NEW TRITYL-ACTIVATED ESTERS FOR PEPTIDE SYNTHESIS*

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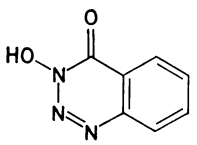
Received January 4, 1990
Accepted March 25, 1990

It has been known for several years that the trityl group (Trt)** is an extremely acid-labile protecting group for peptide synthesis[2-5]. Either the incorporation of N-trityl group or the steric hindrance excercised by this group during peptide coupling has given a confused picture regarding its feasibility in solution or in solid phase synthesis. The fact that this controversial protecting group has been used, however, for special and delicate steps in peptide synthesis and, moreover, its presence imposes stereochemical restrictions[5], has prompted us again to choose another particular set of activated esters[6] in order to achieve satisfactory yields and controlled conditions of coupling. To this end, this communication deals with further efforts for the completion and in general the refinement of the trityl method for peptide synthesis. Thus, it has been found that esters of N^{α}-trityl amino acids with 3,4-dihydro-3--hydroxy-4-oxo-1,2,3-benzotriazine (HODhbt, *I*) are easily prepared using the DCCI procedure[6], as crystalline or solid products, which, as a rule, do not need any further purification and can be stored for several months.



I

As HODhbt esters suppress racemization[7,8], the combination with N^{α}-trityl group may be usefully employed for the preparation of optically pure products. Indeed, the coupling of N^{α}-trityl amino acid-ODhbt esters with amino acid esters is accomplished very easily and in much better yields than with N^{α}-trityl amino acids

* Part of this work was presented at the 4th Symposium on Bio-organic Chemistry of Peptides, Prague, 1989.

** Abbreviations are according to the IUPAC-IUB Commission for biochemical nomenclature[1]; additional abbreviations are explained in the text. All optically active amino acids are of the L-configuration.

activated by DCC/HOBt[6]. No racemization was observed during the coupling step and, moreover, the yields were high. This is not surprising, since it has long been known that "activated" tritylamino acids resist racemization via the well known racemization routes.

The application of these active esters is now further investigated in solid phase synthesis in order to monitor trityl-amino acylation reactions, in analogy with Sheppard's procedure for Fmoc-amino acid HODhbt esters[8]. Earlier efforts, led to the synthesis of Leu[5]-enkephalin in moderate yield[9], while with the attempted synthesis of the linear nonapeptide of oxytocin, bearing also the bulky S-trityl group for Cys protection, on the chloromethylated polystyrene-DVB resin, we experienced difficulties to monitor the completion of the reaction steps. In summary, the N^{α}-tritylamino acid HODhbt esters promise to be useful for peptide synthesis in view of the ease with which they are prepared and react and the sensitivity with which the coupling steps may be monitored.

EXPERIMENTAL

Melting points were determined in open capillary tubes, on a Buchi SMP-20 apparatus, and are reported uncorrected. Thin layer chromatograms were performed on silica gel plates with sample

TABLE I
N-Tritylamino acid-Dhbt esters

Compound	Yield, % M.p., °C	$R_F^{a/b}$	Calculated/Found		
			% C	% H	% N
Trt-Val-ODhbt	80	0·73	73·79	5·59	11·10
	140–141	0·78	73·45	5·65	11·15
Trt-Ala-ODhbt	85	0·71	73·09	5·07	11·75
	142–143	0·77	72·81	5·09	11·69
Trt-Leu-ODhbt	81	0·74	74·11	5·83	10·80
	133–135	0·80	73·70	5·88	10·80
Trt-Phe-ODhbt	79	0·70	76·06	5·10	10·13
	156–158	0·75	76·20	5·11	10·15
Trt-Asn-ODhbt	68	0·68	69·35	4·85	13·48
	133–136	0·80	69·12	4·86	13·52
Trt-Gln-ODhbt	62	0·87	69·77	5·10	13·12
	138–140	0·76	69·44	5·12	13·18

[a] 1-Butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12, v/v); [b] 1-butanol–acetic acid–water (4 : 1 : 1, v/v).

loads of 30–50 μg. The compounds were visualized by reaction with ninhydrin or chlorine followed by tolidine solution. Elemental analyses were done on a Hewlett–Packard model 185 analyzer.

Trt-Val-ODhbt

The following procedure is typical for the preparation of certain tritylamino acid-Dhbt esters which are listed in Table I. To a chilled solution of Trt-Val (0·6 g) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (0·51 g) in THF (10 ml), N,N′-dicyclohexylcarbodiimide (0·35 g) was added. The mixture was kept at 0°C for 20 min and then at room temperature for 1 h. The solvent was removed under reduced pressure and the remaining residue was taken up with AcOEt. The N,N′-dicyclohexylurea which separated out was filtered off and washed with AcOEt. The combined filtrates washed with 5% K_2CO_3 solution and water and dried over Na_2SO_4. The solvent was evaporated under vacuum and the residue crystallized upon addition of petroleum ether.

N-Trityl-Dipeptide Benzyl Esters (General Procedure of Coupling via the Dhbt Esters of N-Trityl-Amino Acids)

Amino acid benzyl ester toluene-*p*-sulphonate (2 mmol) was dissolved in THF (7 ml), neutralized

TABLE II

Physical properties of N-trityl-dipeptide benzyl esters

Compound	Yield, % M.p., °C	TLC[a]			Calculated/Found		
		R_F(A)	R_F(B)	R_F(C)	% C	% H	% N
Trt-Val-Phe-OBzl	80	0·88	0·75	0·35	80·50	6·75	4·69
	153–156[b]				80·10	6·73	4·68
Trt-Ala-Phe-OBzl	82	0·83	0·71	0·40	80·25	6·38	4·92
	110–111[c]				79·98	6·37	4·93
Trt-Leu-Phe-OBzl	78	0·73	0·84	0·45	80·62	6·93	4·58
	155–158[c]				80·27	6·91	4·57
Trt-Phe-Phe-OBzl	85	0·76	0·79	0·40	81·95	6·25	4·34
	oil				81·45	6·25	4·33
Trt-Asn-Phe-OBzl	56	0·84	0·74	0·38	76·57	6·09	6·86
	oil				76·31	6·10	6·87
Trt-Gln-Phe-OBzl	48	0·80	0·70	0·35	76·77	6·28	6·71
	oil				76·47	6·29	6·73

[a] Letters in parentheses indicate solvents system: A, 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12, v/v); B, 1-butanol–acetic acid–water (4 : 1 : 1, v/v); C, chloroform–methanol (4 : 1, v/v). [b] After recrystallization from acetonitrile. [c] After recrystallization from AcOEt–petroleum ether (1 : 1).

TABLE III

Physical properties of dipeptide benzyl ester *p*-toluenesulfonates

Compound	Yield, % M.p., °C	TLC[a]			Calculated/Found		
		R_F(A)	R_F(B)	R_F(C)	% C	% H	% N
PTS.Val-Phe-OBzl	92 118–120	0·71	0·75	0·61	63·85 64·15	6·59 6·52	5·31 5·30
PTS.Ala-Phe-OBzl	95 87–89	0·64	0·68	0·72	62·63 62·85	6·06 6·07	5·61 5·59
PTS.Leu-Phe-OBzl	94 128–129	0·79	0·84	0·55	64·42 64·75	6·71 6·75	5·18 5·16
PTS.Phe-Phe-OBzl	91 138–140	0·71	0·61	0·59	66·87 67·15	5·96 5·98	4·87 4·86
PTS.Asn-Phe-OBzl	85 141–143	0·66	0·56	0·55	59·87 60·20	5·76 5·78	7·75 7·73
PTS.Gln-Phe-OBzl	82 143–145	0·70	0·62	0·54	60·52 60·80	5·98 6·00	7·56 7·57

[a] Meaning of the letters is the same as in Table II.

with triethylamine and allowed to react with N-trityl amino acid-Dhbt ester (2·2 mmol). The mixture was kept at 0°C for 30 min and then at room temperature for 20 h. (Progress of the coupling reaction was followed by TLC and the ninhydrin test). The solvent was removed under reduced pressure and the remaining oily residue was taken up with ethyl acetate (50 ml), washed with 5% $NaHCO_3$ solution (3 × 5 ml) and water and dried over Na_2SO_4. The extract with $NaHCO_3$ becomes yellow at the beginning and turns white at the end of washings. The solvent was evaporated under vacuum and the residue crystallized upon addition of petroleum ether. The yields and the physical properties of N-trityl-dipeptide benzyl esters are given in Table II. Detritylation of the above products (1 mmol) with toluene-*p*-sulfonic acid hydrate (1·1 mmol, 0·208 g) in acetone (3 min boiling under reflux) afforded the dipeptide benzyl ester toluene-*p*-sulfonates which were crystallized from 2-propanol–ether. The yields, as well as, the physical properties are given in Table III.

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