Pergamon

Tetrahedron 54 (1998) 13023–13034

TETRAHEDRON

Selective Detachment of Boc-Protected Amino Acids and Peptides from Merrifield, PAM and Wang Resins by Trimethyltin Hydroxide

Ricardo L. E. Furlán, Ernesto G. Mata and Oreste A. Mascaretti*

Instituto de Química Orgánica de Síntesis (CONICET-UNR). Casilla de Correo 991, 2000 Rosario. Argentina. E-mail: masca@citynet.net.ar

Clara Peña* and Marcelo P. Coba

Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, 1113 Buenos Aires, Argentina. E-mail: clara@qb.ffyb.uba.ar

Received 13 November 1997; revised 19 August 1998; accepted 20 August 1998

Abstract: We describe the development of a new non-acidolytic and non-nucleophilic method for the selective cleavage by trimethyltin hydroxide of amino acids and dipeptides at benzyl ester links attached to resins commonly used in peptide synthesis.

INTRODUCTION

There is an increasing demand for specific cleavage of protected peptides fragments attached by benzyl or phenacyl ester links to resins commonly used in peptide synthesis. The benzyl ester linkage is the most frequent mode of attachment of the first amino acid or non-peptide carboxylic acid functional molecule to the polystyrene chains crosslinked with divinylbenzene as in Merrifield,[1] PAM,[2] Wang,[3] Sasrin® and Riniker[5] resins.

In many ways the final detachment of the completed peptide from the solid support is the key step in Solid-Phase Peptide Synthesis (SPPS).[6] A very fine balance is necessary since conditions that remove the peptide from the resin should be, at the same time, sufficiently mild to allow sensitive structural features to survive. The chemical methods used for detaching peptides from a solid support depend on the type of resin, but for the synthesis of free peptides, it is normally accomplished by acidolysis. The two most widely used protection strategies in SPPS are the *N*-α-Boc/O-benzyl side chain in Merrifield and PAM resin, and the *N*-α-Fmoc/O-tert-butyl side chain in Wang and Sasrin resins.

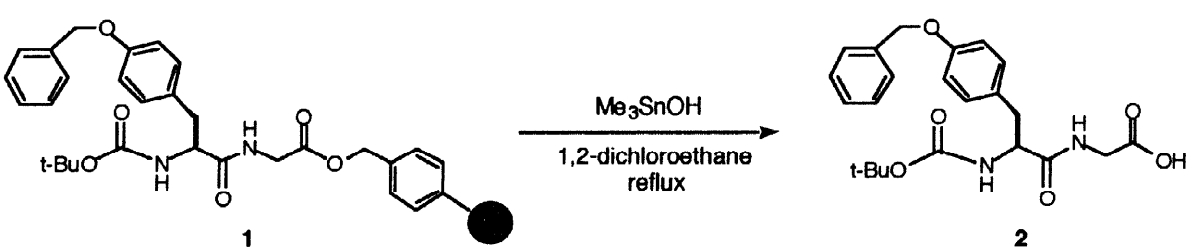
PII: S0040-4020(98)00805-9

Partially protected peptide fragments are useful as intermediates in the synthesis of cyclic peptides in solution and in Convergent Solid-Phase Peptide Synthesis (CSPPS) to build up large sequences or the so-called "difficult" peptides.[7] The protected peptides must be detached from the resin with all the side-chain protecting groups and also that of the *N*-α-amino group in place, so, if that protected peptide is produced by Boc/OBn strategy, acidolysis cannot be used for its cleavage from the resin. For this reason, standard solid supports used for linear SPPS, such as Merrifield, Wang or PAM resins, are rarely applied to the convergent strategy and alternative peptide-resin anchorages are preferred.[7a] Nucleophilic reagents such as tetra-n-butylammonium fluoride[8] and tetra-n-butylammonium carbonate[9] have been used for preparing protected peptide segments from Merrifield type resins; however, the demand for an efficient orthogonal cleavage protocol for the preparation of protected peptide fragments still leaves open possible improvements using new methodology. CSPPS is one of the most promising approaches to the synthesis of peptides and small proteins of 40 amino acid residues or more. The method entails the solid-phase synthesis of protected peptide segments which, after purification, are coupled together on a solid support giving the desired amino acid sequence.

Recently, we have introduced trimethyltin hydroxide (TMTOH) as a non-acidolytic, non-nucleophilic reagent for the selective cleavage of *N*-α-Boc peptide phenacyl esters linked to a polystyrene resin.[10] Here we report the preparation of *N*-α-Boc peptide segments by detachment from the resins of Merrifield, PAM and Wang by TMTOH. This approach will allow the application of standard and low-priced resins to the convergent solid-phase peptide synthesis strategy.

RESULTS AND DISCUSSION

Scheme 1 exemplifies this novel methodology for the cleavage of protected amino acids and peptides from the most commonly used resins in SPPS. Treatment of Boc-Tyr(OBn)-Gly linked to Merrifield resin (**1**) with TMTOH in refluxing 1,2-dichloroethane [$(CH_2Cl)_2$] for 10 h furnished the corresponding protected dipeptide (**2**) in 85% isolated yield.



1 **2**

Scheme 1

The Merrifield resin[1] has historically been the standard support for the synthesis of peptide by Boc-SPPS strategy. Model cleavage studies for this resin are shown in Table 1 demonstrating the efficiency of this method with yields ranging from 85 to 100%. Entry 1 shows that the peptide bond is unaffected during the TMTOH detachment. Entries 2, 3, 4 and 5 illustrate that amino acids can be removed from the resin in very high yield and

a relatively short reaction time. An attractive feature of this methodology is its compatibility with Boc carbamate and side-chain protecting groups such as O-benzyl, S-*p*-methoxybenzyl and N-tosyl, which are conventionally employed in the Merrifield SPPS strategy.

Since SPPS was introduced by Merrifield in 1963, the impressive list of applications of Solid-Phase Organic Synthesis (SPOS)[11] for the preparation of peptide, peptidomimetic and non-peptide libraries has encouraged the development of a very large variety of linkers and polymer supports. The 4-(hydroxymethyl)-phenylacetamidomethyl-resin (PAM resin)[2], introduced by Merrifield in 1976, is the support of choice for the preparation of large peptide by the Boc chain extension methodology. The benzyl ester peptide-PAM resin is 100 times more stable to trifluoroacetic acid (TFA) than the corresponding benzyl ester bond in Merrifield resin, so this also makes this resin more difficult to remove under mild conditions.

Table 1. Release of protected amino acids and peptides from Merrifield resin by TMTOH.

Entry	Substrate	Product[a]	Reaction time[b]	% Isolated yield
1	Boc-Tyr(OBn)-Gly-resin[c] (**1**)	Boc-Tyr(OBn)-Gly-OH (**2**)	10	84
2	Boc-Cys(*Sp*MeOBn)-resin (**3**)	Boc-Cys(*Sp*MeOBn)-OH (**4**)	10	85
3	Boc-Ser(OBn)-resin (**5**)	Boc-Ser(OBn)-OH (**6**)	13	100
4	Boc-Ile-resin (**7**)	Boc-Ile-OH (**8**)	9	100
5	Boc-His(Tos)-resin (**9**)	Boc-His(Tos)-OH (**10**)	9	93

[a]heated at reflux of 1,2-dichloroethane. [b]time in hours. [c]abbreviations: Bn = benzyl, Tos = tosyl (4-toluene sulfonyl); Boc = t-butoxycarbonyl; *Sp*MeOBn = S-*p*-methoxybenzyl.

We tested the usefulness of the cleavage by TMTOH of the benzyl ester linkage in PAM resins for the preparation of Boc/O-benzyl ether/N^G-Tos protected peptide and amino acid segments; the results are summarized in Table 2. Boc-Phe-Met-PAM resin (**11**) and Boc-Ser(OBn)-Ala-PAM resin (**13**) liberate the corresponding protected dipeptides in 80% and 97% yields respectively, without detection of any free amino acid (entries 1 and 2). Different amino acids linked to this resin were also tested and yielded the protected amino acids in yields ranging from 80 to 100% (entries 3 to 7).

p-Benzyloxybenzyl alcohol resins (Wang resins)[3] are the standard support for the preparation of peptide by the Fmoc/*t*-Bu batch SPPS strategy. The Fmoc-group, a base labile *N*-α-protecting group, is removed from peptides and amino acids by secondary amines (e.g. piperidine in DMF), while the side-chain protection and the peptide-resin link are cleaved by mild acidolysis (TFA). We prepared Boc-Thr(OBn)-Leu-Wang resin (**25**) and Boc-Ala-Wang resin (**27**) in order to test the feasibility of the cleavage of this resin linkage by TMTOH (entries 8 and 9, Table 2). Boc-Thr(OBn)-Leu-OH (**26**) and Boc-Ala-OH (**16**) were obtained in quantitative yield. The very high yield of Boc protected segments by detachment with TMTOH and the ready commercial availability of the Wang resins now makes possible the combination Boc-Wang resin as the final step of the synthesis of protected fragments for CSPPS. The Boc-Wang resin combination should be also applicable to SPOS[11] and combinatorial chemistry.[12]

Table 2. Release of protected amino acids and peptides from PAM and Wang resins by TMTOH.

Entry	Substrate	Product[a]	Reaction time[b]	% Isolated yield
1	Boc-Phe-Met-PAM resin[c] (**11**)	Boc-Phe-Met-OH (**12**)	10	80
2	Boc-Ser(OBn)-Ala-PAM resin (**13**)	Boc-Ser(OBn)-Ala-OH (**14**)	9	97
3	Boc-Ala-PAM resin (**15**)	Boc-Ala-OH (**16**)	9	94
4	Boc-Met-PAM resin (**17**)	Boc-Met-OH (**18**)	9	90
5	Boc-Pro-PAM resin (**19**)	Boc-Pro-OH (**20**)	9	80
6	Boc-Arg(Tos)-PAM resin (**21**)	Boc-Arg(Tos)-OH (**22**)	10	100
7	Boc-Thr(OBn)-PAM resin (**23**)	Boc-Thr(OBn)-OH (**24**)	9	90
8	Boc-Thr(OBn)-Leu-Wang resin (**25**)	Boc-Thr(OBn)-Leu-OH (**26**)	11	100
9	Boc-Ala-Wang resin (**27**)	Boc-Ala-OH (**16**)	9	100

[a]heated at reflux of 1,2-dichloroethane. [b]time in hours. [c]abbreviations: Bn = benzyl, Tos = tosyl (4-toluene sulfonyl); Boc = t-butoxycarbonyl.

Cleavage of amino acids and peptides linked to solid supports by TMTOH proceeded without noticeable racemization; five representative products had optical rotations which were in agreement with reported values (see Experimental). Since the enantiomeric purity of protected peptide fragments is critical for convergent solid phase synthesis as well as cyclization, a more sensitive and reliable assay for both D- and L- amino acids was needed. For this purpose Boc-Alanine (**16**) and Boc-O-benzyl-Serine (**6**), were first *N*-deprotected, then derivatized with Marfey's reagent[13] and analyzed by on RP-HPLC. The absence of D-Ala (19 min) and D-Ser(OBn) (27 min) derivatives in the chromatograms suggests that there was no racemization of these amino acids during the cleavage reaction.

We found that TMTOH selectively cleaves Boc-aspartic acid β-cyclohexyl ester and Boc-glutamic acid γ-cyclohexyl ester attached to Merrifield and Pam resins affording Boc-Asp(OcHex)-OH and Boc-Glu(OcHex)-OH in yields ranging from 90 to 100% (see Table 3). These results demonstrate the potential of this new orthogonal deprotection protocol for solid phase peptide synthesis, when aspartic and glutamic side chain carboxylate groups are protected as cyclohexyl esters.

Table 3. Release of *N*-α-Boc-aspartic acid β-cyclohexyl ester and *N*-α-Boc-glutamic acid γ-cyclohexyl ester from Merrifield and PAM resins by TMTOH.

Entry	Substrate	Product[a]	Reaction time[b]	% Isolated yield
1	Boc-Asp(OcHex)-Merrifield resin[c] (**28**)	Boc-Asp(OcHex)-OH (**29**)	12	97
2	Boc-Glu(OcHex)-Merrifield resin (**30**)	Boc-Glu(OcHex)-OH (**31**)	12	97
3	Boc-Glu(OcHex)-PAM resin (**32**)	Boc-Glu(OcHex)-OH (**31**)	10	100
4	Boc-Asp(OcHex)-PAM resin (**33**)	Boc-Asp(OcHex)-OH(**29**)	11	90

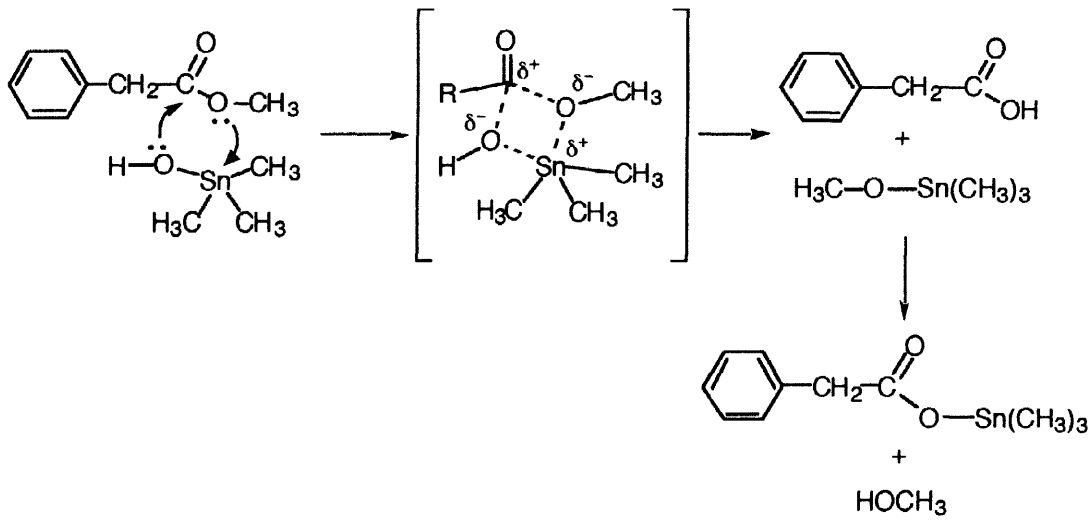
[a]heated at reflux of 1,2-dichloroethane. [b]time in hours.[c]Boc = t-butoxycarbonyl; cHex = cyclohexyl.

The main interest in these semiprotected fragments lies in the application to convergent solid phase synthesis as well as cyclization methods.

A limitation of the TMTOH methodology is the instability of *N*-fluorenylmethoxycarbonyl (Fmoc) and *N*-benzyloxycarbonyl (Cbz or Z) protecting groups.[14]

A mechanism for the cleavage of methyl phenylacetate by TMTOH had been proposed by us based on the identification of trimethyltin phenylacetate.[10] However, we now rather suggest the *in situ* formation of phenylacetic acid and trimethyltin methoxide followed by a further reaction yielding the trimethyltin phenylacetate.

As it was recently described in the literature,[15] carboxylic acids react with tributyltin methoxide to yield tributylesters almost quantitatively. In fact, phenylacetic acid reacted with tributyltin methoxide, under similar conditions of the reaction of ester clevage with TMTOH, to afford tributyltin phenylacetate in a rapid reaction and quantitative yield. Attempts to monitor the progress of the cleavage reaction by ^{1}H NMR spectroscopy failed to reveal the presence of transient intermediates phenylacetic acid and trimethyltin methoxide. A plausible mechansim that is capable of accounting for the formation of phenylacetic acid in this reaction is presented in Scheme 2.



Scheme 2

Unlike other organotin compounds, TMTOH is soluble in water and very insoluble in most organic solvents, this facilitates the removal of excess of TMTOH by acid diluted solution washings.

CONCLUSION

We have developed a very simple and efficient procedure for the preparation of *N*-Boc protected aminoacids and peptide segments without racemization by selective detachment from commercially available Merrifield, PAM and Wang resins, making these resins applicable to Convergent Solid-Phase Peptide Synthesis. We have demonstrated that TMTOH is a highly recommendable reagent for such cleavage being compatible with acid-sensitive side chain protecting groups.

EXPERIMENTAL

General: NMR spectra were measured in $CDCl_3$ or d_6-DMSO at 200 MHz for protons and 20.15 MHz for ^{13}C, and recorded on a Bruker AC-200 spectrometer. Infrared spectra were taken on a Beckman AccuLab spectrometer. Optical rotations were measured with a Jasco DIP-1000 polarimeter at ambient temperature using a 1-mL capacity cell. The HPLC instrument used consisted of two model IIIG Constametric pumps connected to a gradient master from Laboratory Data Control (Riviera Beach, FL, USA). Samples were applied with a Rheodyne 7125 injector (Cotati, CA, USA) and eluant absorption was detected in a 2140 rapid spectral detector (LKB, Sweden). The column used was a Vydac C-18 (218TP54); 250 x4.6 mm id; 5 μm particle diameter, from The Separation Group, Hesperia, CA, USA. TLC and spectral analyses (IR, ^1H and ^{13}C NMR) indicated that all products were identical with commercial authentic material. *N*-protected amino acids were purchased from Calbiochem-Novabiochem Corp. Boc-Tyr(OBn)-Gly linked to Merrifield resin (**1**), Boc-Phe-Met linked to PAM resin (**11**), and Boc-Ser(OBn)-Ala linked to PAM resin (**13**) were prepared according to Merrifield procedure, and Boc-Thr(OBn)-Leu linked to Wang resin (**25**) was synthesized using Fmoc-chemistry.[16]

Cleavage of *N*-Boc amino acids and dipeptides bound to Merrifield resin by TMTOH.
Typical procedure: To a stirred suspension of *N*-Boc-O-benzyl-L-tyrosine-glycine-Merrifield resin (**1**) [0.43 meq Boc-Tyr(O-Bn)-Gly/g of resin (0.174 g, 0.075 mmol)] in 2 mL of $(CH_2Cl)_2$ was added TMTOH (0.050 g, 0.183 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 10 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 25 mL of AcOEt, washed (3 x 10 mL) with aqueous solution of HCl 0.05 N and then extracted with 5% $NaHCO_3$ solution (3 x 10 mL). The combined aqueous solution was acidified to pH 3-4 with 2.5 N H_2SO_4 solution and extracted with AcOEt (3 x 16 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.027 g (84%) of *N*-Boc-O-benzyl-L-tyrosine-glycine (**2**), as a solid. The ^1H NMR analysis of the crude reaction product showed only **2**. No other product was detected by ^1H NMR.

***N*-Boc-O-benzyl-L-tyrosine-glycine (2):** IR (KBr) ν 3400 (N-H, urethane), 3000 (O-H, COOH), 1755 (C=O, carboxylic), 1695-1660 (C=O, urethane, amide I band), 1525 (amide II band), 1260, 830, 740, 700 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.33 (s, 9H, Me), 2.90 (m, 1H, CH_2-Ph), 3.05 (m, 1H, CH_2-Ph), 3.90 (br d, 1H, CH_2), 4.05 (br d, 1H, CH_2), 4.50 (br s, 1H, CH), 4.96 (s, 2H, O-CH_2-Ph), 5.45 (br s, 1H, NH), 6.87 (d, J = 8.15 Hz., 2H, Ar), 7.10 (d, J = 8.15, 2H, Ar), 7.30-7.50 (m, 6H, Ar, NH). ^{13}C NMR ($CDCl_3$) δ 31.32 (Me, Boc), 40.40 (CH2, Cβ, Tyr), 43.60 (CH_2, Cα, Gly), 56.80 (CH, Cα, Tyr), 70.28 (CH_2-Ph), 80.27 (C, Boc), 112.26 (CH, Ar), 123.95 (CH, Ar), 124.40 (CH, Ar), 124.96 (C, Ar), 124.97 (CH, Ar), 126.67 (CH, Ar), 132.81 (C, Ar), 150.58 (C=O, Boc), 152.22 (C, Ar), 165.81(N-C=O).

***N*-Boc-S-p-methoxybenzyl-L-cysteine (4):** $[\alpha]^{20}_D$ -38.0° (c 2.0 in DMF) [lit.[17] -39.0° (c 2.0 in DMF)]. IR (KBr) ν 3370 (NH, urethane), 3000 (OH, COOH), 1730-1715 (C=O, COOH), 1680 (C=O, urethane), 1520 (amide II band), 1255 and 1045 (C=C of vinyl ethers), 825 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H, Me); 2.68 (br s, 2H, CH_2-S); 3.70 (s, 2H, CH_2-Ph); 3.79 (s, 3H, OMe); 4.50 (br s, 1H, CH); 5.28 (br s, 1H, NH); 7.37 (d, J = 8.23 Hz, 2H, Ph); 8.06 (s, 1H, H-Ar). ^{13}C NMR ($CDCl_3$) δ 28.15 (Me, Boc), 33.05 (Cβ), 35.95

(CH_2-Ph), 52.85 (Cα), 55.12 (OMe), 80.41 (C, Boc), 113.90 (CH, Ar), 129.41 (CH, Ar), 129.92 (C, Ar), 155.40 (C=O, Boc), 175.33 (COOH).

***N*-Boc-O-benzyl-L-serine (6):** $[\alpha]^{20}_D$ +22.3° (c 2.0 in EtOH) [lit.[18] +23.5° (c 2.0 in EtOH)]. IR (KBr) ν 3300, 3066 (N-H), 3000 (O-H, COOH), 1745 (C=O, COOH), 1660 (C=O, urethane), 770, 730 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H, Me), 3.70 (dd. *J* = 9.45, 3.95 Hz 1H, CH_2), 3.91 (dd, *J* = 9.45, 3.95 Hz, 1H, CH_2), 4.47 (m, 1H, CH), 4.52 (s, 2H, CH_2-Ph), 5.46 (d, *J* = 8.16 Hz, 1H, NH), 7.29 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$) δ 28.13 (Me, Boc), 53.63 (Cα), 69.50 (Cβ), 73.24 (CH_2, Bn), 80.21 (C, Boc), 127.54 (CH, Ar), 128.30 (CH, Ar), 137.13 (CH, Ar), 155.57 (C=O, Boc), 175.34 (COOH).

***N*-Boc-L-isoleucine (8):** $[\alpha]^{20}_D$ +3.9° (c 2.0 in MeOH) [lit.[18] +3.8° (c 2.0 in MeOH)]. IR (KBr) ν 3340, 3320 (N-H, urethane), 3000 (O-H, COOH), 1760 (C=O, carboxylic, dimer), 1710 (C=O, carboxylic, monomer), 1680 (C=O, urethane), 1530 (amide II band), 1420, 1300 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90-1.00 (m, 6H, Me), 1.10-1.35 (m, 2H, CH_2), 1.45 (s, 9H, Me), 1.90 (br s, 1H, CH), 4.29 (br s, 1H, CH), 5.01 (br s, 1H, NH), 5.90 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$) δ 11.48 (Cδ), 15.37 (Cγ, Me), 24.73 (Cγ, CH_2), 28.15 (Me, Boc), 37.60 (Cβ), 57.70 (Cα), 79.94 (C, Boc), 155.64 (C=O, Boc), 176.83

***N*-Boc-*N*-im-tosyl-L-histidine (10):** IR (KBr) ν 3300, 3070 (N-H, urethane), 3000 (O-H, COOH), 1749, 1725 and 1705 (C=O, COOH, urethane and amide I band); 1530 (amide II band); 1370 and 1180 (SO_2); 1095 cm^{-1}. ^1H RMN ($CDCl_3$) δ 1.44 (s, 9H, Me); 2.45 (s, 3H, Me-Ph); 3.05-3.25 (m, 2H, CH_2); 4.44 (br s, 1H, CH); 5.45 (br s, 1H, NH); 7.10 (s, 1H, H-Ar); 7.37 (d, *J* = 8.23 Hz, 2H, Ph); 7.81 (d, *J* = 8.23 Hz, 2H, Ph); 8.08 (s, 1H, H-Ar). ^{13}C NMR ($CDCl_3$) δ 21.61 (Me-Ar), 33.05 (Cβ), 35.95 (CH_2-Ph), 52.85 (Cα), 55.12 (OMe), 80.41 (C, Boc), 113.90 (CH, Ar), 129.41 (CH, Ar), 129.92 (C, Ar), 155.40 (C=O, Boc), 175.33 (COOH). ^{13}C NMR ($CDCl_3$) δ 28.15 (Me, Boc), 28.16 (Me, Boc), 29.58 (Cβ), 52.37 (Cα), 79.68 (C, Boc), 115.61 (=CH-N,Ar, Cδ), 127.38 (CH, Ar), 130.44 (CH, Ar), 134.16 (=C-N, Ar), 136.79 (-N=CH-N, Ar), 137.96 (C, Ar), 146.62 (C, Ar), 155.08 (C=O, Boc), 172.73 (COOH).

Cleavage of *N*-Boc amino acids and dipeptides bound to PAM resin by TMTOH.

Typical procedure: To a stirred suspension of *N*-Boc-L-phenylalanine-L-methionine-PAM resin (**11**) [0.55 meq Boc-Phe-Met/g of resin (0.151 g, 0.083 mmol)] in 2.5 mL of $(CH_2Cl)_2$ was added TMTOH (0.067 g, 0.245 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 10 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 20 mL of AcOEt, washed with aqueous solution of HCl 0.05 N (3 x 7 mL) and then extracted with 5% $NaHCO_3$ solution (3 x 8 mL). The combined aqueous solution was acidified to pH 3-4 with 2.5 N H_2SO_4 solution and extracted with AcOEt (3 x 13 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.026 g (80%) of *N*-Boc-L-phenylalanine-L-methionine (**12**), as a colorless oil. The ^1H NMR analysis of the crude reaction product showed only **12**. No other product was detected by ^1H NMR.

***N*-α-Boc-L-phenylalanine-L-methionine (12):** IR (film) ν 3340-3300 (N-H, urethane, amide), 3000 (O-H, COOH), 1735-1660 (C=O, COOH, urethane, amide), 1570 (amide II band), 750, 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.38 (s, 9H, Me); 1.95-2.20 (m, 2H, CH_2); 2.06 (s, 3H, H_3C-S), 2.42 (t, *J* = 14.3 Hz, 2H, -CH_2-S); 3.06 (br s, 2H, CH_2-Ph); 4.45 (br s, 1H, CH); 4.63 (br s, 1H, CH); 5.27 (br s, 1H, NH); 5.78 (br s, 1H,

COOH); 7.02 (d, J = 7.60 Hz, 1H, NH); 7.25 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$) δ 15.17 (Me-S), 28.09 (Me, Boc), 29.63 (Cγ, Met), 31.01 (Cβ, Met), 38.09 (Cβ, Phe), 51.66 (Cα), 55.90 (Cα), 80.00 (C, Boc), 126.91 (CH, Ar), 128.54 (CH, Ar), 129.23 (CH, Ar), 136.18 (C, Ar), 155.90 (C=O, Boc), 171.59 (COOH).

***N*-Boc-O-benzyl-L-serine-L-alanine (14):** IR (film) ν 3340 (N-H, urethane, amide), 3000 (O-H, COOH), 1730, 1710, 1660 (C=O, COOH, urethane, amide), 1530 (amide II band), 1260 (amide III band), 770, 730 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H, Me); 1.44 (s, 9H, Me); 3.59 (dd, J = 9.33 Hz., J = 6.27 Hz., 1H, CH_2); 3.88 (dd, J = 9.33, J = 4.02 Hz, 1H, CH_2); 4.29 (br s, 1H, CH); 4.54 (s, 2H, CH_2-Ph); 4.57 (m, 1H, CH); 5.50 (br s, 1H, NH); 7.13 (d, 1H, NH); 7.30 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) δ 17.97 (Me), 28.01 (Me, Boc), 48.16 (Cα), 53.72 (Cα), 69.63 (Cβ, Ser), 73.26 (CH_2-Ph), 80.30 (C, Boc), 127.62 (CH, Ar), 127.74 (CH, Ar), 128.29 (CH, Ar), 137.19 (C, Ar), 155.55 (C=O, Boc), 170.22 (N-C=O), 175.51 (COOH).

***N*-Boc-L-alanine (16):** $[\alpha]^{20}_D$ -22° (c 2.0 in AcOH) [lit.[17] -22° (c 2.0 in AcOH)]. IR (KBr) ν 3410 (N-H, urethane), 3000 (O-H, COOH), 1760 (νC=O, carboxylic, dimer), 1700 (C=O, carboxylic, monomer), 1680 (C=O, urethane), 1530 (amide II band), 1260 cm^{-1} (amide III band). ^{1}H NMR ($CDCl_3$) δ 1.43 (d, J = 6.20 Hz, 3H, Me); 1.45 (s, 9H, Me); 4.34 (br s, 1H, CH); 5.05 (br s 1H, NH); 6.48 (br s, 1H, COOH) ^{1}H NMR (DMSO-d_6) δ 0.97 (d, J = 7.34, 3H, Me); 1.12 (s, 9H, Me); 3.67 (dd, J = 7.34, 7.62 Hz, 1H, CH); 6.76 (d, J = 7.62 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 18.25 (Cβ), 28.14 (Me, Boc), 48.97 and 50.05 (Cα), 80.13 and 81.43 (C, Boc), 155.35 and 158.81 (C=O, Boc), 177.08 y 177.52 (COOH). ^{13}C NMR (DMSO-d_6) δ 17.43(Cβ), 28.61 (Me, Boc), 49.25 (Cα), 78.46 (C, Boc), 155.76 (C=O, Boc), 175.19 (COOH).

***N*-Boc-L-methionine (18):** IR (film) ν 3390 (N-H, urethane), 3000 (O-H, COOH), 1740 (C=O, COOH), 1700 (C=O, urethane), 1530 cm^{-1} (amide II band). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, Me); 2.02 (m, 2H, CH_2); 2.11 (s, 3H, S-CH_3); 2.58 (t, J = 7.45 Hz, 2H, CH_2-S); 4.42 (br s, 1H, CH); 5.17 (br s, 1H, NH); 6.41 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$) δ 15.20 (Me, Cδ), 28.10 (Me, Boc), 29.73 (Cγ), 31.81 and 32.01 (Cβ), 52.48 and 53.32 (Cα), 80.24 and 81.91 (C, Boc), 155.47 and 156.87 (C=O, Boc), 175.89 and 176.53 (COOH).

***N*-Boc-L-proline (20):** IR (KBr) ν 3000 (O-H, COOH), 1750 (C=O, COOH), 1660 (C=O, urethane), 1420, 1210 (both due to coupling between in-plane O-H bending and C-O stretching of dimer), 910 cm^{-1} (O-H out of phase dimer). ^{1}H NMR ($CDCl_3$) δ 1.42 and 1.47 (s, 9H, Me); 1.90-2.30 (m, 4H, CH_2); 3.45 (m, 2H, CH_2-N); 4.30 (br s, 1H, CH); 8.25 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$) δ 23.54 and 24.15 (Cγ), 28.21 (Me, Boc), 30.70 (Cβ), 46.24 and 46.79 (Cδ), 58.89 (Cα), 80.23 and 81.07 (C, Boc), 153.80 and 155.99 (C, Boc), 175.24 and 176.44 (COOH).

***N*-α-Boc-N^G -tosyl-L -arginine (22):** IR (KBr) ν 3440-3340 (N-H, urethane, guanidine), 3000 (O-H, COOH), 1730-1690 (C=O, COOH, urethane and C-N, guanidinium I band), 1650 (C=N), 1595 (C-N, guanidinium II band), 1530 (amide II band), 1420 (both due to coupling between in-plane O-H bending and C-O stretching of dimer), 1370 and 1180 (SO_2), 1095, 850 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, Me); 1.58 (m, 4H, -CH_2-CH_2-); 2.36 (s, 3H, Me); 3.16 (br s, 2H, CH_2-N); 4.17 (br s, 1H, CH); 5.70 (br s, 1H, NH); 6.40 (br s, 2H); 7.20 (d, J = 7.9 Hz, 2H, Ar); 7.69 (d, J = 7.9 Hz, 2H, Ar); 7.80 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 21.28 (Me-Ph), 28.18 (Me, Boc), 29.19 (CH_2,) 40.60 (CH_2,), 46.56 (CH_2,), 53.36 (CH, Cα),

80.02 (C, Boc), 125.86 (CH, Ar), 129.21 (CH, Ar), 140.04 (C, Ar), 142.20 (C, Ar), 156.04 (C=NH), 156.81 (C=O, Boc), 175.94 (COOH).

***N*-Boc-O-benzyl-L-threonine (24):** $[\alpha]^{20}_D$ +16° (c 1.0 in MeOH) [lit.[18] +15.8° (c 1.0 in MeOH)]. IR (KBr) ν 3500 (N-H, urethane), 3000 (O-H, COOH), 1755 (C=O, COOH), 1685 (C=O, urethane), 1540 (amide II band), 1420 (both due to coupling between in-plane O-H bending and C-O stretching of dimer), 770, 730 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.26 (d, *J* = 6.28 Hz, 3H, Me); 1.45 (s, 9H, Me); 4.15-4.21 (m, 1H, O-CH); 4.36 (br d, 1H, CH); 4.39 (dAB, *J* = 11.6 Hz, 1H, CH_2-Ph); 4.59 (dAB, *J* = 11.6 Hz, 1H, CH_2-Ph); 5,33 (d, *J* = 8.57 Hz, 1H, NH); 7.28 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) δ 16.01 (Cγ), 28.12 (Me, Boc), 57.82 (Cα), 70.99 (Cβ), 74.20 (CH_2-Ph), 80.07 (C, Boc), 127.57 (CH, Ar), 128.2 (CH, Ar), 137.41 (CH, Ar), 156.104 (C=O, Boc), 175.62 (COOH).

Cleavage of *N*-Boc-O-benzyl-L-threonine-L-leucine bound to Wang resin (25) by TMTOH: To a stirred suspension of Boc-Thr(OBn)-Leu-resin (**25**) [0.65 meq Boc-Thr(OBn)-Leu/g of resin (0.129 g, 0.084 mmol)] in 1.5 mL of $(CH_2Cl)_2$ was added TMTOH (0.057 g, 0.209 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 11 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 20 mL of AcOEt, washed with aqueous solution of HCl 0.05 N (3 x 10 mL) and then extracted with 5% $NaHCO_3$ solution (3 x 8 mL). The combined aqueous solution was acidified to pH 3-4 with 2.5 N H_2SO_4 solution and extracted with AcOEt (3 x 10 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.035 g (100%) of Boc-Thr(OBn)-Leu-OH (**26**), as a colorless oil. The ^1H NMR analysis of the crude reaction product showed only **26.** No other product was detected by ^1H NMR. IR (film) ν 3340-3300 (N-H, urethane, amide), 3000 (O-H, COOH), 1710, 1665, 1645 (C=O, COOH, urethane, amide), 1530-1510 (amide II band), 1260 (amide III band), 770, 730 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.22 (d, *J* = 11.1 Hz, Me); 1.44 (s, 9H, Me); 2.50-5.65 (m, 3H, CH_2, CH); 4.14 (br s, 1H, CH); 4.36 (br s, 1H, CH); 4.54 (d, *J* = 11.4, 1H, CH_2); 4.64 (d, J = 11.4 Hz, 1H, CH_2); 5.58 (d, *J* = 7.34, 1H, NH); 7.10 (d, J = 7.9 Hz, 1H, NH); 7.32 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) δ 14.72 (Cγ, Thr), 21.45 (Cδ, Leu), 22.88 (Cδ, Leu), 24.59 (Cγ, Leu), 28.14 (Me, Boc), 40.85 (Cβ, Leu), 50.72 (Cα, Leu), 57.10 (Cα, Thr), 71.28 (CH_2-Ph), 74.74 (Cβ, Thr), 80.15 (C, Boc), 127.68 (CH, Ar), 128.28 (CH, Ar), 137.70 (CH, Ar), 155.76 (C=O, Boc), 172.00 (N-C=O), 177.00 (COOH).

Cleavage of *N*-Boc-L-alanine bound to Wang resin (27) by TMTOH: To a stirred suspension of Boc-Ala-resin (**27**) [0.81 meq Boc-Ala/g of resin (0.273 g, 0.22 mmol)] in 2.5 mL of $(CH_2Cl)_2$ was added TMTOH (0.154 g, 0.56 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 9 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 20 mL of AcOEt and washed succesively with diluted aqueous solution of HCl (5 x 6 mL), water (1 x 6 mL) and brine (1 x 6 mL). Then the organic solution was extracted with 5% $NaHCO_3$ solution (3 x 7 mL), the combined aqueous solution was acidified to pH 3-4 with 2.5 N H_2SO_4 solution and extracted with AcOEt (3 x 11 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.042 g (100%) of Boc-Ala-OH (**16**), as a solid. The ^1H NMR analysis of the crude reaction product showed only **16**. No other product was detected by ^1H NMR.

Cleavage of *N*-Boc-aspartate β-cyclohexyl ester bound to Merrifield resin (28) by TMTOH. To a stirred suspension of Boc-Asp(OcHex)-resin (**28**) [0.34 meq Boc-Asp(OcHex)/g of resin (0.331 g, 0.113 mmol)] in 3 mL of $(CH_2Cl)_2$ was added TMTOH (0.071 g, 0.257 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 12 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 16 mL of AcOEt, washed (4 x 6 mL) with diluted aqueous solution of HCl (pH=3-4) and then extracted with 5% $NaHCO_3$ solution (6 x 5 mL). The combined aqueous solution was acidified to pH 3-5 with 2.5 N H_2SO_4 solution and extracted with AcOEt (6 x 10 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.035 g (97%) of Boc-Asp(OcHex)-OH (**29**), as a solid. The ^1H NMR analysis of the crude reaction product showed only **29**. No other product was detected by ^1H NMR. IR (KBr) ν 3420 (N-H, urethane), 3000 (O-H, COOH), 1740 (νC=O, carboxylic ester), 1710 (C=O, carboxylic, urethane), 1530 (amide II band), 1190 cm^{-1} (νC-O, split ester band). ^1H NMR ($CDCl_3$) δ 1.25-1.60 (m, 6H, CH_2); 1.45 (s, 9H, Me); 1.62-1.90 (m, 4H, CH_2); 2.81 (dd, J = 17 Hz, J = 4.33 Hz, 1H, CH_2); 3.00 (dd, J = 17 Hz, J = 4.3 Hz, 1H, CH_2); 4.60 (sa, 1H, CH); 4.77 (m, 1H, CH); 5.55 (d, J = 8.2 Hz, NH); 8.20 (sa, 1H, COOH). ^{13}C NMR ($CDCl_3$) δ 23.48 (CH_2), 25.12 (CH_2), 28.13 (Me, Boc), 31.30 (CH_2), 36.81 (Cβ), 49.78 (Cα); 73.71 (CH); 80.28 (C, Boc), 155.50 (C, Boc), 170.37 (-COO-), 175.67 (COOH).

Cleavage of *N*-Boc-glutamate γ-cyclohexyl ester bound to Merrifield resin (30) by TMTOH. To a stirred suspension of Boc-Glu(OcHex)-resin (**30**) [0.48 meq Boc-Glu(OcHex)/g of resin (0.938 g, 0.45 mmol)] in 7 mL of $(CH_2Cl)_2$ was added TMTOH (0.270 g, 0.988 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 12 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 40 mL of AcOEt, washed (5 x 10 mL) with diluted aqueous solution of HCl (pH=3-4) and then extracted with 5% $NaHCO_3$ solution (5 x 10 mL). The combined aqueous solution was acidified to pH 3-5 with 2.5 N H_2SO_4 solution and extracted with AcOEt (4 x 15 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.143 g (97%) of Boc-Glu(OcHex)-OH (**31**), as a colorless oil. The ^1H NMR analysis of the crude reaction product showed only **31**. No other product was detected by ^1H NMR. IR (film) ν 2990 (O-H, COOH), 1710-1750 (νC=O, carboxylic ester, carboxylic acid, urethane), 1530 (amide II band), 1190 cm^{-1} (νC-O, ester band). ^1H NMR ($CDCl_3$) δ 1.19-1.62 (m, 6H, CH_2); 1.55 (s, 9H, Me); 1.62-1.92 (m, 4H, CH_2); 1.93-2.35 (m, 2H, CH_2); 2.44 (m, 2H, CH_2); 4.31 (sa, 1H, CH); 4.77 (q, 4.0 Hz, 1H, CH); 5.21 (sa, NH).

Cleavage of *N*-Boc-glutamate γ-cyclohexyl ester bound to Pam resin (32) by TMTOH. To a stirred suspension of Boc-Glu(OcHex)-resin (**32**) [0.45 meq Boc-Glu(OcHex)/g of resin (0.344 g, 0.155 mmol)] in 3 mL of $(CH_2Cl)_2$ was added TMTOH (0.098 g, 0.359 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 10 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 20 mL of AcOEt, washed (4 x 7 mL) with diluted aqueous solution of HCl (pH=3-4) and then extracted with 5% $NaHCO_3$ solution (3 x 8 mL). The combined aqueous solution was acidified to pH 3-5 with 2.5 N H_2SO_4 solution and extracted with AcOEt (3 x 10 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.051 g (100%) of Boc-

Glu(OcHex)-OH (**31**), as a colorless oil. The ^{1}H NMR analysis of the crude reaction product showed only **31**. No other product was detected by ^{1}H NMR.

Cleavage of *N*-Boc-aspartate β-cyclohexyl ester bound to Pam resin (33) by TMTOH. To a stirred suspension of Boc-Asp(OcHex)-resin (**33**) [0.44 meq Boc-Asp(OcHex)/g of resin (0.444 g, 0.195 mmol)] in 3.5 mL of $(CH_2Cl)_2$ was added TMTOH (0.117 g, 0.429 mmol) at room temperature under nitrogen. The reaction mixture was then refluxed (83°C) for 11 h and the resulting suspension filtered and washed succesively with $(CH_2Cl)_2$, CH_2Cl_2, MeOH and AcOEt. The combined organic solution was evaporated to dryness under reduced pressure and the resultant residue was dissolved in 16 mL of AcOEt, washed (4 x 6 mL) with diluted aqueous solution of HCl (pH=3-4) and then extracted with 5% $NaHCO_3$ solution (6 x 5 mL). The combined aqueous solution was acidified to pH 3-5 with 2.5 N H_2SO_4 solution and extracted with AcOEt (6 x 10 mL). The AcOEt solution was dried (Na_2SO_4) and evaporated to dryness to yield 0.055 g (90%) of Boc-Asp(OcHex)-OH (**29**) as a solid. The ^{1}H NMR analysis of the crude reaction product showed only **29**. No other product was detected by ^{1}H NMR.

Study of the Optical Purity and Racemization-free Cleavage of amino acids by TMTOH
Boc-Ala-OH (**16**) and Boc-Ser(OBn)-OH (**6**) were deprotected by treating with 50% (v/v)TFA/DCM for 20 min at room temperature and the resulting solutions evaporated to obtain the free amino acids. Derivatization of the amino acids was performed with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (DNPA) (Calbiochem-Novabiochem Corp.)(Marfey's reagent).

Typical procedure: 1 mL of a 10 mM solution of amino acid in 0.1 M $NaHCO_3$ was added to 2 mL of freshly prepared 10 mM 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone and the solution kept at 40 °C for 1h with frequent mixing. 1 mL of 0.2 N HCl was then added to the cooled solution. After degassing and filtration, 10 µL samples were injected into HPLC. Conditions: 0.8 mL/min of 10 to 60% acetonitrile in 30 min; 0.1% TFA was maintained throughout elution. Elution times: L-Ala-DNPA 17 min, D-Ala-DNPA 19 min, L-Ser(OBn)-DNPA 25 min, D-Ser(OBn)-DNPA 27 min.

Acknowledgements. We would like to thank Professor Sir Alan R. Battersby (Cambridge University, U.K) for his assistance in the preparation of the manuscript. We would also like to thank CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas - Argentina) for financial support and fellowship (R. F.). One of us (C. P.) thanks the University of Buenos Aires (Argentina) for a grant.

REFERENCES AND NOTES

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
2. a) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976**, *98*, 7357. b) For a new synthetic route to PAM resin, see: Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43*, 2845.
3. a) Wang. S.S. *J. Am. Chem. Soc.* **1973**, *95*, 1328. b) Wang, S. S. *J. Org. Chem.* **1975**, *40*, 1235. c) Chang, J. K.; Shimizu. M.; Wang. S. S. *J. Org. Chem.* **1976**, *41*, 3255.
4. a) Mergler, M.; Tanner, R.; Gostelli, J.; Grogg, P. *Tetrahedron Lett.* **1988**, *29*, 4005. b) Mergler, M.; Nyfeler, R.; Tanner, R.; Gostelli, J.; Grogg, P. *Tetrahedron Lett.* **1988**, *29*, 4009.
5. Riniker, B.; Flörsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307.

6. For recent reviews on Solid-Phase Peptide Synthesis, see: a) Williams, P. L.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton, **1997**, Chapter 2. b) Merrifield, B. In *Peptides Synthesis, Structures and Applications:* Gutte, B. Ed. Academic Press, San Diego, **1995**, pp. 93-169.
7. For recent reviews on convergent SPPS, see: a) ref. 6b) pp. 139-207. b) Williams, P. L.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065. c) Benz, H. *Synthesis* **1994**, 337.
8. Ueki, M.; Kafi, K.; Amemiya, M.; Horino, H.; Oyamada, H. *J. Chem. Soc. Chem. Commun.*, **1988**, 414.
9. Anwer, M. K.; Spatola, A. F. *Tetrahedron Lett.* **1992**, *33*, 3121.
10. Furlán, R. L. E.; Mata, E. G.; Mascaretti, O. A. *J. Chem. Soc. Perkin Trans. 1*, **1998**, 355.
11. For recent reviews on Solid-Phase Organic Reactions, see: a) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527. b) Früchtel, J. S.; Jung, G. *Angew. Chem, Int. Ed. Engl.* **1996**, *35*, 17.
12. For recent reviews on combinatorial libraries and molecular diversity, see: a) Terrett, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135. b) Thompson, L.A.; Ellman, J.A. *Chem. Rev.* **1996**, *96*, 555. c) Patel, D.V.; Gordon, E.M. *Drug Discovery Today* **1996**, *1*, 134. d) Balkenhohl, F.; Bussche-Hünnefeld, Ch.; Lansky, A.; Zechel, Ch. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288. For recent highlights, see *Acc. Chem. Res.* **1996**, *29*, No. 3.
13. Gordon Adamson, J.; Hoang, T.; Crivici, A.; Lajoie, G.A. *Analytical Biochemistry* **1990**, *202*, 210.
14. When Fmoc-Ser(O^{t}Bu) linked to Wang resin is treated with TMTOH in 1,2-dichloroethane for 10 h. a mixture of free Ser(O^{t}Bu)-OH and Fmoc-Ser(O^{t}Bu)-OH is obtained.
15. Thibonet, J.; Abarbri, M.; Parrain, J. L.; Duchêne, A. *Main Group Metal Chemistry* **1997**, *20.*, 195. See also: Valade, J; Pereyre M. *Académie des Sciences*, **1962**, 235.
16. Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd. Edition Pierce Chemical Company, Rockford, **1984**, pp 76-83.
17. Taken from the Analytical Data Sheet of Bachem Feinchemikalien AG (Switzerland).
18. Reference 16, page 146.