Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 386–392
Published online 10 May 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.856

JOURNAL OF PEPTIDE SCIENCE

Comparison of procedures for directly obtaining protected peptide acids from peptide-resins‡

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Received 3 January 2007; Revised 22 February 2007; Accepted 23 February 2007

Abstract: The preparation of small-sized protected peptide acids related to cholecystokinin and gomesin was attempted using peptide-Kaiser oxime resins (KOR) as starting materials. For comparison, peptide-2-Cl-trityl resin (CLTR) was also employed. While peptide detachment from KOR was achieved through the oxime ester bond hydrolysis mediated by DBU, hydroxide ion or Ca^{+2} ion, peptide release from CLTR was accomplished through acid-catalysed hydrolysis of the peptide-resin ester linkage. Amino acid analysis of the peptide-resins before and after peptide release allowed the calculation of the reaction yields. RP-HPLC and LC/ESI-MS of the resulting crude peptides allowed estimation of their quality. The data collected indicated that: (i) among the procedures used for peptide displacement from KOR, the one employing DBU was the most efficient since it furnished all model protected peptide acids with the highest quality in a very short time; (ii) although slow, Ca^{+2}-assisted peptide detachment from KOR was selective and was suitable for generating high-quality protected peptide acids containing up to five residues; (iii) even though the protocols employed for peptide release from CLTR have shown to be appropriate, the one employing 1% TFA/DCM was the most productive; (iv) in terms of product quality, DBU-catalysed peptide detachment from KOR was similar to TFA-catalysed peptide release from CLTR although the latter was more productive. These findings are relevant to peptide chemists in general, but especially to those interested in preparing acyl donors for convergent peptide syntheses by the t-Boc chemistry. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: solid-phase peptide synthesis; acyl donors for convergent synthesis; Kaiser oxime resin; 2-chlorotrityl resin

INTRODUCTION

The stepwise solid-phase method has been considered highly efficient for the synthesis of a wide variety of peptides [1,2]. Nevertheless, it is well known that some peptides are difficult, or even impossible, to produce by means of this method owing to their high tendency to aggregate and/or undergo undesirable reactions during the peptide build-up process [3–5].

Convergent peptide synthesis has been described as an interesting and straightforward approach to prepare such 'difficult sequences' and long peptides [6]. It consists of forming the desired molecule by condensing its fully protected segments, the acyl donors and acyl acceptors [7].

In principle, any polymeric resin applicable to stepwise solid-phase peptide synthesis (SSPPS) can be employed to produce the acyl acceptors for convergent solid-phase peptide synthesis (CSPPS). As to the acyl donors, they are usually synthesized by SSPPS using resins and/or linkers that form a highly labile ester linkage with the growing peptide chain [7].

The Kaiser oxime resin (KOR; *p*-nitrobenzophenone oxime resin) [8], the *N*-[9-(hydroxymethyl)-2-fluorenyl] succinamic acid (HMFS) linker [9] and the bromophenacyl resin [10] are examples of polymeric resins and linkers proven to be useful for synthesizing protected peptide acids by Boc chemistry. Among those compatible with the Fmoc chemistry, the 2-chlorotrityl resin (CLTR) [11], the handle 4-hydroxymethyl-3-methoxyphenoxybutyric acid (HMPB) [12] and the 2-methoxyl-4-alkoxybenzyl alcohol resin (SASRIN; super acid sensitive resin) [13] are frequently employed. Intriguingly, attempts to prepare protected peptide acids using those polymeric resins and linkers, as well as peptide release procedures recommended by the manufacturers, can be troublesome. In addition, relatively little research has been done on the preparation of acyl donors for CSPPS containing several trifunctional amino acids. Therefore, in the present work we attempted to synthesize small-sized protected peptide acids of varied sequences starting from the corresponding peptide-KOR and using three different procedures for peptide detachment through hydrolysis of the oxime ester bond. In each case, the peptide detachment yield was determined and the resulting crude peptide was inspected. For comparison, we also employed peptide-CLTR as starting material and employed the protocols based on acid catalysis usually recommended for peptide detachment. We chose KOR as the main polymeric resin to be used because,

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‡ Preliminary accounts of certain aspects of this work were described earlier as a communication in: Moraes, C. M.; Vieira, L. A. O.; Silva, K. N.; Miranda, M. T. M. In *Peptides 2000, Proceedings of the 26th European Peptide Symposium*; and published in Martinez, J. & Fehrentz, J.-A., Eds. Methanolysis and hydrolysis on peptidyl-Kaiser oxime resin assisted by metal ions. EDK: Paris, 2001; p. 255–256.

although employing Fmoc chemistry, we still regularly use the Boc strategy.

MATERIALS AND METHODS

General

Protected amino acids and coupling reagents were obtained from Bachem California and Peptide Institute Inc. Analytical or chromatographic grade solvents and reagents were from Merck, Riedel-De Haën AG, Sigma Chemical Co. and Applied Biosystem Inc. KOR was purchased from Advanced Chemtech or prepared in our laboratory according to DeGrado and Kaiser's procedure [8]. CLTR was obtained from Novabiochem Corp.

Total acid hydrolysis of the amino acid- or peptide-resin was performed on a Waters Pico-Tag workstation (Milford, USA). Amino acid analysis of the hydrolysate was performed on a Beckman automated analyser, model 7300 (Palo Alto, USA). Peptide RP-HPLC analysis was performed on a Waters system (two Waters 510 pumps, a Waters 680 automated gradient controller and a Waters 486 tunable absorbance UV-vis detector (Milford, USA)) or on an LDC Analytical system (a Constametric 3500 pump, a Constametric 3200 pump, a TSP 3100 UV-vis detector (San Jose, USA)) equipped with a 7125 Rheodyne injector and a 745B Waters integrator and connected to a Vydac C_{18} or C_4 column (5 μm, 300 Å, 25 cm). The elution conditions were: solvent A, 0.1% TFA in H_2O; solvent B, ACN/0.09% TFA in H_2O in appropriate proportions; flow rate, 1 ml/min; detection wavelength: 210 nm. Liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) analysis was performed using an on-line coupling of a Shimadzu RP-HPLC system VP Series (Kyoto, Japan) (two LC-10AD pumps, a SDP-10-AV detector and a 7125 Rheodyne injector) to the Micromass Quatro II triple quadrupole mass spectrometer (Altrincham, UK). LC was achieved using the same column and the RP-HPLC conditions described above. The cone voltage used for ESI-MS was 37 V.

First Residue Loading on KOR Resin

KOR (1.0 g, 1.0 mmol) was suspended in 10 ml of DCM containing 2.5 mmol of Boc-amino acid and equimolar amount of DIPCI or equimolar amounts of DIPCI and HOBt. The reaction vessel was shaken for 22 h at room temperature. The reaction mixture was drained. The aminoacyl resin was washed with 15 ml of DCM and dried. No end-capping step was performed. The picric acid assay [14] was used to determine the average loading of the first amino acid (triplicates).

First Residue Loading on CLTR

The Fmoc-amino acid (87.15 mg, 0.29 mmol) and DIPEA (41 μl, 0.23 mmol, corresponding to 1/3 of the total amount of base required for 2.5equiv. relative to the Fmoc-amino acid) were added to 0.6 g of the resin (1.6 mmol/g) suspended in 2.5 ml of DCM/DMF (4 : 1, v/v). The resulting mixture was stirred for 5 min. The remainder of the DIPEA (82 μl, 0.47 mmol) in DCM (3.5 ml) was then added and the stirring was continued for a further 60 min. The end capping was performed by adding HPLC grade MeOH (0.5 ml) and stirring the reaction mixture for 10 min more, and then the resin was filtered, successively washed with DCM, DMF, 2-propanol, MeOH and diisopropyl ether and, finally, dried. The average loading of the first amino acid was determined through total hydrolysis followed by amino acid analysis of the hydrolysate (triplicates).

General Procedure for Manual Solid-phase Assembly of Protected Peptide-KOR

The Boc protective group was removed by a 20-min treatment with 40% TFA/DCM. Coupling reaction was carried out in DMF by *in situ* activation for 1 h of 2.5-fold molar excess of Boc-amino acid in the presence of TBTU and DIPEA sufficient to provide an apparent pH ~8. An exception was made for Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly-KOR and Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly-KOR, which were built up employing BOP/HOBt in the third amino acid coupling to minimize DKP formation [15]. *N*-terminal acetylation was performed using 50% acetic anhydride in DCM after removal of the Boc group.

After each step of the chain assembly, the filtered resin was sequentially washed with DMF, MeOH and DCM and submitted to the Kaiser test [16]. The initial substitution level (SL) of the resultant dried peptide-KOR was determined through total hydrolysis followed by amino acid analysis of the hydrolysate (triplicates).

General Procedure for Solid-phase Assembly of Protected Peptide-CLTR

The synthesis was performed as described above, except obviously for the Fmoc removal (carried out with 20% piperidine/DMF for 10 min followed by alternate washings with DMF and MeOH) and use of only TBTU/DIPEA as the coupling system (no DKP formation was expected during the synthetic process most likely due to the CLTR hindrance [17]). The initial SL of the resultant dried peptide-CLTR was determined through total hydrolysis followed by amino acid analysis of the hydrolysate (triplicates).

Peptide Detachment from Resins

Promoted by DBU (adapted from (18)). The peptide-KOR (17.5 μmol) was suspended in 1 ml of 15% H_2O/THF in the absence or presence of DBU (35.0 μmol). The resulting suspension was incubated for 4 h at room temperature or at 55 °C under orbital shaking at 300 rpm. The supernatant was separated by filtration from the solid resin, which was washed alternately with MeOH and DCM.

Promoted by hydroxide ion (19). Approximately 20 mg of the peptide-KOR was pre-swollen in 0.3 ml of dioxane for 15 min. A volume of 0.1 ml of chilled 1 M NaOH was added and the suspension was left to stand for 15 min in an ice bath. The supernatant was separated from the solid resin by filtration and collected in 0.2 ml of 0.1 M HCl in an ice bath. The pH of the resulting solution was adjusted to 7.0. The solid resin was washed alternately with MeOH and DCM.

Assisted by Ca^{+2} ion (adapted from (20)). The peptide-KOR (17.5 μmol) was suspended in 1 ml of 15% H_2O/THF in the

DOI: 10.1002/psc

absence or presence of calcium acetate, $Ca(OAc)_2$, (35.0 μmol). The resulting suspension was incubated for 50 h at room temperature or 55 °C under orbital shaking at 300 rpm. The supernatant was separated by filtration from the solid resin, which was washed alternately with MeOH and DCM.

Catalysed by TFA (21). Approximately 40 mg of the peptide-CLTR was suspended in 500 μl of 1% TFA/DCM. The resulting suspension was shaken for 2 min at room temperature at 300 rpm. The supernatant was separated from the solid resin by filtration and collected in a flask containing 10% pyridine/MeOH (100 μl). The entire procedure was repeated twice and then the solid resin was washed with DCM.

Catalysed by AcOH (11). Approximately 40 mg of the peptide-CLTR was suspended in 500 μl of AcOH/TFE/DCM (2 : 2 : 6, 1 : 2 : 7 or 1 : 1 : 8, v/v/v) and shaken for 2 h at room temperature. The supernatant was separated by filtration from the solid resin, which was washed with DCM.

In all cases, the supernatants containing the crude products were analysed by RP-HPLC and LC/ESI-MS. The final SLs of the dried remaining peptide-resins were determined through total hydrolysis followed by amino acid analysis of the hydrolysate (triplicates). The difference between the initial and final SLs allowed the calculation of the yields of peptide detachment from the resins.

RESULTS AND DISCUSSION

Owing to difficulties encountered during the SSPPS of CCK-33 [22], CCK-33 fragments [23] and gomesin (Gm) analogues [24], we attempted to prepare short protected fragments of such biologically active peptides that could act as acyl donors in CSPPS. Therefore, the present investigation focusses on the CCK-(22–24)-peptide [25], the CCK-(1–5)-peptide and the Gm-(9–16)-peptide.

KOR has been used successfully for preparing *C*-terminal free and modified protected peptides because it forms with the growing peptide an oxime ester susceptible to attack by any nucleophile under acid or base conditions [8,18,26,27]. Among the procedures used to prepare protected peptide acids [18,19,28,29], we chose those comprising a single step: hydroxide-ion-promoted hydrolysis of the oxime ester bond (procedure 1; [19]) and DBU-promoted hydrolysis of the oxime ester linkage (procedure 2; [18]). We also employed procedure 3, which is based on our previous observations that hydrolysis [20] and methanolysis [25,30] of the oxime ester bond of a peptide-KOR can be assisted by Ca^{+2}.

Peptide-CLTR has been used to directly provide the corresponding protected peptide acids [31] because CLTR binds to the peptide growing chain through an ester linkage labile to diluted solutions of TFA (procedure 4; [21]), acetic acid/TFE (procedure 5; [11]) or HFIP [32] in DCM. Therefore, we also attempted to prepare the protected peptide acid related to Gm, the Gm-(9–16)-peptide, starting from the corresponding peptide-CLTR using procedures 4 and 5.

As seen in Table 1, the analytical data for the peptide-resins obtained and for the major components

Table 1 Characterization of the peptide-resins (**A**) and of the major components of the crude protected peptide acids (**B**) obtained

Peptide sequence	Resin	A		B	
		Amino acid analysis found (theor.)[a]	SL (mmol/g)	RP-HPLC t_R (min)	ESI-MS $[MH]^+$ found (calcd)
Ac-Ile-Ser(Bzl)-Asp(OcHx)	KOR	Ile: 1.00 (1.00); Asp: 1.00 (1.00)	0.49	22.3	548.1 (548.6)
Ac-Ile-Ser(Bzl)-Lys[Z(2Cl)]	KOR	Ile: 1.10 (1.00); Lys: 0.90 (1.00)	0.25	22.8	647.9 (648.2)
Ac-Ile-Ser(Bzl)-Leu	KOR	Ile: 1.00 (1.00); Leu: 1.00 (1.00)	0.23	20.0	463.9 (464.6)
Ac-Ile-Ser(Bzl)-Phe	KOR	Ile: 1.00 (1.00); Phe: 1.00 (1.00)	0.22	20.7	498.3 (498.6)
Ac-Ile-Ser(Bzl)-Gly	KOR	Ile: 1.10 (1.00); Gly: 0.90 (1.00)	0.24	16.5	408.4 (408.5)
Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly	KOR	Lys: 1.00 (1.00); Ala: 1.23 (1.00); Pro: 1.00 (1.00); Gly: 1.00 (1.00)	0.37	22.6	817.6 (817.3)
Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly	KOR	Arg: 1.74 (2.00); Val: 1.00 (1.00); Tyr: 0.94 (1.00); Gly: 0.94 (1.00)	0.28	31.9	1878.5 (1877.1)
Boc-Arg(Tos)-Cys(SBu^t)-Val-Thr(Bu^t)-Tyr(Bu^t)-Cys(SBu^t)-Arg(Pmc)-Gly	CLTR	Arg: 2.01 (2.00); Val: 1.00 (1.00); Tyr: 1.05 (1.00); Gly: 1.28 (1.00)	0.11	18.8[b]	1765.4 (1766.4)

[a] Ser, Thr and Cys are partially or totally destroyed under acid conditions.
[b] A C_4 column was used instead of a C_{18} column.

DOI: 10.1002/psc

of the resulting crude protected peptide acids are in agreement with the theoretical ones.

Preparation of Protected Peptide Acids from the Corresponding Peptide-KOR

CCK-(22–24)-peptide: Ac-Ile-Ser(Bzl)-Asp(OcHx)-OH. The RP-HPLC profiles shown in Figure 1 indicated that: (i) despite the high rate at low temperature (15 min at 0–5 °C), the hydroxide-ion-promoted reaction generated a product containing a few contaminants, including an epimer; whether the epimer was formed during peptide elongation [7], oxime ester hydrolysis [29] or simple exposure to alkali [33] remains to be seen; (ii) the reaction promoted by DBU was slower (4 h at room temperature) but provided a higher-quality product; (iii) despite being very slow (50 h at 55 °C), the Ca^{+2}-assisted reaction was efficient and selective (no by-product was detected in the crude protected peptide); (iv) because of the elevated susceptibility of the oxime ester linkage to nucleophiles, a relatively low percentage of peptide detachment occurred in 15% H_2O/THF at 55 °C for 50 h with no additive.

It is noteworthy that the experimental conditions employed for procedure 3 were previously determined as follows: at first, the swelling properties of Ac-Ile-Ser(Bzl)-Asp(OcHx)-KOR were examined at room temperature and at 55 °C in mixtures of H_2O with DMF, DMSO, NMP or THF capable of dissolving $Ca(OAc)_2$; as THF led to the highest swelling degree, the tripeptide-KOR was then incubated in 5, 15 and 25% H_2O/THF mixtures containing $Ca(OAc)_2$ at 37 °C for 72 h resulting in peptide detachment yields of 1, 36 and 33%, respectively; finally, reactions were performed in 15% H_2O/THF that furnished the desired product with yields of 36% (42 h at 37 °C) and 60% (72 h at 50 °C).

CCK-(22–24)-peptide analogues: Ac-Ile-Ser(Bzl)-X-OH, where X is Lys(Z(2Cl)), Leu, Phe or Gly. Aiming to further compare procedures 1–3, we used Ac-Ile-Ser(Bzl)-Asp(OcHx)-KOR analogues as starting materials. The results described in Table 2 indicated that: (i) as expected, the oxime ester bond was labile even in the absence of base or metal ion; (ii) glycine was the most suitable amino acid to bind to KOR leading to the protected tripeptide acid with very high yields independent of the procedure used; (iii) regardless of the nature of the *C*-terminal amino acid, the hydroxide-ion-promoted reactions were the most productive; (iv) independent of the *C*-terminal amino acids, oxime ester hydrolysis was more efficiently promoted by DBU than by Ca^{+2}; (v) the substitution of THF by NMP in the Ca^{+2}-assisted reactions at 70 °C significantly increased the peptide displacement yields.

The monitoring of the reactions by RP-HPLC and LC/ESI-MS (not shown) indicated that the Ca^{+2}-mediation provided the best-quality crude protected peptides, although the peptide detachment was the most time consuming. In addition, it revealed that the reactions promoted by DBU occurred with satisfactory rates and furnished products of good quality. Moreover, it showed that, despite being fast, the hydroxide-ion-promoted reactions generated crude protected peptides of poor quality.

It should be stressed that the superiority of glycine as the *C*-terminal amino acid residue for the preparation of acyl donors for CSPPS starting from the corresponding peptide-KOR (Table 2) is accentuated by the absence of epimerization not only during the loading of this amino acid to KOR but also during the peptide detachment through the oxime ester hydrolysis. On the other hand, the synthesis of such acyl donors requires greater care since glycine as *C*-terminal residue facilitates the formation of DKP [15].

CCK-(1–5)-peptide: Boc-Lys(Z(2Cl))-Ala-Pro-Ser(Bzl)-Gly-OH. As shown in Table 2, procedures 1 and 2 were

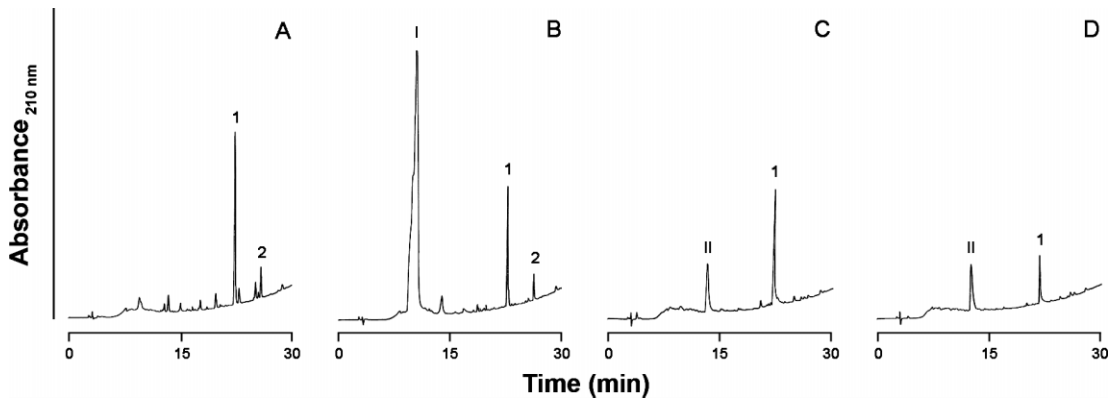
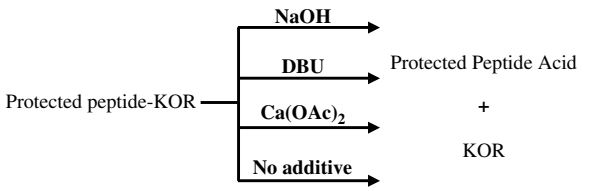


Figure 1 RP-HPLC monitoring of the oxime ester bond hydrolysis of Ac-Ile-Ser(Bzl)-Asp(OcHx)-KOR in 25% NaOH (1 M)/dioxane at 0–5 °C for 15 min (A), promoted by DBU in 15% H_2O/THF at room temperature for 4 h (B), assisted by Ca^{+2} in 15% H_2O/THF at 55 °C for 50 h (C) and with no additive in 15% H_2O/THF at 55 °C for 50 h (D). Peaks I and II: DBU and THF, respectively; peak 1: Ac-Ile-Ser(Bzl)-Asp(OcHx)-OH; peak 2: unidentified by-product.

DOI: 10.1002/psc

Table 2 Percentages of peptide detachment from KOR using different procedures



Protected peptide-	Temperature (°C)	Additive			
		NaOH[a]	DBU[b]	Ca^{+2}[c]	None[c]
Ac-Ile-Ser(Bzl)-Asp(OcHx)-	0–5	93	—	—	—
	RT	—	87	—	—
	55	—	—	64[d]	18
Ac-Ile-Ser(Bzl)-Gly-	0–5	92	—	—	—
	RT	—	90	—	—
	55	—	—	90	41
Ac-Ile-Ser(Bzl)-Leu-	0–5	87	—	—	—
	RT	—	32	—	—
	55	—	—	11	7
Ac-Ile-Ser(Bzl)-Phe-	0–5	82	—	—	—
	RT	—	24	—	—
	55	—	—	28	21
Ac-Ile-Ser(Bzl)-Lys[Z(2Cl)]-	0–5	94	—	—	—
	RT	—	58	—	—
	55	—	—	19[d]	5
Boc-Lys[Z(2Cl)]-Pro-Ala-Ser(Bzl)-Gly-	0–5	95	—	—	—
	RT	—	92	49	18
	55	—	95	59	20

[a] 15 min, 25% NaOH (1 M)/dioxane.
[b] 4 h, 15% H_2O/THF.
[c] 50 h, 15% H_2O/THF.
[d] Using 15% H_2O/NMP at 70 °C, these yields increased 0.5 (Asp) and 4 times (Lys).

more efficient than procedure 3. Indeed, the hydroxide-ion-promoted hydrolysis was again the most productive. Temperature elevation clearly improved the peptide detachment assisted by Ca^{+2}, but not when it was promoted by DBU.

RP-HPLC and LC/ESI-MS analyses of the crude products showed that in all cases the major component corresponded to the desired protected pentapeptide acid (Figure 2 and Table 1). Two by-products resulted from the synthetic process: Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-OH formed because no end-capping step was done after the loading of first glycine to KOR; Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly-Gly-OH possibly formed during the coupling or recoupling of glycine to KOR [7,34].

Gm-(9–16)-peptide: Boc-Arg(Tos)-Cys(Bzl(Me))-Val-Thr(Bzl)-Tyr(Z(2Br))-Cys(Bzl(Me))-Arg(Tos)-Gly-OH.

DBU-promoted peptide detachment (procedure 2) at room temperature or at 55 °C was more efficient (84% and 100% in 4h, respectively) than that promoted by the hydroxide ion (procedure 1; 60%) and that assisted by Ca^{+2} at room temperature or at 55 °C (yields lower than 20%).

Procedure 2 supplied a crude product whose major component was the desired protected peptide acid (Figure 3(A) and (B)). Although to a lesser extent, it also contained the analogue Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr-Cys[Bzl(Me)]-Arg(Tos)-Gly-OH, whose formation is consistent with previous observations that 2-bromobenzyloxycarbonyl is reasonably labile under base conditions. In this context, base-stable protecting groups such as 3-pentyl for tyrosine and cyclohexylocarbonyl for tryptophan should be used [35].

Interestingly, the crude product resulting from procedure 1 contains the desired protected peptide acid only as a minor component and the by-product cited above among the major ones (Figure 3(C)). In addition, we also noticed among the other minor components an epimer of the desired product, probably resulting from the peptide exposition to the inorganic alkali [33]. Collectively, these observations revealed that, in the case of Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly-KOR, procedure 1 was not suitable for preparing the corresponding protected peptide acid.

Preparation of Boc-Arg(Tos)-Cys(SBu^t)-Val-Thr(Bu^t)-Tyr(Bu^t)-Cys(SBu^t)-Arg(Pmc)-Gly-OH from the Corresponding Peptide-CLTR

All reaction conditions employed led to quantitative peptide detachment. The RP-HPLC and LC/ESI-MS analyses of the resulting crude peptides revealed that they were practically identical and of good quality: the major component was the desired protected peptide acid, as shown in Figure 3(D); the by-products Boc-Arg(Tos)-Cys(SBu^t)-Val-Thr(Bu^t)-Tyr(Bu^t)-Cys(SBu^t)-OH and Boc-Arg(Tos)-Cys(SBu^t)-Val-Thr(Bu^t)-Tyr(Bu^t)-OH, probably present owing to DKP formation during the peptide assembling process, were also detected as minor components.

Overall, these results indicated that procedures 4 and 5 were equally suitable for the preparation of Boc-Arg(Tos)-Cys(SBu^t)-Val-Thr(Bu^t)-Tyr(Bu^t)-Cys(SBu^t)-Arg(Pmc)-Gly-OH. Nevertheless, as the ester linkage hydrolysis catalysed by TFA occurs in 2 min and the same reaction catalysed by AcOH is carried out in 2 h, the productivity of the former is higher.

DOI: 10.1002/psc

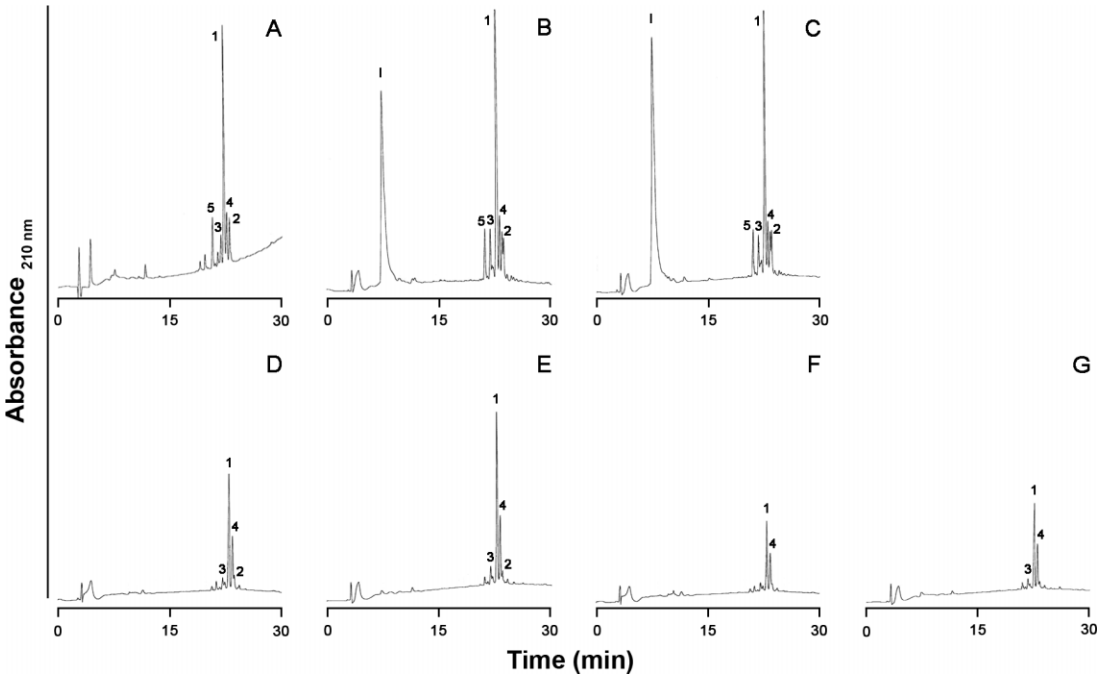


Figure 2 RP-HPLC monitoring of the oxime ester bond hydrolysis of Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly-KOR in 25% NaOH (1 M)/dioxane at 0–5 °C for 15 min (A), promoted by DBU in 15% H_2O/THF at room temperature (B) and at 55 °C (C) for 4 h, assisted by Ca^{+2} ion in 15% H_2O/THF at room temperature (D) and at 55 °C (E) for 50 h and with no additive in 15% H_2O/THF at room temperature (F) and at 55 °C (G) for 50 h. Peak I: DBU; peak 1: Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly-OH; peak 2: Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-OH; peak 3: Boc-Lys[Z(2Cl)]-Ala-Pro-Ser(Bzl)-Gly-Gly-OH; peaks 4 and 5: unidentified by-products.

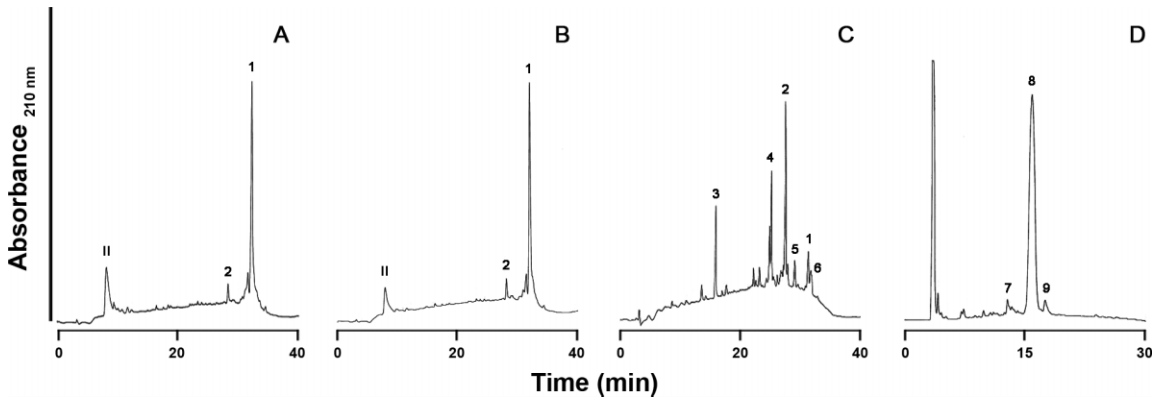


Figure 3 RP-HPLC monitoring of the oxime ester bond hydrolysis of Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly-KOR promoted by DBU in 15% H_2O/THF at room temperature (A) and at 55 °C (B) for 4h, in 25% NaOH (1 M)/dioxane at 0–5 °C for 15 min (C). RP-HPLC monitoring of peptide cleavage from Boc-Arg(Tos)-Cys(SBut)-Val-Thr(But)-Tyr(But)-Cys(SBut)-Arg(Pmc)-Gly-CLTR in 1% TFA/DCM at room temperature for 2 min (D). Peak I: DBU; peak 1: Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly-OH; peak 2: Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr-Cys[Bzl(Me)]-Arg(Tos)-Gly-OH; peaks 3–5: unidentified by-products. Peak 6: epimer of Boc-Arg(Tos)-Cys[Bzl(Me)]-Val-Thr(Bzl)-Tyr[Z(2Br)]-Cys[Bzl(Me)]-Arg(Tos)-Gly-OH; peak 7: Boc-Arg(Tos)-Cys(SBut)-Val-Thr(But)-Tyr(But)-Cys(SBut)-OH; peak 8: Boc-Arg(Tos)-Cys(SBut)-Val-Thr(But)-Tyr(But)-Cys(SBut)-Arg(Pmc)-Gly-OH; peak 9: Boc-Arg(Tos)-Cys(SBut)-Val-Thr(But)-Tyr(But)-OH.

CONCLUSIONS

Our results indicated that the procedure based on the use of DBU as promoter of peptide detachment from peptide-KOR through oxime ester hydrolysis (Boc strategy) was the most efficient since it furnished crude products with reasonable quality in a relatively short time. In fact, the quality of the crude protected octapeptide acid derived from Gm, the most complex model studied, provided by such a procedure is equivalent to that resulting from the peptide-CLTR ester linkage hydrolysis catalysed by TFA (Fmoc strategy).

DOI: 10.1002/psc

Acknowledgements

This work was supported by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (00/05410-2). P.B.P. was an undergraduate fellow of FAPESP (01/01368-4), C.R. was a graduate fellow of CNPq (133023/2001-0). The authors thank L.A.O. Vieira for preliminary experiments related to Ca^{+2}-assisted oxime ester bond hydrolysis, C.W. Liria for the amino acid analyses and F.M. Prado for the help with the mass spectrometry analyses

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