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Protection of Some Peptides and Amino Acids by Tritylation

*John Halstrøm and Kay Brunfeldt**

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Summary: The results from the tritylation of five peptides and three amino acids by trityl chloride and triethylamine in anhydrous pyridine are presented. The yields are considerably dependent on the solubility in pyridine. This is especially noticeable in the tritylation of free amino acids. The procedure allows the derivatization of side-chain-protected peptides for use in fragment coupling by the carbodiimide or related methods, and eliminates the need for saponification of esters, which can lead to imide formation.

Tritylierung einiger Peptide und Aminosäuren

Zusammenfassung: Fünf Peptide und drei Aminosäuren wurden durch Behandlung mit Tritylchlorid und Triäthylamin in trockenem Pyridin in die entsprechenden Trityl-Peptide und -Aminosäuren überführt. Die Ausbeuten sind von der Löslichkeit in Pyridin sehr abhängig, was besonders bei der Tritylierung von freien Aminosäuren auffällt. Seitenkettengeschützte Peptide können auf diese Weise ohne eine Verseifungsstufe, die zu Imidbildung führen kann, für das Carbodiimid-Fragmentkondensationsverfahren eingesetzt werden.

Previously, we described the selective, temporary *N*-protection of a free peptide by tritylation under anhydrous conditions[1]. It was shown that the side-chain *p*-nitrobenzyl esters of aspartic and glutamic acid remained intact during this treatment, indicating the absence of imide formation[2,3]. That tritylation of peptides in pyridine may be generally feasible is indicated by the present results, comprising two additional synthetic intermediates and two naturally occurring peptides. For purposes of comparison, three trifunctional amino acids were included in the investigation.

The reaction of the amino group and, to some extent, the carboxyl group of a peptide with trityl chloride and triethylamine in pyridine solution at room temperature produces an *N*-tritylated peptide trityl ester (Table 1) together with some *N*-tritylpeptide acid. In the presence of methanol, the trityl ester undergoes selective cleavage at room temperature to give the *N*-tritylpeptide acid (Table 2) and trityl methyl ether[4]. This observation is consistent with the finding of Zervas[5,6], that the direct tritylation of an amino acid is accompanied by the formation of some *N*-tritylamino acid trityl ester, which can be decomposed by brief boiling with alcohol. Although in the present experiments the solubility in pyridine appears to be a limiting factor, the yield for peptides with a reasonable solubility seems equal or superior to that obtained in the tritylation of amino acids in aqueous solution[7] (Table 2). Thus the tritylation

Abbreviations: Trt = trityl = triphenylmethyl;
DEA = diethylammonium;
TLC = thin-layer chromatography.

* *Address:* Dr. K. Brunfeldt, The Danish Institute of Protein Chemistry, 33, Finsensvej, DK-2000, Copenhagen F, Denmark.

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Table 1. Data of N^{α}, *C*-ditritylated peptides.

N-Tritylpeptide trityl ester	Molecular weight	Microanalysis (upper theory, lower found) C	H	N	O	M. p. [°C]	$[\alpha]_D^{22}$ ($c=1$ in ethyl acetate)	R_F in S1[a]	R_F in S5
NO_2 NO_2 NO_2 Bzl Bzl Bzl O O O Glu-Asp-Ala-Asp-Pro	1435.5	66.9	5.2	7.8	20.1				
		66.9	5.2	7.8	20.2	110–120	− 60.4°	0.55	0.35
Bzl Ser-Gly-Pro-Ala	905.1	77.0	6.2	6.2	10.6				
		77.0	6.2	6.2	10.7	107–110	− 84.1°	0.56	0.41
Phe-Phe-Pro-Pro-Phe-	1686.1	72.7	6.7	8.3	12.3				
-Phe-Val-Pro-Pro-Ala, 2 H_2O		72.5	6.8	8.5	12.1	135–140	−102.0°	0.54	0.35

[a] In S1 the R_F values for the *N*-tritylpeptide acid and the corresponding *N*-tritylpeptide trityl ester were in all three cases found to be identical. In S5, however, each *N*-tritylpeptide acid had R_F 0. The methanolysis could therefore be conveniently followed in S5.

Table 2. Data of *N*- and *S*-tritylated products.
+ + + Means good, + means poor.

Starting material	Solubility in pyridine	Tritylation yield [%][a]	Unreacted material [%]	Final product isolated	Over-all yield [%]	Characterization: Microanalysis (upper theory or lit., lower found) C	H	N	O	S	Neutral. equiv.	$[\alpha]_D^{22}$ ($c=1$ in methanol)	TLC R_F using S7	M. p. [°C]
NO_2 NO_2 NO_2 Bzl Bzl Bzl O O O Glu-Asp-Ala-Asp-Pro, AcOH	+ + +	74		N^{α}-Trt	53	61.4 61.0	5.1 4.9	9.4 9.3	24.1 24.6		1193 1370	− 35.0°	0.75	90 − 110
Bzl Ser-Gly-Pro-Ala, HCl	+ + +	67		N^{α}-Trt	−[b]	70.7 70.9	6.4 6.4	8.5 8.1	14.5 14.3		663 687	− 71.2°	0.61	100 − 105
Phe-Phe-Pro-Pro-Phe- -Phe-Val-Pro-Pro-Ala, HBr	+ + +	61		N^{α}-Trt, H_2O	−[b]	69.9 69.8	6.8 6.7	9.8 10.3	13.5 13.3		1426 1236	−128.0°	0.77	140 − 145
Asn-Arg-Val-Tyr- -Val-His-Pro-Phe	+ +			N^{α},N^{im}-diTrt	45	68.9 69.0	6.5 6.6	12.9 12.6	11.6 11.7		1516 1604	− 35.0°[e]	0.47	211 − 214
Cys-Gly[c] Glu	+		56	*S*-Trt[d]	28	63.4 63.1	5.7 5.9	7.7 7.5	17.5 17.3	5.8 5.7		+ 5.5°[e]	0.38	127 − 130
Trp	+ +			N^{α}-Trt, DEA-salt	35							+ 4.5°[7] + 3.8° ($c=5$)	0.75	150 − 151[7] 151 − 152
Arg, HCl	+		81 15[f]	N^{α}-Trt	10 38[f]	72.1 71.5	6.8 6.9	13.5 13.7	7.7 7.8			+ 45.7°[e]	0.00	>250[8] >250
His[g]	+		73[f]	N^{im}-Trt	3 10[f]	75.5 75.4	5.8 6.2	10.6 10.5	8.1 8.0			+ 8.7°[e]	0.50	188 − 190

[a] The yield of *N*-tritylpeptide trityl ester plus *N*-tritylpeptide acid.
[b] Prepared only for analysis.
[c] Reduced form, Sigma Chemical Company.
[d] Substitution of the SH-function by a trityl group was confirmed by PMR.
[e] Addition of 5 − 10 drops of glacial acetic acid was necessary to dissolve the substance.
[f] Starting material lyophilized.
[g] The product precipitated from the acidified aqueous phase; it was ninhydrin-positive and Pauly-negative.

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yield, i.e. the yield of *N*-tritylpeptide trityl ester plus *N*-tritylpeptide acid was found to vary from 61 to 74%. For two amino acids, tryptophan and arginine, the yield of N^{α}-trityl derivative was of the same order of magnitude as that generally obtained in aqueous solution[6]. From glycine and valine, practically no reaction was observed under identical conditions. In the case of histidine, the yield was very small, and, as for glutathione, no N^{α}-trityl derivative was isolated. That yields may be improved by previous lyophilization of poorly soluble starting material is shown in the cases of histidine and arginine. A considerable quantity of unchanged material may in many cases be recovered.

Tritylation of a free peptide leads to a protected intermediate, whose carboxyl group may be liberated smoothly and without danger to other protecting groups to give a derivative with greatly enhanced solubility in organic solvents. The potential usefulness of commercially available peptides and synthetic intermediates as building blocks in the synthesis of biologically important larger peptides and proteins is therefore indicated.

Experimental

Melting points are uncorrected. Optical rotation was measured with the Perkin-Elmer model 141 photoelectric polarimeter (tube length 1 dm). Thin-layer chromatography was carried out on commercial plates (Kieselgel F_{254}, Merck); solvents: S1 (chloroform/methanol/acetic acid, by volume 90:5:5); S2 (2-butanol/formic acid/water, by volume 75:15:10); S5 (tert. butanol/pyridine/heptane, by volume 33:13:54) and S7 (1-butanol/acetone/diethylamine/water, by volume 37:37:7:19).

General procedure: The substance (2−10 mmol) is dissolved in pyridine (50−100 ml), and the solution is concentrated to half volume *in vacuo* on a rotatory evaporator. The process of dissolving and concentrating is repeated twice, and to the final solution is added trityl chloride (9.5 g, 34 mmol) and triethylamine (5.0 ml, 36 mmol), and the resulting solution is left to stand in the dark at room temperature for 16−48 h, in the course of which it assumes a reddish colour. The solution is then concentrated to dryness *in vacuo* on the rotatory evaporator, and the remaining solvent removed by drying in high vacuum. The solid residue is dissolved in a mixture of ethyl acetate (60 ml) and water (30 ml), the mixture acidified under vigorous stirring to pH 4.5 by dropwise addition of acetic acid, and the aqueous phase extracted with ethyl acetate (40 ml). The combined organic layers are washed with water, dried over magnesium sulfate and concentrated to a small volume (ca. 10 ml) *in vacuo*. The product is isolated by precipitation with petroleum ether and purified by reprecipitation from ethyl acetate or chloroform solution by addition of ether and/or petroleum ether. Separation of *N*-monotritylated product from *N,C*-ditritylated product is carried out by sodium hydrogencarbonate extraction of an ethyl acetate solution of the mixture.

N^{α}-Trt-L-Arginine

L-Arginine × 1 HCl (2.1 g, 10 mmol), lyophilized from acetic acid (60 ml) containing a few drops of water, was treated with pyridine as above. To the resulting suspension (50 ml) was added trityl chloride (9.5 g, 34 mmol) and triethylamine (9.0 ml, 64 mmol). After stirring for 22 h at room temperature the reaction mixture was worked up as above. The solid, yellow residue (10 g) resulting from evaporation of the ethyl acetate solution *in vacuo* was redissolved in chloroform (50 ml), and the solution was poured into a mixture of ether (50 ml) and petroleum ether (400 ml). The yellow precipitate (3 g) was triturated with hot methanol (30 ml), the mixture diluted with ether (50 ml) and filtered, the reddish-yellow colour remaining in the filtrate. After washing with ether, the product was dried at 0.2 mm. Yield: 1.6 g (38%); R_F in S2 = 0.53. Spotted on paper, the product was Sakaguchi-positive[7], and ninhydrin-negative.

N^{α},N^{im}-diTrt-[Val^5]Angiotensin II β-amide

The reprecipitation product (see general procedure) was methanolyzed directly by dissolving it in a mixture of methanol (20 ml) and methylene chloride (5 ml) and letting the solution stand for 4 days in the dark at room temperature. In S7, conversion of the initial tritylation product (R_F = 0.55) was found to be complete, and the product was isolated by concentration to dryness *in vacuo*, redissolving in methanol (2 ml) and methylene chloride (8 ml) and precipitation as a yellow oil by addition of petroleum ether (50 ml). Reprecipitation from the same solvents by dilution with ethyl acetate (50 ml) afforded an almost white, Sakaguchi-positive and ninhydrin-negative solid, homogeneous in S7 (R_F = 0.47), except for a trace impurity (R_F = 0.35). Addition of sodium hydroxide to a cold, dilute acetic acid solution of the product resulted in the appearance of a strong absorption at 296 nm due to the phenoxide ion of the tyrosine residue. On treatment for 30 min at room temperature with a dry, bromine-free, saturated solution of hydrogen bromide in trifluoroacetic acid/methylene chloride, by volume 1:1, the full pressor activity of the starting material in rats was regenerated.

S-Trt-Glutathione

By crystallizing the reprecipitation product (see general procedure) from 80% aqueous ethanol, a quantity of

trityl ethyl ether, m. p. 80–81 °C identified by its mass spectrum, was isolated. The mother liquor was concentrated to dryness and the residue crystallized from 60% aqueous ethanol to give a chromatographically (ninhydrin) homogeneous product. Remaining trityl ethyl ether was completely removed by precipitating the product from chloroform/ethyl acetate (by volume 1:1) by addition of petroleum ether.

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