One-Pot Liquid-Phase Synthesis of DSIP and 5-Phe-DSIP Using Fluoren-9-ylmethoxycarbonyl-Protected Amino Acid Pentafluorophenyl Esters

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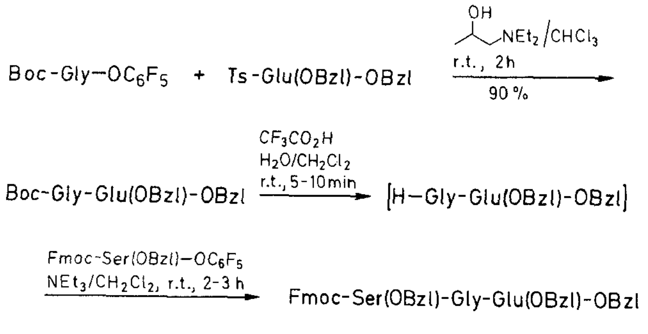
Fmoc-protected amino acid pentafluorophenyl esters are used in a one-pot liquid-phase synthesis of Delta Sleep-Inducing Peptide (DSIP) and 5-Phe-DSIP. Operational simplicity, fast performance high yield, and purity of product are advantages of this procedure which may represent an improved approach towards polypeptide synthesis.

Considerable work has been done on the improvement of peptide synthesis,[3,4] but there are still many problems. For example, in liquid-phase syntheses the times required for reaction and purification may be long and the yields low, and in solid-phase syntheses the final products are often difficult to purify.

The use of *N*-(fluoren-9-ylmethoxycarbonyl)amino acid (Fmoc-amino acid) derivatives in liquid and solid-phase peptide synthesis leads to better yields and purity than the use of Boc-amino acid derivatives.[1,5] For the peptide-coupling step, amino acid pentafluorophenyl esters have been found to be the most reactive derivatives.[2,6] However, only limited work has been done on their application in peptide synthesis.

DSIP (Delta Sleep-Inducing Peptide) is the only endogeneous peptide with obvious delta sleep-inducing activity.[9] Some work has also been done on the synthesis of DSIP analogs.[7,8] We here present an improved, highly efficient one-pot procedure for the synthesis of DSIP and 5-Phe-DSIP, utilizing the synthetic properties of Fmoc-amino acid pentafluorophenyl esters.



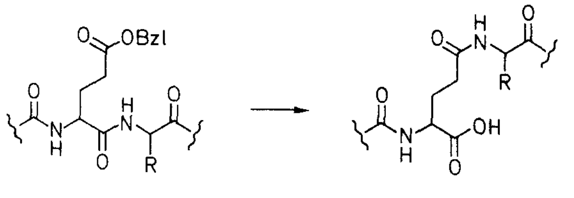
In our first attempt, we applied the classical liquid-phase two-step procedure to the synthesis of the protected tripeptide Fmoc-Ser(OBzl)-Gly-Glu(OBzl)-OBzl, but it was obvious that the reaction components are appropriate for one-pot procedure. Firstly, the peptide-coupling step using the pentafluorophenyl ester is quantitative and very rapid, without formation of any detectable impurity; secondly, the difference in solubilities of the protected amino acids and the resultant peptides in diethyl ether is large enough so that product purification after coupling can be simply performed by washing and precipitation with diethyl ether. Further, the whole synthesis can be carried out in one suitable reaction vessel, i.e., as a one-pot procedure.

One serious problem was encountered in the synthesis of the above tripeptide from Fmoc-Gly-Glu(OBzl)-OBzl. Deprotection of this latter compound with piperidine gave a product which was unsoluble even in dimethylformamide and which was found to be a cyclic lactam, as reported.[10] Later studies proved that this undesired cyclization reaction was rapid and unavoidable; we therefore used Boc-Gly-Glu(OBzl)-OBzl instead of the Fmoc derivative for the synthesis of the tripeptide.

In the present work, we applied the above one-vessel technique to the synthesis of the two nonapeptides:

H-Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu (short spelling: WAGGDASGE)	DSIP
H-Trp-Ala-Gly-Gly-Phe-Ala-Ser-Gly-Glu (short spelling: WAGGFASGE)	5-Phe-DSIP

According to Lit.[8], dipeptides of the type Asp(OBzl)-NH-R may undergo an isomerization as follows:



Both acidic and basic conditions favor this side reaction; however, it can be avoided by protecting the side chain of Asp by a bulky group. In the present syntheses, we did not find this side reaction (by HPTLC analysis of the coupling product, C,H,N analysis, and carboxylase cleavage). There are two possible explanations: firstly, the residue next to Asp is Ala, which has been reported to be sufficiently bulky to hinder the isomerization; secondly, the peptide-coupling and deprotection reactions together require less than 10 minutes in our procedure, this probably being the critical factor in inhibiting isomerization.

By tracing all deprotection and coupling reactions with HPTLC and identifying all intermediates by element and amino acid analysis, we did not observe any UV-detectable side products. In view of the very strong UV absorption of the Fmoc group it may be said that all coupling and deprotecting reactions are quantitative. HPTLC analysis of both deprotected DSIP and 5-Phe-DSIP did not show the presence of side products and this is also in agreement with the assumption of quantitative conversions in all of the coupling and deprotecting reactions.

The total yield, from dipeptide to nonapeptide, is > 87%. According to Lit.[8] the total yield of the synthesis of DSIP using Boc-protected amino acid pentafluorophenyl esters as monomers are 26% and 13% in solid-phase and liquid-phase procedures, respectively. Chemical syntheses of DSIP and its analogs[7] afforded similar results. Thus, our syntheses of DSIP and 5-Phe-DSIP represent a considerable improvement and it may be expected that our one-pot methodology can be advantageously applied to the syntheses of other peptides.

Each cycle of the sequence, consisting of coupling, washing, and deprotection, requires only 30 minutes or even less time whereas in the liquid-phase and solid-phase procedures using Boc-amino acids and other reagents several hours are required for each cycle. Since all coupling and deprotecting reactions of our sequence are nearly quantitative, the purities of the intermediate and final peptides are excellent. In the usual liquid-phase and solid-phase procedures, purification of the intermediate and final products in general is time-consuming whereas in our procedure, the time needed for purification has been reduced to a minimum: only simple washing and precipitation is required.

All reagents were of commercial quality and were taken from freshly opened containers. Reagent-quality solvents were used without further purification. All Boc- and Fmoc-protected amino acid pentafluorophenyl esters were prepared in this laboratory according to Carpino Lit.[1,2], with some modifications. Melting points are uncorrected. Observed rotations were measured for the Na D-line at 25°C using a Perkin-Elmer 141 polarimeter. Amino acid analyses were preformed on a Hitachi 835 analyzer. HPLC analyses were performed on a Varian 5000 liquid chromatograph. TLC was carried out on silica gel plates (Merck) using BuOH/AcOH/H_2O (4:1:1) as solvent system.

Boc-Gly-Glu(*O*Bzl)-*O*Bzl:

Boc-Gly-OC_6F_5 (2.00 g, 5.7 mmol), Ts-Glu(OBzl)-OBzl (2.20 g, 4.4 mmol), and 1-diethylamino-2-propanol (0.82 mL, 4.4 mmol) are dissolved in $CHCl_3$ (15 mL) and this mixture is stirred at room temperature for about 2 h. More $CHCl_3$ is then added and the solution is washed with 5% aq Na_2CO_3 (3 × 50 mL) and water H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated at reduced pressure to give a yellow oil; yield: 1.90 g (90%); $[\alpha]_D^{25}$ $-10.5°$ (DMF).

$C_{26}H_{32}N_2O_7$	calc.	C 64.45	H 6.66	N 5.78
(484.5)	found	63.84	6.93	5.71

Fmoc-Ser(*O*Bzl)-Gly-Glu(*O*Bzl)-*O*Bzl:

Boc-Gly-Glu(OBzl)-OBzl(4.845 g, 10 mmol) is deprotected by stirring with 50% CF_3CO_2H (5 mL) in CH_2Cl_2 (5 mL) for 5–10 min. The solvent is then evaporated to dryness at reduced pressure. The residue is again dissolved in CH_2Cl_2 (5 mL), Fmoc-Ser(OBzl)-OC_6F_5 (6.5 g, 11 mmol) and Et_3N (1.11 g, 11 mmol) are added, and this mixture is stirred at r.t. for 2–3 h. The solvent is then evaporated at reduced pressure and the remaining solid product is recrystallized from EtOAc/petroleum ether.

$C_{46}H_{45}N_3O_9$	calc.	C 70.47	H 5.74	N 5.35
(783.8)	found	69.86	5.72	5.18

One-Pot Synthesis of Peptides; General Procedure:

The Fmoc-protected first unit of the desired peptide chain [Fmoc-amino acid benzyl ester or Fmoc-peptide benzyl ester or, in certain cases, the Boc analog, e.g. Boc-Gly-Glu(OBzl)-OBzl or Fmoc-Ser(OBzl)-Gly-Glu(OBzl)-OBzl; 0.50 mmol] is stirred with piperidine (5 mL)/DMF (5 mL) at r.t. for 5 min. The solvent is then evaporated at reduced pressure and the residue is washed with petroleum ether (bp 30–60°C; 3 × 20 mL). The deprotected peptide is dissolved in DMF (5 mL) and the Fmoc-protected pentafluorophenyl ester (0.6 mmol) of the next amino acid is added. The solution is stirred at room temperature for 5–10 min. The solvent is evaporated at reduced pressure to a volume of 2 mL. To this, Et_2O (20 mL or more) is slowly added dropwise, with stirring. The precipitated product is isolated by suction, washed with Et_2O (3 × 10 mL), and dried with N_2. It can then be deprotected and submitted to the next peptide coupling. All products thus obtained are suitable for analysis without further purification.

Amino Acid Analyses of Protected Intermediates:

	Amino Acid per Molecule					
	D (Asp)	S (Ser)	E (Glu)	G (Gly)	A (Ala)	F (Phe)
Fmoc-DASGE	0.90	0.98	1.00	1.00	0.90	
Fmoc-FASGE		0.95	0.98	1.00	0.90	0.85
Fmoc-GDASGE	1.10	0.98	1.02	2.00	1.17	
Fmoc-GFASGE		0.87	1.03	2.00	0.99	1.05
Fmoc-GGDASGE	1.10	1.09	1.00	3.00	1.02	
Fmoc-GGFASGE		0.87	1.02	3.00	0.99	0.97
Fmoc-AGGDASGE	1.09	1.08	1.13	3.00	2.44	
Fmoc-AGGFASGE		0.94	1.09	3.00	2.30	1.07
Fmoc-WAGGDASGE	1.39	1.26	1.45	3.00	2.22	
Fmoc-WAGGFASGE		1.02	1.20	3.00	2.00	1.14

Deprotection and Purification of DSIP and 5-Phe-DSIP:

The protected peptide DSIP or 5-Phe-DSIP (0.5 mmol) is dissolved in DMF (5 mL) and hydrogenolysed over Pd–C catalyst for 2–4 h. The catalyst is filtered off and the solvent is evaporated to dryness at reduced pressure. The peptide thus obtained is washed with Et_2O (3 × 20 mL) and submitted to HPLC and HPTLC analyses without purification.

DSIP; yield: 87%; powder or solid.

HPLC analysis carried out on a nucleosil 5C 18 column; eluent (gradient): MeCN/0.3% aq CF_3CO_2H (1:9 → 6:4 over 30 min) at a flow rate of 1.0 mL/min at 25°C; retention time 7.04 min; optical detection at $\lambda = 230$ nm.

HPTLC analysis carried out using the solvent system BuOH/AcOH/H_2O (4:1:1) and chlorine/tolidine for coloring; R_f 0.27.

5-Phe-DSIP; yield: > 92%; powder or solid.

HPLC analysis carried out on a nucleosil 5C18 column; eluent (gradient): MeCN/0.3% aq CF_3CO_2H (1:9 → 6:4 over 30 min) at a flow rate of 1.0 mL/min at 25°C; retention time 6.69 min; optical detection at $\lambda = 210$ nm.

HPTLC analysis carried out using the solvent system BuOH/AcOH/H_2O (4:1:1) and chlorine/tolidine for coloring; R_f 0.29.

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