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Solid phase insertion of diamines into peptide chains

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Abstract—Diamines derived from naturally occurring aminoacids were inserted into peptide chains by the reaction of the monophthaloyl diamines with amino acid 1-benzotriazolyl esters, bound through their amino functions onto trityl-type resins. The phthaloyl group was removed and peptide chains using *N*-Fmoc amino acids, were assembled on the liberated amino function. The peptidyl diamides obtained, were cleaved from the resins with ^{t}Bu-side chain protection remaining intact, or fully deprotected.

Besides polyamines, linear and cyclic diamines are often contained in drugs and biologically active compounds. Examples are the radioprotector, ethyol **1**,[1–3] the anti-HIV drug ritonavir **2**,[4] the neurotoxin NPTX-11 **3**[5] (Fig. 1), the atypical anti-psychotic clozapine,[6,7] the peptide nucleic acids (PNAs),[8,9] and several enzyme inhibitors.[10–12] Due to their pharmaceutical importance, we were interested in developing methods for the simple solid phase synthesis of diamine-containing compounds and peptides. In particular, diamines derived from naturally occurring amino acids could lead to pharmaceuticals with higher selectivity to individual receptors, as compared to linear symmetrical diamines. Examples are found in the case of PNAs.[13]

To insert a chiral diamine into peptide chains we reacted the monophthaloyl diamines **4** with the benzotriazolyl-esters of the resin-bound amino acids **5**. The phthaloyl protection of the diamines was chosen because of the good crystallinity of **4**, which allows for their simple purification.[14,15] Benzotriazolyl esters of *N*-trityl protected amino acids have proved to be effective acylating agents[16] which do not racemize even at high temperatures in the presence of tertiary amines.[17] The corresponding benzotriazolyl esters of the resin bound amino acids **5** have been successfully applied to syntheses of amino acid derivatives and peptides.[18]

Indeed, **5** reacted quantitatively within 4 h at rt or within 30 min at 50°C with a two-fold molar excess of diamines **4** in dimethylformamide (DMF) in the presence of excess diisopropylethylamine (DIPEA) (Scheme 1).[19] The formation of the resin bound derivatives **6** was followed by FT-IR.[20] Thus, during the reaction, the disappearance of the benzotriazolyl-ester band at 1814 cm^{-1} was correlated with the concurrent appearance and gradual increase of the characteristic phthaloyl amide band at 1712 cm^{-1}. The reactivity of **5** was found to be independent of the resin used for *N*-protection.

To determine the conditions required for the complete cleavage of **7** from the corresponding resins **6**, these

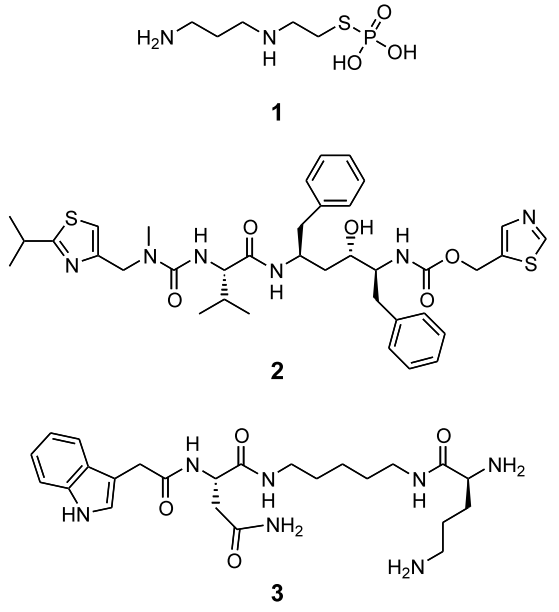

Figure 1.

Keywords: chiral diamines; peptide synthesis; protected peptides; trityl resin.

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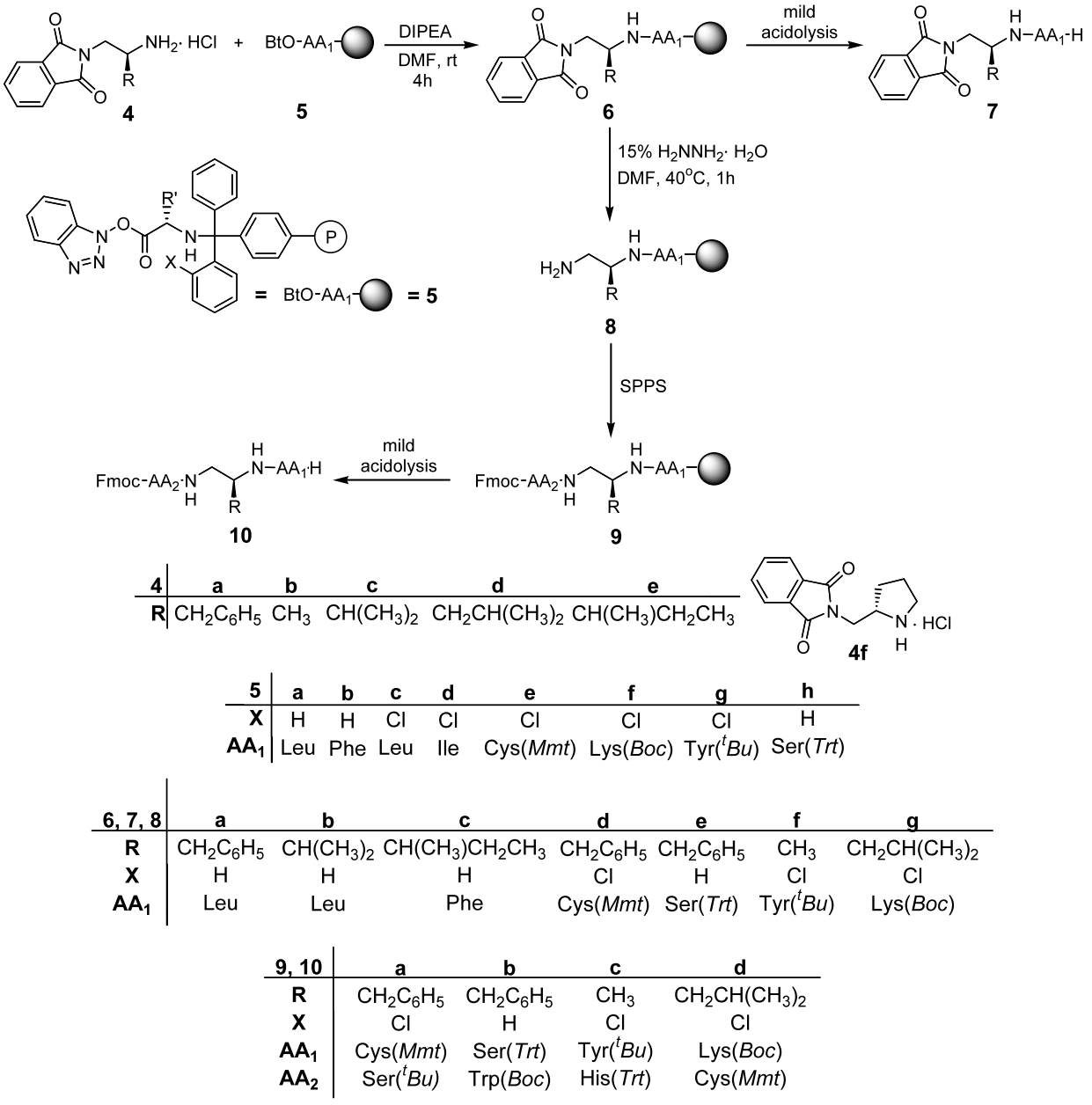

4	a	b	c	d	e
R	$CH_2C_6H_5$	CH_3	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$

5	a	b	c	d	e	f	g	h
X	H	H	Cl	Cl	Cl	Cl	Cl	H
AA_1	Leu	Phe	Leu	Ile	Cys(*Mmt*)	Lys(*Boc*)	Tyr(^{t}*Bu*)	Ser(*Trt*)

6, 7, 8	a	b	c	d	e	f	g
R	$CH_2C_6H_5$	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$	$CH_2C_6H_5$	$CH_2C_6H_5$	CH_3	$CH_2CH(CH_3)_2$
X	H	H	H	Cl	H	Cl	Cl
AA_1	Leu	Leu	Phe	Cys(*Mmt*)	Ser(*Trt*)	Tyr(^{t}*Bu*)	Lys(*Boc*)

9, 10	a	b	c	d
R	$CH_2C_6H_5$	$CH_2C_6H_5$	CH_3	$CH_2CH(CH_3)_2$
X	Cl	H	Cl	Cl
AA_1	Cys(*Mmt*)	Ser(*Trt*)	Tyr(^{t}*Bu*)	Lys(*Boc*)
AA_2	Ser(^{t}*Bu*)	Trp(*Boc*)	His(*Trt*)	Cys(*Mmt*)

Scheme 1.

Table 1. Yields and analytical results from the cleavage of diamine containing crude protected peptides **10–13** from resins of the trityl-type

	Cleavage Method	Yield (%)	Retention time (min)	HPLC purity (%)	m/z[d]	
					Calculated	Found
10a	B	92	17.62[a]	94	891.13	891.4
10b	B	91	20.71[a]	94	988.18	988.6
10c	A	93	14.53[a]	95	895.10	894.7
10d	B	93	18.61[a]	95	941.48	941.7
11	A	90	23.65[b]	97	765.98	767.5
12	C	94	27.01[b]	96	1088.54	1090.8
13	C	91	23.26[c]	95	1430.72	1431.5

[a] Column: Zorbax SB-C18, 3.5 μm, 2.1×30 mm; gradient: from 20 to 100% acetonitrile (B) in water (A) within 30 min, flow rate 0.4 ml/min; detection at 265 nm.
[b] Column: Lichrospher RP-8, 5 m; 4×150 mm; gradient: from 20 to 100% B in A within 30 min; flow rate 1 ml/min; detection at 265 nm.
[c] Column: Nucleosil C-8, 5 m; 4×125 mm; gradient: from 20 to 100% B in A within 30 min; flow rate 1 ml/min; detection at 265 nm.
[d] ES-MS of the corresponding purified peptide.

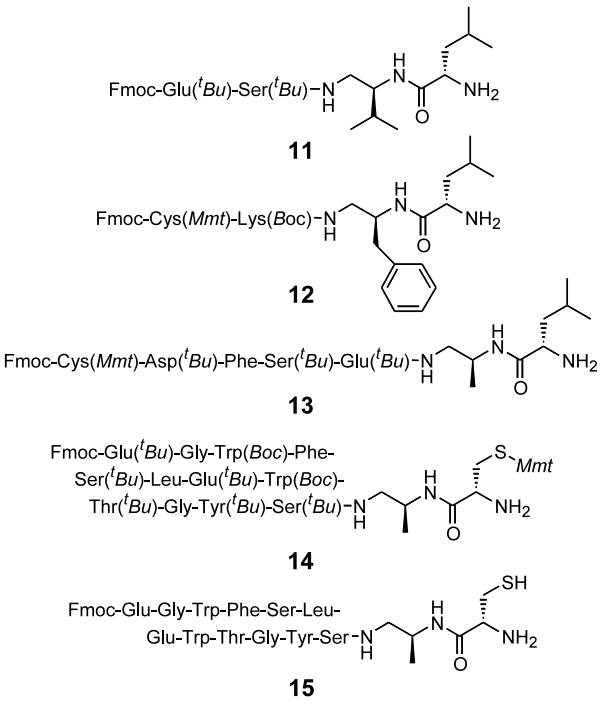

Figure 2.

resins were treated with 1% trifluoroacetic acid (TFA) in dichloromethane (DCM) (mixture A), acetic acid (AcOH)/trifluorethanol (TFE)/DCM (1:2:7) (mixture B), and 35% hexafluoroisopropanol (HFIP) in DCM (mixture C). In all cases tested the cleavage was complete within 30 min at rt. Under these conditions, protecting groups of the ^{t}Bu-type remained intact. In contrast, the more acid-sensitive *S*-Mmt and *O*-Trt groups in **7d** and **7e** were removed by mixture A in 18 and 10%, and by mixture C in 0.5 and 2%, respectively, concurrently with cleavage from the corresponding resin.

The complete removal of the phthaloyl group of **6** was performed by treatment with 15% hydrazine hydrate in DMF for 1 h at 40°C (Scheme 1). As expected, under these conditions, the acid labile ^{t}Bu, Boc, Trt and 4-methoxytrityl (Mmt) side chain protecting groups remained unaffected. The progress of the dephthaloylation was monitored by FT-IR, by the decrease of the phthaloyl band at 1712 cm^{-1}.

In order to evaluate the utility of resins **8** in the solid phase synthesis of partially protected and free peptidyl aminoalkyl diamides, we synthesized the peptides **10**–**14** (Fig. 2). Starting from resins **8**, peptide chain elongation to the corresponding resin bound derivatives was performed in all cases, using Fmoc/^{t}Bu-amino acids in *N*-methyl-pyrrolidone (NMP). The coupling reaction was performed using a solution of *N*-[(1*H*-benzotriazol-1-yl) (dimethyl-amino) methylene]-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide (HBTU)/1-hydroxybenzotriazole (HOBt) (0.50/0.45 M), in DMF.[21] The resin bound peptides obtained were cleaved from the resin by treatment with the mixtures A, B and C for 30 min at rt. Their purity was determined to be >94%. The protected peptide **14** was obtained by treatment with mixture C, for 30 min at rt. Cleavage with TFA/DCM/triethylsilane (TES) (65:32:3) for 45 min at rt afforded the

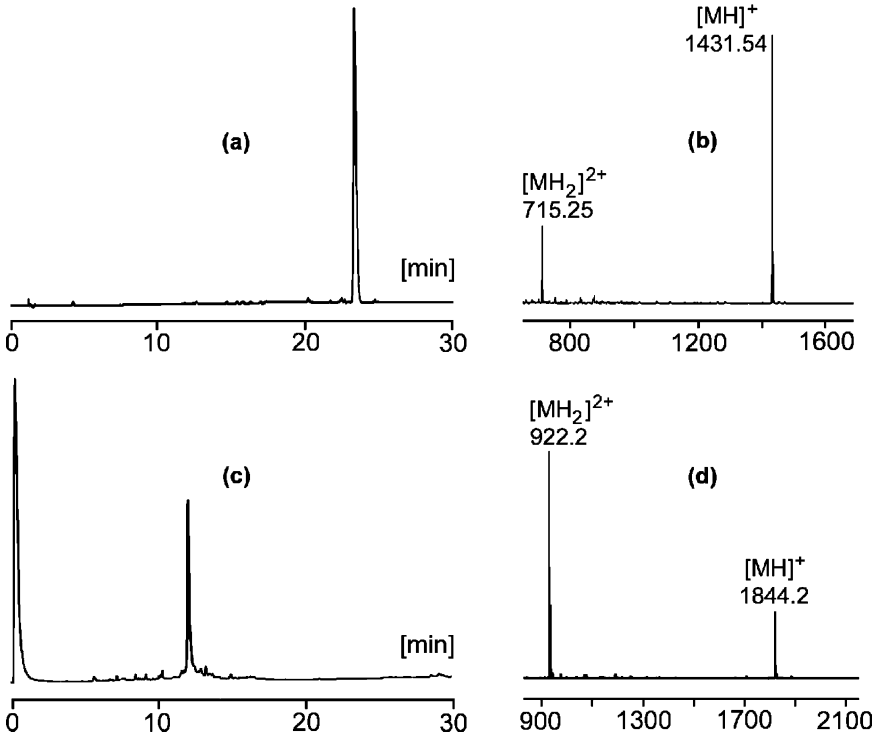

Figure 3. **(a)** Analytical HPLC of crude peptide **13** (conditions reported in Table 1). **(b)** ES-MS of **13** at 30V. **(c)** Analytical HPLC of crude peptide **15** (Column: Zorbax SB-C18, 3.5 m, 2.1×30 mm; gradient 20–100% acetonitrile (B) in water (A), within 30 min; flow rate 0.4 ml/min; detection at 220 nm). **(d)** ES-MS of **15** at 30 V.

corresponding fully deprotected peptide **15**. Crude peptides **10**–**15** were precipitated from ether and their purity was determined by HPLC analysis (Table 1). The correct molecular weights of the purified compounds were identified by ES-MS. As examples the HPLC chromatograms of the crude protected heptapeptide **13**, and the crude fully deprotected tetradecapeptide **15**, as well as the ES-MS spectra of the purified compounds are presented in Fig. 3(a–d).

Acknowledgements

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19. The synthesis of 4-polystyryltriphenylmethyl-*N*-leucine-(2-amino-1-benzyl-ethyl)amide **8a** is representative. To a suspension of 1 g **5a** (0.6 mmol HOBt/1 g resin) in 10 ml DMF were added 0.25 ml (1.5 mmol) DIPEA and 0.379 g (1.2 mmol) **4a**. The resulting mixture was agitated at rt for 4 h. The obtained resin **6a** was filtered and washed with DMF (6×10 ml). The characteristic band at 1814 cm^{-1} disappeared completely, indicating 100% coupling. The resin was then suspended in 8 ml DMF, 1.2 ml (250 mmol) hydrazine hydrate were added and the mixture was agitated on a rotary evaporator for 1 h at 40°C. The resin **6a** was filtered and washed with DMF (6×10 ml), isopropanol (6×10 ml), *n*-hexane (6×10 ml) and dried in vacuo, affording 0.96 g of resin **8a**.
20. FT-IR spectra were collected on a Thermo-Nicolet, Avatar™ 360 E.S.P™ spectrometer using attenuated total reflection (ATR) sampling technique. HPLC analysis was performed either on a Waters 600S multisolvent delivery system, combined with a Waters 717plus autosampler and a Waters 996 photodiode array detector or on an Agilent 1100 series system combined with an Agilent mass spectrum detector.
21. Peptide synthesis was performed using an Applied Biosystems 433A automatic synthesizer, equipped with a conductivity based monitoring system. Fmoc-deprotection was performed by treatment with piperidine in DMF. The coupling and the Fmoc-deprotection time were 30 and 5 min, respectively.