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SPPS of Protected Peptidyl Aminoalkyl Amides

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Abstract: Monophthaloyl diamines derived from naturally occurring amino acids were attached through their free amino functions to resins of the trityl type. The phthaloyl groups were removed by hydrazinolysis, and peptide chains were assembled using Fmoc/*t*Bu-amino acids on the liberated amino functions. The peptidyl aminoalkyl amides obtained were cleaved from the resins by mild acidolysis, with the *t*Bu-side chain protection remaining intact. Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

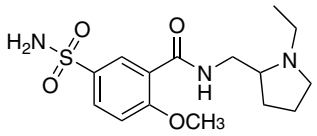
Keywords: chiral diamines; combinatorial chemistry; peptide synthesis; protected peptides; trityl resin

INTRODUCTION

The solid phase synthesis (SPPS) of protected peptide segments using the 2-chlorotrityl resin has enabled the industrial scale synthesis of peptide pharmaceuticals, such as the anti-HIV 36mer peptide T-20 [1]. In addition, this synthesis demonstrated the effectiveness of convergent methods, in which independently synthesized protected peptide fragments are condensed in solution or on solid phase to larger and more complex peptides [2]. On the other hand, several biologically active compounds contain in their structure linear or cyclic diamines. Examples of such compounds are the naturally occurring polyamines [3], the antipsychotic sulpiride **1** [4,5], and the wide range cytoprotectand amifostine **2** [6–8].

We were therefore interested in the solid phase synthesis of protected peptidyl aminoalkyl amides. These could be useful for application in the convergent synthesis of modified peptide diagnostics and pharmaceuticals [9,10].

In particular, we were interested in the synthesis of protected peptides containing the chiral diamines **3**, which are derived from naturally occurring amino acids. Such diamines could give the individual pharmaceutical compound a higher specificity of action than the linear unsubstituted diamines. Peptide aminoalkyl amides were synthesized by SPPS using the 2-chlorotrityl resin [11] or resins of the alkoxycarbonyl type [12,13]. In both cases, the conditions reported for the cleavage of the diamino-compounds from the resins led to the concurrent removal of the acid-labile side chain protecting groups. On the other hand, trityl- [14] and polymer-bound trityl-groups [15] were used for α-amino protection of amino acids allowing the cleavage of the amino acid derivatives synthesized on it under milder conditions.



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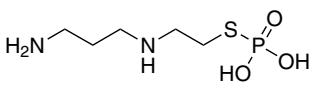

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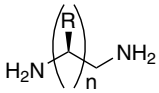

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Linear symmetric diamines can be loaded unprotected onto resins **5** of the trityl type (Scheme 1). But the chiral diamines **3** have to be selectively

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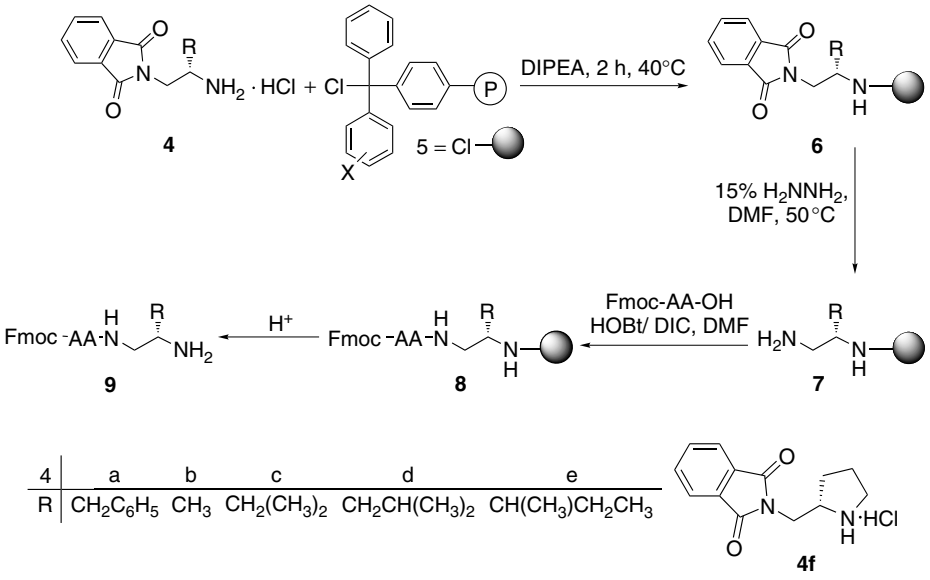
protected prior to their application in the preparation of resins **6**. Due to the high acid-sensitivity of the amino-trityl resin bond, the protecting group applied for the selective protection of the N^2-function of the diamine must be chosen from among the base labile groups such as the Fmoc- and the phthaloyl-group. We preferred the phthaloyl-group because of the high crystallinity of derivatives **4** which allows their easy preparation in high purity [16].

MATERIALS AND METHODS

Chemicals

2-Chlorotrityl, trityl, 4-methoxytrityl and 4,4′-dimethoxytrityl resins and Fmoc-amino acids were obtained from CBL-Patras (Patras, Greece). N^2-phthaloyl-1,2-diamines were used as hydrochloride salts, synthesized by the procedure of Burgess *et al.* [16]. SPPS was carried out by the Fmoc-strategy as described previously [17]. TLC was performed on precoated silica gel 60 F_{254} (Merck) aluminium sheets. All HPLC runs were performed on a Waters 600E multisolvent delivery system, combined with a Waters 991 photodiode array detector, using a Lichrosphere RP-8 column (4 × 250 mm, 5 μm) (column A), or Nucleosil C-8 column (4 × 125 mm) (column B) using a linear gradient from 20% to 100% B over 30 min, where A = 0.1% TFA in water and B = 0.1% TFA in MecN, at a flow rate of 1 ml/min, monitored at 265 nm. ES-MS spectra were recorded on a Micromass Platform LC at 30 V. All IR spectra were collected on a Avatar™ 360 E.S.P™ FT-IR spectrometer using the attenuated total reflection (ATR) sampling technique.



4	a	b	c	d	e
R	$CH_2C_6H_5$	CH_3	$CH_2(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$

5	a	b	c	d
X	2-Cl	H	4-OCH_3	4,4'-OCH_3

6	a	b	c	d	e	f	g	h
R	$CH_2C_6H_5$	$CH_2C_6H_5$	$CH_2C_6H_5$	$CH_2C_6H_5$	CH_3	$CH_2(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$
X	2-Cl	H	4-OCH_3	4, 4'-OCH_3	H	H	H	H

7, 8, 9	a	b	c	d	e	f	g	h	i
R	$CH_2C_6H_5$	$CH_2C_6H_5$	$CH_2C_6H_5$	$CH_2C_6H_5$	CH_3	CH_3	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$
X	2-Cl	H	H	H	H	2-Cl	H	H	H
AA	Leu	Val	Trp(Boc)	His(Trt)	Asp(tBu)	Cys(Mmt)	Ser(tBu)	Lys(Boc)	Glu(tBu)

Scheme 1 Loading of diamines onto resins of the trityl-type.

Solid Phase Peptide Synthesis

General procedure for the loading of the trityl chloride resins 5 with diamines 4. To a suspension of 1 g trityl chloride resin **5** (1.3–1.9 mmol chloride/g resin) in 10 ml of dichloromethane (DCM), were added 0.54 ml (3.5 mmol) diisopropylethylamine (DIEA) and 1.5 mmol diamine **4**. The resulting mixture was agitated in a rotary evaporator for 3 h at 35 °C. The resin was filtered off, suspended in 20 ml of a 80 : 15 : 5 mixture of DCM-MeOH-DIPEA and shaken for 1 h at room temperature, in order to destroy excess trityl chloride. The resin **6** obtained was filtered and washed once more with the same mixture, and then with DMF (6 × 10 ml), isopropanol (6 × 10 ml) and dried *in vacuo*. The loading results are summarized in Table 1.

Loading determination of resins 6a–h. 10 mg of resin **6** was treated for 1 h at 24 °C, with 0.2 ml 65% TFA in DCM. DMF was then added and the resin was filtered off and washed with 3 × 2 ml DMF. The filtrates were combined, and diluted until the total volume was 25 ml. The peak area, obtained after HPLC analysis of 0.01 ml from the above solution, at 300 nm was compared with an external standard (a solution of 15.7 mg of the corresponding diamine **4** in 250 ml DMF).

General procedure for the removal of the phthaloyl group. Synthesis of N^1-trityl diamine resins 7. 1 g of resin **6** was suspended in 8 ml DMF and 0.12 ml (25 mmol) hydrazine hydrate was added. The mixture was agitated for 45 min at 50 °C. The resulting resin-bound diamine **7** was filtered off and washed with DMF (6 × 10 ml), ^{i}PrOH (6 × 10 ml), *n*-hexane (3 × 10 ml) and dried *in vacuo*.

Table 1 Loading of the Trityl Chloride Resins **5** with Diamines **4**

Resin	mmol[a]	Diamine	yield (%)[b]	(mmol/g)[c]
5a	1.4	**4a**	65	0.91
5b	1.5	**4a**	84	1.25
5c	1.8	**4a**	86	1.28
5d	1.9	**4a**	90	1.32
5a	1.3	**4b**	60	0.87
5b	1.5	**4b**	87	1.30
5b	1.5	**4c**	88	1.32
5a	1.3	**4d**	67	0.94
5b	1.5	**4d**	91	1.36
5b	1.5	**4e**	85	1.27
5b	1.5	**4f**	89	1.33

[a] Chloride loading of the resin.
[b] Calculated in respect to the applied diamine **4**.
[c] Resin substitution on phthaloyl diamine.

General procedure for the cleavage of peptidyl aminoalkylamides 9–15 from the resins of the trityl type. The resin-bound peptidyl aminoalkyl amide (1 g) was treated with 15 ml of a 1% mixture of TFA in DCM (mixture A), or a 1 : 2 : 7 mixture of AcOH-trifluoroethanol (TFE)-DCM (mixture B), or a 35% mixture of hexafluoroisopropanol (HFIP) in DCM (mixture C), for 30 min at 24 °C, and washed with DCM (3 × 10 ml). The combined filtrates were concentrated *in vacuo*. The protected crude peptides were precipitated by the addition of diethylether (DEE), filtered, washed with DEE and dried *in vacuo*. Table 2 summarizes the synthetic and analytical results obtained for compounds **9a–f**.

Table 2 Results of the Solid Phase Synthesis of the Model Dipeptides **9** Derived from Chiral Diamines

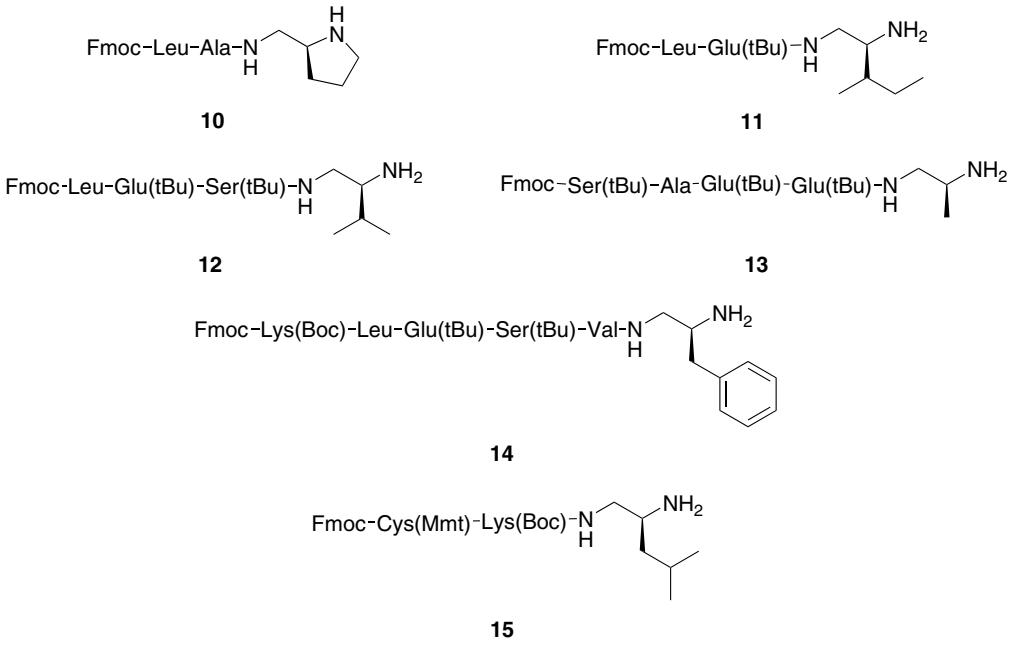
	Cleavage method	t_R		Crude yield (%)	HPLC purity (%)	*m/z*	
		Column A	Column B			Calculated	Found
9a	B	—	12.6	94	97	486.28	486.63
9b	A	19.3	—	93	97	472.18	472.20
9c	A	—	18.6	92	97	659.32	659.30
9d	A	—	17.2	93	98	752.92	753.30
9e	B	—	14.8	95	99.3	468.24	468.35
9f	C	20.1	—	95	98.7	672.29	672.86
9g	B	19.2	—	94	98.2	468.28	468.55
9h	C	19.7	—	92	99.5	567.18	567.35
9i	B	—	20.0	93	96	524.30	524.66

RESULTS AND DISCUSSION

For the attachment of phthaloyl diamines **6** to the solid support, resin-chlorides **5** were treated with an equimolar amount of **4** and excess DIEA in DCM for 2 h at 35 °C. The formation of **6** was confirmed using FT-IR spectroscopy, by the characteristic absorbance of the phthaloyl amide at 1712 cm^{-1}. Unreacted remaining trityl chloride was converted to the corresponding tritylmethyl ether, by washing the resin with a (85 : 10 : 5) mixture of DCM-MeOH-DIPEA.

The attachment yield, obtained based on the amount of **4** used, was determined spectrophotometrically by HPLC to be 65%, 84%, 86% and 90%, respectively, for the various resins **5a–d** (Table 1).

We next investigated the conditions required for the complete and selective removal of the phthaloyl group from the resins **6a–d**. Application of 15%–60% of hydrazine hydrate in mixtures of MeOH in DMF, for 3–12 h at room temperature, resulted in incomplete deprotection. We found that 15% hydrazine in DMF for 45 min at 50 °C was necessary for the complete removal of the phthaloyl group from **6a** and **6b**. The progress of the phthaloyl removal was followed by FT-IR spectroscopy. Under the same conditions complete dephthaloylation of **6c–d** occurred, but concurrent cleavage of the diamines **3** from the resins was observed. Due to



Scheme 2 Protected peptidyl amino alkyl amides.

Table 3 Results of the Solid Phase Synthesis of the Representative Peptidyl Aminoalkyl Amides **10–15** Derived from Chiral Diamines

	Cleavage method	t_R		Crude yield (%)	HPLC purity (%)	*m/z*	
		Column A	Column B			Calculated	Found
10	C	—	13.3	88	98.7	507.3	507.58
11	B	22.1	—	90	98.5	637.4	637.82
12	C	23.7	—	92	99.5	766.5	767.52
13	B	—	18.0	95	96	881.5	881.19
14	B	—	21.4	93	95	1141.69	1141.26
15	C	26.5	—	92	98.9	942.48	943.57

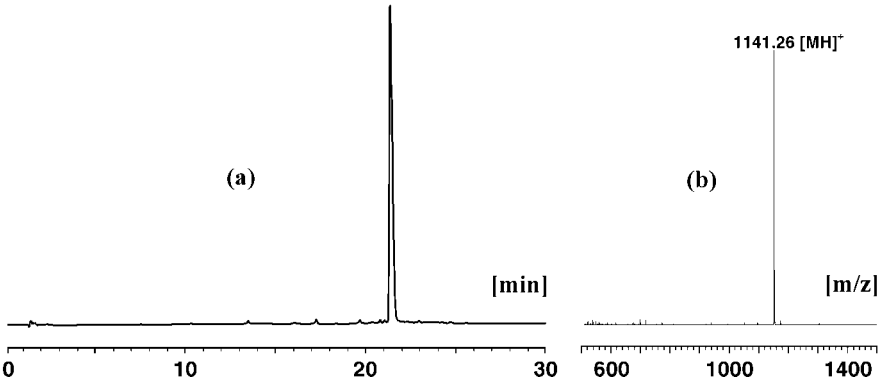


Figure 1 (a) Analytical HPLC of crude peptide **14** obtained by treatment of resin **5b** with 35% HFIP/DCM for 30 min at RT (b) ES-MS of **14** at 30 V.

the thermal lability of **6c–d**, all further experiments were performed using only the resins **6a–b**.

Using resin **7**, we synthesized the model peptides **9**, chosen to contain the acid-labile *t*Bu, Boc, Trt and Mmt-amino acid side chain protecting groups. The resin-bound dipeptides **8** obtained were then treated with solutions known to cleave peptides from **5a** without affecting side chain protection of the *t*Bu-type, in order to determine the selectivity and completion of the cleavage from resins **5a–b**. These mixtures were 1% TFA in DCM (mixture A), AcOH-TFE-DCM (1 : 2 : 7) [17] (mixture B) and a 30% HFIP in DCM (mixture C) [18]. In all three cases, the cleavage was completed within 30 min at 24 °C. Mixtures A, B and C were proven to cleave **9** from the resins **5** with >95% selectivity. Trp(Boc) in **9c** and His(Trt) in **9d** were slightly more sensitive, and 2%–3% deprotection occurred concurrently with the cleavage from the resin. Higher sensitivity towards mixture A was observed with the *S*-Mmt-group containing **9f**, where 18% deprotection occurred.

To evaluate the utility of resin bound diamines **7** in the SPPS of partially protected peptidyl aminoalkyl amides, we synthesized fragments **10–15** (Scheme 2). Starting from **7a–b**, peptide chain elongation to the resin bound **9–15** diamines was performed using Fmoc/*t*Bu protected amino acids in DMF, and DICI/HOBt as the coupling agent. The Fmoc-group was removed by treatment with 25% piperidine in DMF. Finally, peptide fragments **10–15** were cleaved from the resins by treatment with mixtures B and C for 30 min at RT. All peptides were cleaved quantitatively from the resin; the purity was >95% in all cases. The results obtained on characterization of the peptide segments were by HPLC and ES-MS are summarized in Table 3. As an example, the analytical HPLC and ES-MS of the hexapeptide **14** are presented in Figure 1a and 1b, respectively.

It is concluded that resins **5a–b** are well suited for the SPPS of protected diamine derivatives.

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