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A New Facile One-Pot Preparation of Pentafluorophenyl (Pfp) and 3,4-Dihydro-4-oxo-1,2,3-benzotriazine-3-yl (Dhbt) esters of Fmoc Amino Acids.

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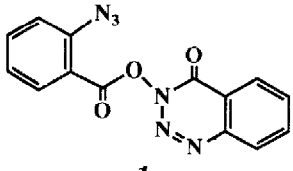
Abstract: A new one-pot procedure for the preparation of pentafluorophenyl and 3,4-dihydro-4-oxo-1,2,3-benzotriazine-3-yl esters of N^{α}-9-fluorenylmethyloxycarbonyl amino acids bearing no side chain protecting groups is described. The method gives the desired activated esters in high yield and purity without use of the highly allergenic DCC.

The use of 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl (Dhbt)[14] esters of N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids has been described to be very advantageous in peptide synthesis.[1-5] However, the preparation of these esters by N,N'-dicyclohexylcarbodiimide (DCC) couplings, was followed by a side reaction which led to the formation of an azido-benzoyl derivative[2,5] (**1**), which itself is a powerful acylating agent. To avoid this disturbing side reaction, conditions had to be controlled rigorously and the activated esters recrystallized carefully. In our work with substituted Dhbt esters[6,7] we found it impossible to obtain the Fmoc amino acid 7-nitro-Dhbt ester by the conventional DCC coupling procedure without the formation of the corresponding azidobenzoyl derivative. This let us to investigate alternative synthetic routes to prepare these activated esters. Carpino et. al.[11] had previously reported the preparation of stable Fmoc amino acid chlorides as reagents for peptide synthesis, and we found these to be promising candidates as intermediates in the preparation of the new activated esters. Thus refluxing of the subtituted HODhbt derivatives with Fmoc valine and thionyl chloride afforded the desired substituted Fmoc valine Dhbt esters in high yield and purity.[7]



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We have now developed this into a general procedure that gives the Dhbt esters of Fmoc amino acids, bearing no acid labile side chain protecting groups, in high yield and purity (Table I). IR-spectra of the crude Dhbt esters dit not exhibit the azide absorption at 2120 cm^{-1}. In the attempt to use the same procedure to prepare Pfp esters a mixture of the desired activated ester and Fmoc amino acid chloride was obtained. However, in a slightly modified procedure, where the intermediate acid chloride is isolated by evaporation of solvent and residual thionyl chloride and then reacted with PfpOH in the precence of a base (pyridine), the Pfp esters were obtained in high yield and purity (Table II).

In conclusion the described method is easy, fast, efficient, and should be well suited for scale up. The desired activated esters is obtained in high yield and purity without the use of DCC - which is a powerfull allergen - thus also avoiding the serious side reaction leading to the azido benzoyl derivative (**1**).

Preparation of Fmoc amino acid Dhbt esters (Scheme I). The Fmoc amino acid (2 mmol), HODhbt (2.2 mmol) and $SOCl_2$ (20 mmol) was suspended in methylene chloride (12 ml) and the mixture refluxed until complete convertion to the Dhbt ester (the reaction is followed by TLC with petroleum ether/EtOAc/AcOH 75:25:5 as eluent). Residual thionyl chloride and solvent was removed *in vacuo* and the crude Fmoc amino acid Dhbt ester crystallized either by adding dry ethyl acetate to the resultant oil (Ala, Met and Phe) or by adding a solution of the crude product in ethyl acetate petroleum ether while stirring (Val, Leu, Ile and Gly). The crude products were analysed by HPLC and contained no byproducts except some residual Fmoc amino acid. Analytical data (table I) are given for compounds that - if necessary - had been freed from residual Fmoc amino acid by passing through a short column of silicagel 60 (230-400 mesh, dried over night at 110° C) with dry EtOAc or EtOAc/petrolcum ether as eluent.

SCHEME I.

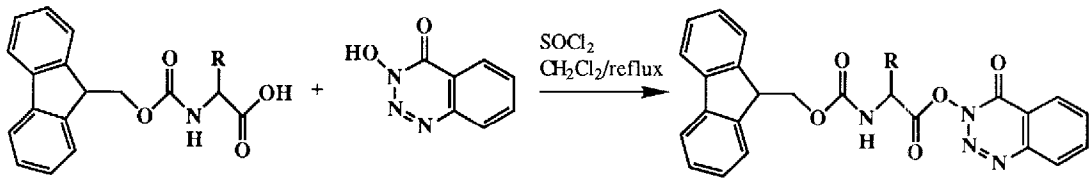

TABLE I.

Compound[a]	Yield (%)	Residual Fmoc amino acid[b] (%)	m.p.[c,d] (°C)	Lit. m.p.[4] (°C)	$[\alpha]_D$[d,e]	Lit. $[\alpha]_D$[4]
Fmoc-Ala-ODhbt	90	0.3	155	156-158	-136.4	-137.0
Fmoc-Gly-ODhbt	90	2.1	157	156-159	----	----
Fmoc-Ile-ODhbt	89	0.3	121-123	122-125	-109.5	-111.0
Fmoc-Leu-ODhbt	91	0.2	82.5-83	83-84	-114.0	-116.0
Fmoc-Met-ODhbt	92	<0.05	139-140	136-138	-106.0	-107.0
Fmoc-Phe-ODhbt	97	1.9	196-199	194-199	-109.7	-110.9
Fmoc-Val-ODhbt	94	1.4	116-116.5	116-118	-124.8	-125.5

a All starting Fmoc amino acids were purchased form MilliGen.

b HPLC of the crude products showed no impurities except for residual free Fmoc amino acid.

c All melting points are uncorrected.

d Analytical data are given for compounds that - if necessary - have been freed from residual Fmoc amino acid by passing the crude product through a short column of silica gel 60 (230-400 mesh dried over night at 110°C) with dry EtOAc or EtOAc/petroleum ether as eluent. Elemental analysis were in agreement with theory; C: ±0.2% H: ±0,2% N: ±0.2%.

e In DMF; c=1.

Preparation of Fmoc amino acid Pfp esters (Scheme II). The Fmoc amino acid (2 mmol) and thionyl chloride (20 mmol) was dissolved/suspended in methylene chloride (12 ml) and the mixture refluxed until complete convertion to the acid chloride (a small sample is diluted with dry methanol thus giving the derived methyl ester and then analysed by TLC with petroleum ether/EtOAc/AcOH 75:25:5 as eluent). Residual thionyl chloride and solvent was removed in vacuo and the acid chloride redissolved together with PfpOH (2.1 mmol) in dry THF (10 ml). The solution was cooled in an ice bath and pyridine (3 mmol) added to the stirred solution which was then diluted to 50 ml with THF. The THF solution was washed twice with a cold NaCl solution and immediately dried over $MgSO_4$-sicc. The solvent was removed *in vacuo* and the resultant oil triturated with petroleum ether. The crude products were analysed by HPLC and contained no byproducts except for some residual Fmoc amino acid. Analytical data (Table II) are given for compounds that - if necessary - had been freed from residual Fmoc amino acid by recrystallization.

SCHEME II.

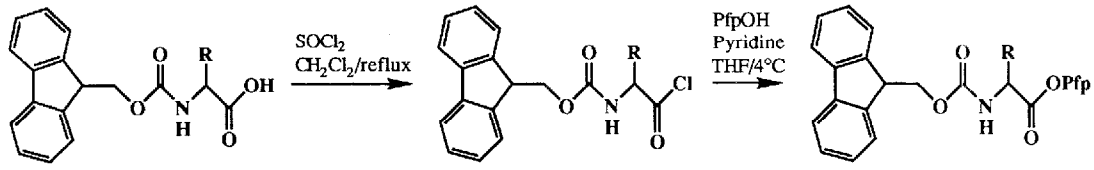

TABLE II.

Compound[a]	Yield (%)	Residual Fmoc amino acid[b] (%)	m.p.[c,d] (°C)	Lit. m.p.[8] (°C)	$[\alpha]_D$[d,f]	Lit. $[\alpha]_D$[8]
Fmoc-Ala-OPfp	92	1.5	179-180	171-173	-22.2	-22.7
Fmoc-Gly-OPfp	93	5.4	157-158	160-161	------	-----
Fmoc-Ile-OPfp	81	0.6	93-94	96-98	-13.5	-13.4
Fmoc-Leu-OPfp	83	1.5	113-114	114-116	-25.4	-25.7
Fmoc-Met-OPfp	90	1.0[d]	109-112	102-104	-13.2	-12.6
Fmoc-Phe-OPfp	88	5.8	153-153.5	154-157	-20.4	-20.3
Fmoc-Pro-OPfp	84	0.4	125-126	127-129	-58.9	-59.2
Fmoc-Val-OPfp	91	4.8	120-121	122-123	-23.1	-21.9

[a] All starting Fmoc amino acids were purchased form MilliGen.

[b] HPLC of the crude products showed no impurities except for residual Fmoc amino acid.

[c] All melting points are uncorrected.

[d] Analytical data are given for compounds that - if necessary - have been freed from residual free Fmoc amino acid by recrystallization. Elemental analysis were in agreement with theory; C: ±0.2% H: ±0,2% N: ±0.2%.

[e] One minor impurity besides Fmoc-Met-OH was seen in the crude product.

[f] In $CHCl_3$; c=1.

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14. Abbrevations used in the text: HODhbt, 3,4-dihydro-5-hydroxy-4-oxo-1,2,3-benzotrazin; PfpOH, pentafluorophenol; Fmoc, 9-fluorenylmethoxycarbonyl, EtOAc, ethyl acetate; DMF, dimethyl

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