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Synthesis and Applications of a New Base-Labile Fluorene Derived Linker for Solid-Phase Peptide Synthesis[1,2]

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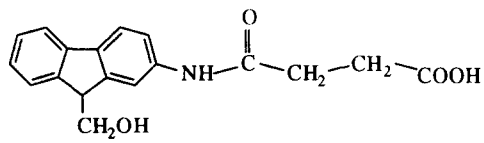
Abstract: *The handle N-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid (HMFS) is reported for the preparation of protected peptide segments in combination with a Boc/Bzl protection scheme. Treatment of peptide-resins with morpholine in DMF renders protected peptides in high yields and purities.*

Introduction

The chemical synthesis of peptides and proteins is devoted to a series of scientific advances that have made the preparation of small and intermediate sized (up to 30 residues) peptide molecules quite straightforward. Among these advances, the advent of Merrifield's solid phase peptide synthesis (SPPS) methodology[4] and the availability of reliable chromatographic techniques such as high performance liquid chromatography (HPLC)[5] is of outstanding importance. In addition, the design and development of a series of protecting groups and bifunctional linkers (*handles*)[6] have also facilitated and extended enormously the scope and application of the SPPS approach[7] for the preparation of large peptides *via* a convergent strategy[8] and of hybrids of peptides with other biomolecules containing labile structures.[9]

In the present article we report the design and development of a new handle, *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid (HMFS) (**1**). This compound belongs to the 9-hydroxymethylfluorene type of protecting groups that are designed to be cleaved by bases through a β-elimination reaction.[10,11] The HMFS handle can be compatibly applied with a *tert*-butyloxycarbonyl (Boc)/Benzyl (Bzl) scheme for the synthesis of protected peptides, and with a Boc/Fluorenyl (Fm) based side-chain protecting groups[12] scheme for the synthesis of unprotected peptides. Finally, appropriate derivatization of the HMFS handle can provide a convenient method for the solid-phase synthesis of other biopolymers such as oligonucleotides.[13]

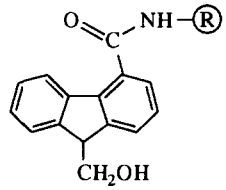


(1)

Results and Discussion

Previous resins containing handles based in the fluorene ring (Scheme 1) present some drawbacks. Thus, resin **2**[14] is also slightly labile to bases such as diisopropylethylamine (DIEA)–CH_2Cl_2 (1:9) (*ca* 10% cleavage after three hours of treatment), which is generally used for the neutralization of trifluoroacetate salt after Boc removal. Even if *N,N*-dimethylformamide (DMF) is not freshly destilled, a 4% cleavage was observed within 12 hours. Moreover and surprisingly, incomplete removal of protected peptides from the resin **2**[14b] has been described. On the other hand, resin **3**[15], which was designed to be more stable by including a

methylene group between the fluorene nucleus and the carboxamide function, showed similar problems. This resin was found to be deprotected by the basicity of the free amino group of the growing peptide chain mainly in DMF. The addition of an acidic compound such as 1-hydroxybenzotriazole (HOBt) solved partially the problem of peptide losses throughout the synthesis, because the free amino group of phenylalanine still promoted cleavage of the handle.[15a] A reasonable explanation for this phenomenon is that the electron-withdrawing effect provided by substituents such as -CONH (into **2**) or -CH_2-CONH (into **3**) increase the acidity of the hydrogen at position 9 of the fluorene ring. Therefore, the elimination process is facilitated even for bases such as DIEA, dimethylamine formed from decomposition of stored DMF, and amino groups present in growing peptide chains.[16]



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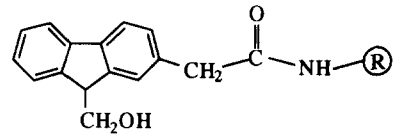
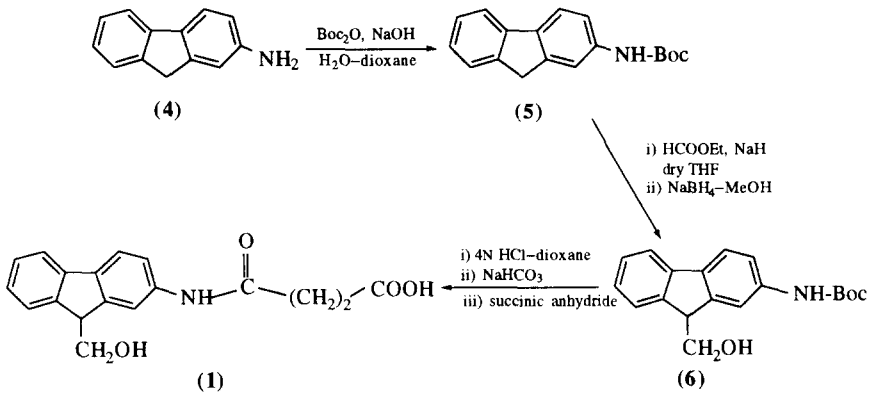


(3)

In contrast, the base lability of the resin derivatized with the HMFS handle (**1**) has been conveniently *fine tuned* by means of an electron-donating *N*-acylamido group. Thus, stability to those bases is achieved and consequently, the growing peptide chain is not prematurely lost. We have found that the HMFS handle is fully stable to DMF (24 hours at 25 °C). Furthermore, it is also stable to DIEA–CH_2Cl_2 (1:19) for 3 hours at 25 °C, and only less than 8% loss is detected after 24 hours at room temperature. However, lability towards secondary amines such as piperidine and morpholine is preserved, allowing the release with excellent yields of the target peptide from the resin. Finally, the HMFS handle is easily synthesized from the commercially available 2-aminofluorene.

Synthesis of HMFS handle. 2-Aminofluorene is a suitable compound since the amino function provides a starting point to incorporate easily a carboxylic acid group to be later attached to a varied set of commercial solid supports containing amino groups [*p*-methylbenzhydrylamino (MBHA)-, aminomethyl-polystyrene[17] and aminomethylpolyethyleneglycol-polystyrene[18]].

Hydroxymethylation at position 9 was essentially carried out according to the method described by Carpino[19] for the preparation of fluorenylmethanol. This procedure required the protection of the amino function with a Boc group. The introduction of the Boc was carried out with di-*tert*-butyldicarbonate (Boc_2O) following the method reported by Moroder *et al.*[20] The formylation reaction required a careful monitoring of conditions to avoid formation of a secondary byproduct of unknown structure upon long reaction times. The conditions that rendered the best yields involved the used of anhydrous conditions under argon atmosphere and a reaction time of 20 minutes. Once the aldehyde was formed and without isolation or characterization, the crude product was quickly reduced with sodium borohydride to yield the desired hydroxy derivative. The Boc group was removed and the resulting ammonium salt was neutralized to be acylated with a cyclic symmetrical anhydride (succinic anhydride) to furnish the title molecule. The overall yield of this scheme after crystallization was 45-55%. It is important to note that only one purification step is needed throughout to all synthetic process to obtain the HMFS handle in a homogeneous state as demonstrated by TLC, HPLC, ^{1}H-NMR, and FAB-MS. A single crystallization in tetrahydrofurane (THF):hexane after coupling with succinic anhydride is sufficient since intermediate products were pure enough for further use.[21]



Use of HMFS in SPPS. An "internal reference" amino acyl-MBHA resin was used to facilitate accurate monitoring of coupling and cleavage yields.[22] Handle **1** (1.5 equiv.) was smoothly introduced onto the amino resin with diisopropylcarbodiimide (DIPCDI)[23] and HOBt in DMF at 25 °C for at least 5 hours. A qualitative ninhydrin test was used to ascertain the completion of the coupling.[24]

The *C*-terminal Boc-amino acid was anchored to the hydroxymethyl group of the HMFS-resin using DIPCDI[21] in the presence of catalytic amount of *N,N*-dimethyl-4-aminopyridine (DMAP) in DMF for only one hour to reduce risk of racemization. Coupling was repeated once more to assure a quantitative yield. Peptides were then built up by a Boc/Bzl strategy following standard protocols used in our laboratory.[25]

A kinetic study of the cleavage of a rather hindered model amino acid [Boc-Tyr(BrZ)-OH] was performed in order to establish the lability of HMFS-resin. These experiments showed that a 30 min treatment with either piperidine–DMF (7:93) or morpholine–DMF (1:4) was sufficient to fully cleave the model amino acid from the resin (Figure 1). Protected peptides were cleaved with a little longer time or more concentrated piperidine solutions [1-2 hours, (1:9) to (1:4) mixtures of secondary amine and DMF] to reach quantitative cleavage yields.

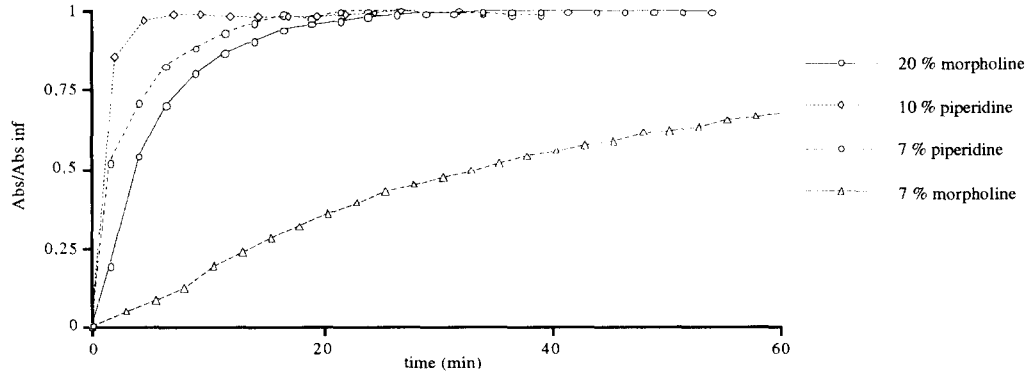


Figure 1. Kinetic study of the cleavage of Boc-Tyr(BrZ)-OH from the corresponding HMFS-Phe-MBHA-resin at different concentrations of piperidine and morpholine in DMF (see experimental for details).

The usefulness of the new handle was demonstrated by the synthesis of several protected peptides. Thus, the protected Merrifield peptide, Boc-Leu-Ala-Gly-Val-OH; the peptide corresponding to the sequence

31-38 of uteroglobin, Boc-Asp(OcHex)-Asp(OcHex)-Thr(Bzl)-Met-Lys(ClZ)-Asp(OcHex)-Ala-Gly-OH; and the peptides corresponding to the sequences 188-193 and 194-203 of maize γ-zein, Boc-Leu-Gln-Gln-Pro-Thr(Bzl)-Pro-OH and Boc-Cys(Acm)-Pro-Tyr(BrZ)-Ala-Ala-Ala-Gly-Gly-Val-Pro-OH, were manually synthesized. The first two peptides were cleaved with both piperidine– and morpholine–DMF (1:4) for 2 h, at 25 °C.[26] Although yields in both cases were superior to 98%, as indicated by amino acid analysis of the corresponding peptide-resins before and after the cleavage, the morpholine-based cleavages rendered cleaner HPLC profiles than those afforded by piperidine treatments. Thus, purities of > 95% and 93% were achieved with morpholine for Merrifield tetrapeptide and Uteroglobin (31-38), respectively, whereas 92% and 85% purity were obtained with piperidine. The two peptides corresponding to γ-zein protein were cleaved only with morpholine–DMF. HPLC chromatograms corresponding to the four peptides (morpholine cleavages) (Figure 2) shown that both processes, the bulding up of the peptidic chain and the cleavage of the protected peptides from the resins, were carried out in optimal conditions, allowing the preparation of protected peptides with a high level of purity.[27]

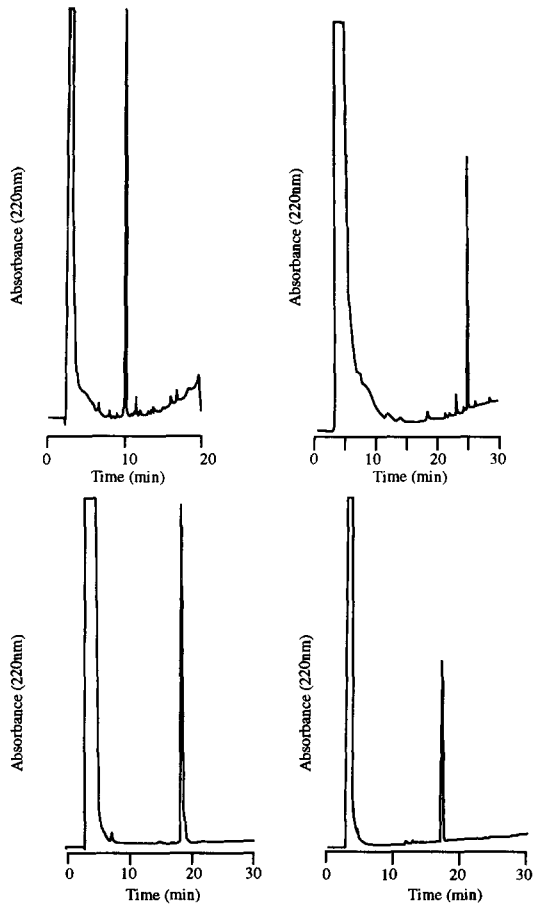


Figure 2. Analytical HPLC of crude protected peptides synthesized with HFMS-Phe-MBHA-resins. HPLC was performed on a Vydac C-18 reversed-phase column (4.6 x 250 mm) with a linear gradient using 0.045% aqueous TFA and 0.036% TFA in CH_3CN, flow rate 1.0 mL/min. a) Boc-Leu-Ala-Gly-Val-OH, gradient over 25 min from (9:1) to (0:1). b) Boc-Asp(OcHex)-Asp(OcHex)-Thr(Bzl)-Met-Lys(ClZ)-Asp(OcHex)-Ala-Gly-OH, gradient over 25 min from (9:1) to (0:1) and then 5 min isocratic (0:1). (c) Boc-Leu-Gln-Gln-Pro-Thr(Bzl)-Pro-OH, gradient over 30 min from (9:1) to (3:7). (d) Boc-Cys(Acm)-Pro-Tyr(BrZ)-Ala-Ala-Ala-Gly-Gly-Val-Pro-OH, gradient over 30 min from (9:1) to (3:7).

Conclusions

The synthesis and applications of the new HMFS handle, *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid, is reported. This handle has been synthesized in multigram scale with only one purification step at the end of the process and with an overall yield of 45-55%. HMFS linker has proved to be stable throughout SPPS approach following a standard Boc/Bzl strategy, even to the neutralization step with tertiary amines such as DIEA. Moreover, kinetic experiments have shown that a treatment with morpholine in DMF renders protected peptides in high yields and purities. Work is also underway to extent the use of this handle to other SPPS protecting strategies and oligonucleotide synthesis. as well.

Experimental Section

Some of the materials and general synthetic and analytical procedures have been described in earlier publications from this laboratory.[2,12,25]

Protected Boc-amino acid derivatives were from Propeptide (Vert-le Petit, France), Novabiochem (Läufelfingen, Switzerland), Bachem Feinchemikalien (Bubendorf, Switzerland), and Advanced ChemTech (Louisville, KY). 4-Methylbenzhidrylamine resin (0.81 meq/g) was also from Bachem. DMF was peptide synthesis grade from Scharlau (Barcelona, Spain) and was flushed with nitrogen for at least two hours, and maintained over molecular sieves (3 Å). Piperidine was purchased from Aldrich, TFA from Kali-Chem, CH_2Cl_2 from Scharlau, and HOBt and DIPCDI from Fluka. Peptide resins were hydrolyzed in 12 N HCl–propionic acid (1:1, v/v) at 115 °C for 48 hours or at 155°C for 2 hours. Amino acid analyses were carried out in a Biotronik LC-6001 analyzer.

Analytical HPLC was performed using Vydac C-18 reverse-phase columns (0.46 x 25 cm, 10 μm particle size) on a Shimadzu system configured with two LC-6A pumps, a SPD-A UV-spectrophotometric detector, a SIL-6B autoinjector, a SCL-6B controller and C-R6A chromatopac integrator. Peptide samples were chromatographied at 1mL/min using gradients of H_2O and CH_3CN containing 0.045% and 0.036% of TFA, respectively.

Positive-ion fast atom bombardment mass spectrometry (FAB-MS) was carried out on a Fisons VG-Quattro instrument with matrices of glycerol, thioglycerol or *m*-nitrobenzyl alcohol. H^1-NMR spectra were recorded in a 200-MHz Varian XL-200 instrument. Ultraviolet spectra were performed in a Perkin-Elmer Lambda 5 spectrophotometer.

2-(Boc-amino)fluorene: 2-aminofluorene (20 g, 110 mmol) was suspended in a mixture of dioxane–water (2:1) (200 mL) and 2 N NaOH (60.5 ml) in an ice bath with magnetic stirring. Boc_2O (26.5 g, 121 mmol) was then added and the stirring was continued at 25 °C for 24 hours. The suspension was acidified with 1M $KHSO_4$ to pH 3, filtered and the solid washed with water, dioxane–water (1:1), hexane and dried in vacuo over P_2O_5. The product, a pale yellow powder (28.3 g, 91% yield), was pure by TLC. Rf (CMA) 0.77; ^{1}H-NMR ($CDCl_3$) δ 7.2-7.8 (m, 7 H, Ar), 6.6 (broad s, 1H, NH urethane), 3,9 (s, 2H, CH_2), 1.6 (s, 9H, Boc); FAB-MS: 282.1 [$(M+H)^+$], 281,0 [M^+].

9-Hydroxymethyl-2-aminofluorene: To a suspension of sodium hydride (80% in paraffin oil, 6.15 g) in dry THF (recently destilled from sodium in paraffin) under argon atmosphere, a solution of 2-(Boc-amino)fluorene (18 g, 64 mmol) in dry THF was carefully added. Gas evolution and spontaneous warming occurred. Ethyl formate (12.12 ml, kept over anhydrous K_2CO_3) was then also carefully added in order to avoid vigorous bubbling of hydrogen. After 20 minutes, the reaction was quenched with ice chips, diluted with

water (100 mL) and the THF was rotatory evaporated. The aqueous layer was washed with diethyl ether (3 x 100 mL), cooled in an ice bath and acidified with glacial acetic acid to pH=5. A yellow solid precipitates, which was extracted with ethyl acetate (3 x 100 mL) and the organic layer was washed with saturated aqueous sodium bicarbonate, brine, dried over Na_2SO_4 a the solvent rotatory evaporated. The product was finally dried in vacuo over P_2O_5. The resulting product (19 g) was suspended in dry methanol (300 ml) and $NaBH_4$ (2.5 g, 66 mmol) was added portionwise. The solution, that rapidly clarifies, was magnetically stirred for 3-4 hours. Then, it was diluted with water (800 mL) and brought to pH=5 by addition of glacial HOAc. The solid precipitated was extracted with EtOAc, dried over Na_2SO_4 , rotatory evaporated, and dried in vacuo to yield a pale yellow product (18.9 g) which was pure enough for further use. Part of this solid (9 g) was suspended in 4.5 N HCl–dioxane (50 mL) in an ice bath for two hours and an additional hour at 25 °C. The solution was rotatory evaporated to partially eliminate the HCl. Then, a large excess of CH_2Cl_2 (*ca* 1 L) was added onto the solution at 0°C. The precipitated was filtered, washed thoroughly with CH_2Cl_2 and dried. This product was dissolved in water, and the pH was brought to 8 by addition of $NaHCO_3$. The oil separated was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated, and dried in vacuo over P_2O_5. A light brown solid was obtained (4.0 g, 63 % yield) which was homogeneous by HPLC and TLC: Rf (CMA) 0.42; ^{1}H-NMR ($CDCl_3$) δ 6.7-7.7 (m, 7 H, Ar), 3.9-4.1 (m, 3H, CH-CH_2), 2.5-3.4 (broad s, 2H, NH_2); FAB-MS: 212.7 [$(M+H)^+$], 211.7 [M^+]

***N*-[(9-Hydroxymethyl)-2-fluorenyl]succinamic acid (HMFS handle)** : 9-Hydroxymethyl-2-aminofluorene (4 g, 19 mmol) was dissolved in a minimum amount of recently destilled THF and succinic anhydride (1.9 g, 19 mmol) was added portionwise over a period of 20 minutes. The reaction was allowed to proceed for two hours. A light yellow solid precipitated spontaneously, which was filtered and washed with THF–hexane (1:1), hexane, and crystallized in THF hexane with some drops of methanol. Up to three crops may be obtained rendering finally 4.6 grams (78 %) of a TLC and HPLC homogeneous product. Rf(CMA) 0.21; ^{1}H-NMR (CD_3OD) δ 7.2-8.0 (m, 7 H, Ar), 3.8-4.1 (m, 3H, CH-CH_2), 2.7 (broad s, 4H, CH2-CH2) ; FAB-MS: 312.0 [$(M+H)^+$], 311.0 [M^+], 294.0 [$(M-OH)^+$], 276 [$(M-OH-H_2O)^+$], 210.9 [$(M-CO(CH_2)_2COOH)^+$].

General procedures for solid-phase peptide synthesis with HMFS handle. Syntheses were carried out manually in polypropylene syringes fitted with a polyethylene disc. HMFS handle (380 mg, 1.22 mmol) and HOBt (188 mg, 1.22 mmol) were dissolved in DMF (10 mL) and added to a aminoacyl ("internal reference") MBHA-resin (1 g, 0.81 mmol NH_2/g). Then, DIPCDI (0.2 ml, 1.22 mmol) was added and the mixture was shaken at 25 °C for a minimum of 5 hours. The reaction proceeds with quantitative yield as shown by the ninhydrin test. The resin was then filtered and washed with DMF (5 x 5 mL). Next, the *C*-terminal Boc-amino acid (5 equiv.) was anchored to the hydroxymethyl resin using DIPCDI (5 equiv.) in the presence of DMAP (0.5 equiv.) in DMF for one hour at 25 °C. A double coupling was carried out in the same conditions to ensure high yields of attachment of the first amino acid. Usually, this protocol renders quantitative incorporation of the first amino acid. The following amino acid residues of the sequence were incorporated manually in a stepwise manner according to standard Boc/Bzl protocols used in our laboratory and described elsewhere.[25] Boc removal was accomplished with TFA–CH_2Cl_2 (4:6), 1 min prewash + 20 min treatment, after CH_2Cl_2 washings, neutralization was carried out with DIEA–CH_2Cl_2 (1:19), 3 times for 2 min, followed by CH_2Cl_2 washings, and finally the coupling was carried out adding Boc-amino acids (4 equiv.) dissolved in DMF to the resin and then the DIPCDI (4 equiv.). The mixture was shaken for 1 h and the extend

of the coupling was checked by the ninhydrin test. Cleavages of the completed peptide-resins were performed by soaking and washing the peptide-resins previously in DMF and then by treatment with freshly solutions of piperidine or morpholine in DMF at 25 °C. Resins were removed by filtration through a disposable pipette with a glass wool plug, and the cleaved resins were washed with DMF. The combined filtrates were rotary evaporated to dryness under high vacuum and analyzed by HPLC without further treatment.

Kinetic studies on the stability and lability of HMFS-resin. Boc-Val-HMFS-Phe-MBHA-resin (50 mg, 30 μmol) were separately treated with neat DMF and DIEA–CH_2Cl_2 (1:19) (1 mL) at 25°C for 3 and 24 h. After the corresponding time, resins were removed by filtration as described above and washed with additional solvents (3 x 1 mL). Aliquots of the combined filtrates were rotary evaporated and subjected to acid hydrolysis as well as samples of resins before and after the treatment. The loss of Val and, therefore, the stability of aminoacyl-HMFS bond to these reagents was assessed by comparison of amino acid analysis of both resins and confirmed by the amino acid analysis of the filtrate.

The lability of HMFS handle to secondary bases (piperidine and morpholine) at different concentrations was established by UV spectroscopy. Boc-Tyr(BrZ)-OH was attached to HMFS-Phe-MBHA-resin and the corresponding Boc-amino acyl resin (5-6 mg, 0.56 meq/g) was soaked in the corresponding amount of DMF for 10 minutes in a quartz cuvette (3 mL) and finally treated with the amount required of either piperidine or morpholine to reach the desired concentration of base in each kinetic experiment [(7:93) and (1:9) for piperidine and for (7:93) and (1:4) for morpholine]. UV absorption of the solution at 280 nm was then measured every 2.5 minute following manual agitation. This time was found enough to allow the resin to settle down. The kinetic experiment was considered to be finished when at least three measures were coincident. The final measure was taken as the maximum absorbance (A_∞) and the intermediate measures were normalized to this value for comparison between the experiments (Figure 1). Amino acid analysis of the acid hydrolizates of the resin after the experiment confirmed that the cleavage of the Boc-Tyr(BrZ)-OH took place in all case quantitatively.

Boc-Leu-Ala-Gly-Val-OH (Merrifield peptide). Synthetic details were essentially as already given in the General Procedures. The cleavage was performed by both piperidine– and morpholine–DMF (1:4) treatments. The yield of cleavage was superior to 98 % in both cases according to the amino acid analyses of the resin before and after the cleavage step. The amino acid composition of the crude cleaved peptide was Gly, 1.10; Ala, 0.88; Val, 1.01; Leu, 1.00. Analytical HPLC revealed a purity of >95% for the morpholine (Figure 2a) treatment and 92% for the piperidine one. The peptide coeluted into HPLC with another sample of the same peptide independently prepared.

Uteroglobin (31-38) [Boc-Asp(OcHex)-Asp(OcHex)-Thr(Bzl)-Met-Lys(ClZ)-Asp (OcHex)-Ala-Gly-OH]. Chain assembly and cleavage were carried as outlined above. Met was incorporated as its free form, and no oxidation was detected during the synthetic protocol. Although cleavage yields were in both cases [base–DMF (1:4)] superior to 95%, treatment with morpholine (Figure 2b) gave a purer product than that obtained with piperidine (92% *versus* 85%). The morpholine crude cleaved peptide showed an amino acid composition as follows: Asp, 2.70; Thr, 0.74; Gly, 1.21; Ala, 0.85; Met, 0.96; Lys, 0.96. FAB-MS: 1478.7 [$(M+Na)^+$], 1494.9 [$(M+K)^+$].

γ-Zein (188-193) [Boc-Leu-Gln-Gln-Pro-Thr(Bzl)-Pro-OH]. The two residues of Gln were incorporated with the side-chain unprotected in the presence of equimolar amount of HOBt to prevent the formation of the corresponding nitrile. Cleavage with piperidine–DMF (1:9) (> 95% yield based on amino acid

analyses both on the isolated product and on the cleaved resin) for 1 h yielded a homogeneous product as shown by HPLC (Figure 2c). Amino acid analysis of the peptide-resin: Thr, 0.74; Glx, 1.95; Leu, 1.05; Pro, 2.26. FAB-MS: 873.4 [(M+H)$^+$], 895.6 [(M+Na)$^+$], 911.2 [(M+K)$^+$], 773.4 [(M-Boc+H)$^+$].

γ-Zein (194-203) [Boc-Cys(Acm)-Pro-Tyr(BrZ)-Ala-Ala-Ala-Gly-Gly-Val-Pro-OH]. The peptide assembly and the cleavage were performed similarly as described in General Procedures. Cleavage with morpholine–DMF (1:4) (> 95% yield) for 1 h gave a homogeneous product as shown by HPLC (Figure 2d). Amino acid analysis corresponding to the acid hydrolysis of the peptide-resin gave: Gly, 2.08; Ala, 3.13; Val, 1.09; Tyr, 0.36 (no phenol was added to prevent the destruction of Tyr); Pro, 2.33. FAB-MS: 1290.2 [(M+H)$^+$], 1312.1 [(M+Na)$^+$], 1190.1 [(M-Boc+H)$^+$], 1076.1 [(M-BrZ+H)$^+$], 1098.2 [(M-BrZ+Na)$^+$].

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References and Notes

1. Abbreviations used are: Acm, acetamidomethyl; Boc, *tert*-butyloxycarbonyl; $(Boc)_2O$, di-*tert*-butyldicarbonate; BzZ, 2-bromobenzyloxycarbonyl; Bzl, benzyl; ClZ, 2-chlorobenzyloxycarbonyl; CMA, chloroform–methanol–acetic acid (95:5:3); DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; EtOAc, ethyl acetate; FAB-MS, Fast atom bombardment mass spectrometry; Fm, fluorenylmethyl; Fmoc, 9-fluorenylmethyloxycarbonyl; HMFS, *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MBHA, 4-methylbenzhydrylamine (resin); MeOH, methanol; OcHex, *O*-cyclohexyl; SPPS, solid-phase peptide synthesis; THF, tetrahydrofuran. Amino acid symbols denote L-configuration unless indicate otherwise.
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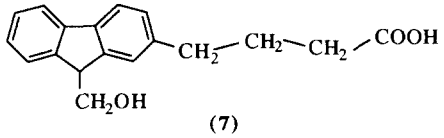
16. During the preparation of this manuscript a communication has appeared [W. Lin, L. Chen, Y-Z. Liu, and C-I. Niu, in *Peptides , Biology and Chemistry. Proceedings of the 1992 Chinese Peptide Symposium* (Y-C. Du, J.P. Tam, Y-S. Zhang eds.) ESCOM Sci. Publ., Leiden, The Netherlands, pp. 299-300 (1993)] where handle (**7**) is proposed for overcoming some of the problems of resin (**3**). We still think that our approach is better in terms of both stability (-NH-CO- is better electron-withdrawing than $-CH_2-CH_2-$) and synthetic scheme [besides the common hydroxymethylation reaction, the preparation of handle (**7**) requires both a Friedel-Crafts and a Clemmensen reaction].



(**7**)

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