-*t*)	121–123°	ethyl acetate/*n*-hexane	85	0.83	−25.2°	$C_{30}H_{26}F_5NO_6$ (591.54)
Ser	125–130°	ethyl acetate/*n*-hexane	84	0.65	−21.3°	$C_{24}H_{16}F_5NO_5$ (493.39)
Thr	126–128°	ethyl acetate/*n*-hexane	77	0.75	−33.0°	$C_{25}H_{18}F_5NO_5$ (507.42)

[a] All compounds were prepared with the general procedure with exception of Fmoc-Asn-OC$_6$F$_5$.
[b] New derivatives represented in Table 2 were synthesized as described in Ref.[7].
[c] T.L.C. was performed using a mixture of chloroform/methanol/acetic acid = 80:10:1.
[d] *c* 1.0, chloroform, unless otherwise stated.
[e] All compounds gave satisfactory microanalyses: C ±0.30; H ±0.35; N ±0.29; S ±0.15.
[f] The product decomposes during chromatography.
[g] *c* 1.0, dioxan.
[h] *c* 1.0, ethyl acetate.

Table 2. Preparation of the New 9-Fluorenylmethyloxycarbonyl Amino Acid Derivatives[a]

Starting Amino Acid Derivative	9-Fluorenylmethyloxycarbonyl Amino Acid Derivative					
	m.p. [°C]	Recrystallization solvent	Yield [%]	R_f[b]	$[\alpha]_D^{25}$ (*c* 1.0, methanol)	Molecular Formula[c,d]
Thr	amorphous	—	62	0.30	−4.8°	$C_{19}H_{19}NO_5$ (341.37)
Cys(Acm)	amorphous	—	81	0.18	−34.4°	$C_{21}H_{22}N_2O_5S$ (414.49)
Cys(Bzl)	125–126°	ether/*n*-hexane	90	0.60	−40.6°	$C_{25}H_{23}NO_4S$ (433.53)
Asp-OBzl	112–115°	ethanol/water	64	0.60	+4.0°	$C_{26}H_{23}NO_6$ (445.45)
Asp(OBzl)	113–115°	ether/*n*-hexane	81	0.50	−3.5°	$C_{26}H_{23}NO_6$ (445.45)
Pip	147–150°	ether/*n*-hexane	45	0.65	−24.0°	$C_{21}H_{21}NO_4$ (351.40)
Lys(Z)	108–110°	ether/*n*-hexane	81	0.50	−2.0°	$C_{29}H_{30}N_2O_6$ (502.58)

[a] All compounds were prepared as described in Ref.[7].
[b] T.L.C. was performed with a mixture of ethyl acetate/(pyridine/acetic acid/water = 20:6:11) = 9:1.
[c] I.R. and ^{1}H-N.M.R. spectra are in agreement with the structures.
[d] All compounds gave satisfactory microanalyses (C ±0.27; H ±0.33; N ±0.22; S ±0.12).

mmol) is added and stirring is continued for 1 h at 0 °C. The work-up of the reaction mixture is carried out according to the general procedure; yield: 1.0 g (96%); m.p. 150–160 °C.

Fmoc-Gly-Trp-Leu-Asp-Phe-NH_2:

Fmoc-Asp(OC_4H_9-*t*)-Phe-NH_2:

To a stirred suspension of H-Phe-$NH_2 \cdot HBr$[16] (1.67 g, 6.8 mmol) and triethylamine (1.90 ml, 13.6 mmol) in dimethylformamide (10 ml) Fmoc-Asp(OC_4H_9-*t*)-OC_6F_5 (3.92 g, 6.8 mmol) is added and stirring is continued for 10 min. The mixture is then concentrated in vacuo, and a solution of the residue in chloroform (50 ml) is washed with 1 molar hydrochloric acid (3 × 15 ml), then 5% sodium hydrogen carbonate solution (3 × 15 ml), and dried with sodium sulfate. The solvent is evaporated, the residue is recrystallized from methanol (10 ml) resulting in Fmoc-Asp(OC_4H_9-*t*)-Phe-NH_2; yield: 2.94 g (77%); m.p. 174–175 °C; R_f: 0.67 (S1); $[\alpha]_D^{25}$: −27.4° (*c* 1.0, DMF).

Fmoc-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2:

Fmoc-Asp(OC_4H_9-*t*)-Phe-NH_2 (0.56 g, 1.0 mmol) is treated with a 10% dimethylamine solution in dimethylformamide (10 ml) for 5 min, then the solution is concentrated in vacuo. The residue crystallizes by trituration with *n*-hexane to give H-Asp(OC_4H_9-*t*)-Phe-NH_2[16,17]; R_f: 0.35 (S1); m.p. 116–117 °C.

To a stirred solution of this free dipeptide amide in dimethylformamide (5 ml), Fmoc-Leu-OC_6F_5 (0.57 g, 1.1 mmol) and triethylamine (0.14 ml, 1.0 mmol) are added and stirring is continued for 10 min, then the solution is concentrated in vacuo. The residue crystallizes by trituration with ether, giving Fmoc-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2; yield: 0.62 g (93%; calculated on the protected dipeptide amide); m.p. 178–180 °C; R_f: 0.60 (S1). An analytical sample of another batch is recrystallized from methanol; m.p. 193–194 °C; $[\alpha]_D^{25}$: −36.3° (*c* 1.01, dimethylformamide).

Fmoc-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2:

The protected tripeptide amide (0.62 g, 0.93 mmol) is treated with a 10% dimethylamine solution in dimethylformamide (5 ml) for 5 min, then the solution is concentrated in vacuo. The residue is triturated with petroleum ether, then filtered off to give H-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2[16] [R_f: 0.1 (S1)].

To a solution of this compound in dimethylformamide (5 ml), Fmoc-Trp-OC_6F_5 (0.65 g, 1.1 mmol) and triethylamine (0.14 ml, 1.0 mmol) are added. The reaction mixture is stirred for 10 min, then concentrated in vacuo, and trituration of the residue with ethyl acetate results in Fmoc-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2; yield: 0.70 g (88%; based on the protected tripeptide amide); m.p. 186–189 °C; R_f: 0.60 (S1). An analytical sample of another batch triturated with a hot mixture of ethanol (9 ml) and acetic acid (1 ml) melts at 195–196 °C (dec); $[\alpha]_D^{25}$: −32.1° (*c* 0.40, dimethylformamide).

Fmoc-Gly-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2:

Fmoc-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2 (0.70 g, 0.82 mmol) is treated with a 10% dimethylamine solution in dimethylformamide (5 ml) for 5 min, then the solution is concentrated in vacuo. The residue is triturated with petroleum ether, then filtered off to give H-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2; yields: 0.47 g; m.p. 181–184 °C; R_f: 0.20 (S2).

To a solution of the above product (0.47 g) in dimethylformamide (5 ml), Fmoc-Gly-OC_6F_5 (0.50 g, 1.1 mmol) and triethylamine (0.14 ml, 1.0 mmol) are added. The reaction mixture is stirred for 10 min, then concentrated in vacuo. Trituration of the residue with ethyl acetate results in Fmoc-Gly-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2; yield: 0.64 g (85%; based on the protected tetrapeptide amide); m.p. 173–179 °C; R_f: 0.50 (S1). An analytical sample of another batch recrystallized from 90% aqueous ethanol melts at 177–179 °C; $[\alpha]_D^{25}$: −26.1° (*c* 0.82, dimethylformamide).

Fmoc-Gly-Trp-Leu-Asp-Phe-NH_2:

Fmoc-Gly-Trp-Leu-Asp(OC_4H_9-*t*)-Phe-NH_2 (0.37 g, 0.405 mmol) is treated with 3.5 molar hydrochloric acid solution in acetic acid (10 ml) in the presence of mercaptoethanol (1.1 ml) for 30 min, then the solvent is removed in vacuo, and recrystallization of the residue from methanol/water (3:1, 40 ml) results in Fmoc-Gly-Trp-Leu-Asp-Phe-NH_2; yield: 0.29 g (84%); m.p. 204–206 (dec.); R_f: 0.28 (S2). An analytical sample recrystallized from 90% aqueous ethanol melts at 206–208 (dec); $[\alpha]_D^{25}$: −31.8° (*c* 1.0, dimethylformamide). The product contains two impurities of R_f: 0.15 (S2) and R_f: 0.70 (S2), amounting to less than 2% as estimated by T.L.C.

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