A Novel and Versatile Silicon-Derived Linkage Agent, 4-[1-Hydroxy-2-(trimethylsilyl)ethyl]benzoic Acid, Compatible with the Fmoc/*t*-Bu Strategy for Solid-Phase Synthesis of C-Terminal Peptide Acids

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Abstract: Tryptophan alkylation by resin-derived carbocations and formation of diketopiperazine are two major yield reducing side reactions observed in Fmoc/*t*-Bu based solid-phase peptide synthesis. With the development of a versatile silicon-based linkage agent, "SAC", 4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoic acid (**2**), for the production of peptide C-terminal acids, these problems have been successfully solved. Demonstration of the advantages of using SAC linker was provided by synthesizing a C-terminal tryptophan-containing dodecapeptide and a C-terminal proline-containing undecapeptide. The pure peptides were isolated in 30–40% yields, a dramatic improvement compared to the syntheses using the conventional HMPA linker. SAC linker was prepared in two steps in 70% overall yield. Attachment of the Fmoc–amino acid–SAC linker derivatives to amino functionalized solid supports was best carried out using the preformed 2,4-dichlorophenyl ester derivatives. The amino acid–SAC linkage was tested and found to be stable under typical peptide synthesis conditions. An additional feature of SAC linker is the ability to generate protected peptide fragments using either fluoride ions or 1% TFA/CH_2Cl_2 solution. The versatility of using SAC linker was demonstrated by preparation of several Boc- or Fmoc-protected tetrapeptides and a cyclic heptapeptide by either fluoridolysis methodology or treatment with dilute acid. Furthermore, formation of succinimide, a side reaction resulting from cyclization of *β-tert*-butyl ester protected aspartyl residues, under fluoridolysis conditions, was also investigated.

Introduction

Solid-phase synthesis is a rapid and powerful technique for the preparation of peptides. An important aspect of the process is the use of a linkage agent. The structure and chemical properties of the linkage agent, which serves as a C-terminal protecting group that covalently links the peptide undergoing synthesis to an insoluble solid support, ultimately contribute to the yield and purity of the final product and also dictate the product's structure (e.g., C-terminal carboxylic acid or amide). Conventional linkage agents commonly used when the Fmoc/*t*-Bu protection strategy is employed can suffer from serious sequence dependent, yield reducing side reactions such as alkylation of tryptophan residues[1] and diketopiperazine (DKP) formation.[2,3] The development of novel linkage agents may therefore be a valuable approach to solving such problems.

Recently this laboratory reported the novel SAL linker,[4] designed to take advantage of the chemistry of silicon, for the production of peptide C-terminal amides by the Fmoc/*t*-Bu strategy. Improved yields of tryptophan containing peptides were clearly demonstrated when SAL linker was employed. These results led to the design, synthesis and application of a similar silicon-based linkage agent "SAC" (acronym for *S*ilyl *AC*id linker), or 4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoic acid for the synthesis of C-terminal peptide acids by the Fmoc/*t*-Bu strategy. The use of the novel SAC linker reported here was shown to have several attractive features such as (1) lack of tryptophan alkylation by the linker hence improved yields of tryptophan-containing peptides, (2) cleavability with dilute acid or fluoride ion under mild conditions to generate protected peptide fragments, and (3) suppression of diketopiperazine formation.

Results and Discussion

Preparation of SAC Linker and Attachment to the Solid Support. The SAC linker was prepared in two steps in 70% overall yield (Scheme 1). Treatment of *p*-bromobenzaldehyde with ((trimethylsilyl)methyl)magnesium chloride gave crude 1-bromo-4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzene (**1**) in quantitative yield. The crude product was greater than 95% pure as determined by ^{1}H NMR and used for the following reaction without further purification. The subsequent carboxylation reaction of the lithium anion of **1**, generated by lithium–halogen exchange with solid

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(3) Abbreviations for amino acids and nomenclature of peptide structures follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follow: AAA = amino acid analysis; Boc = *tert*-butyloxycarbonyl; BOP = benzotriazolyl *N*-oxytris(dimethylamino)phosphonium hexafluorophosphate; BTAF = benzyltrimethylammonium fluoride; BTAHF = benzyltrimethylammonium hydrogen difluoride; *n*-BuLi = *n*-butyllithium; DCC = *N,N'*-dicyclohexylcarbodiimide; DCU = dicyclohexyl urea; DIEA = diisopropylethyl amine; DKP = diketopiperazine; DMAP = 4-(*N,N*-dimethylaminopyridine; DMF = *N,N*-dimethylformamide; DPPA = diphenylphosphoryl azide; DTT = dithiothreitol; EDT = 1,2-ethanedithiol; MS/FAB = mass spectrometry/fast atom bombardment; Fmoc = 9-fluorenylmethyloxycarbonyl; GITC = 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate; HMPA = 4-(hydroxymethyl)phenoxyacetic acid; HOAc = acetic acid; HOBt = 1-hydroxybenzotriazole; HPLC = high-performance liquid chromatography; Pfp = pentafluorophenyl; SAC = 4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoic acid; SAL = 4-[(1-amino)-2-(trimethylsilyl)ethyl]phenoxyacetic acid; TBAF = tetrabutylammonium fluoride; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin-layer chromatography.

(4) Chao, H.-G.; Bernatowicz, M. S.; Matsueda, G. R. *J. Org. Chem.* **1993**, *58*, 2640.

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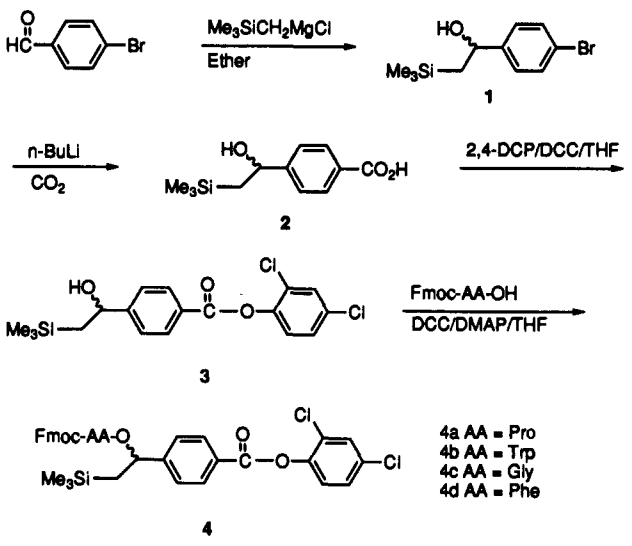
Scheme 1



carbon dioxide, proceeded smoothly to give 4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoic acid (**2**) as a crystalline solid in 70% yield.

Although attachment of linker derivative **2** directly to the solid support followed by coupling of the Fmoc-protected C-terminal amino acid can be carried out sequentially by the conventional methods, it was found that a more efficient and reliable way of attachment was through the coupling of the preformed Fmoc-amino acid–SAC linker derivatives **4**. These were successfully prepared by coupling of Fmoc–amino acids with the SAC linker derivative **3** using DCC/DMAP (catalytic amount). The products were isolated in yields of 75–80% after silica gel chromatography. Alternatively formation of **4** can be carried out using an Fmoc–amino acid chloride[5] and SAC linker derivative **3** under the conditions described by Akaji et al.[6] The Fmoc–amino acid fluorides,[7] however, were found to be less satisfactory. It is noteworthy that the 2,4-dichlorophenyl ester of compound **3** serves dual roles: protection of the linker carboxyl function while protected amino acids are coupled to **3** and, in the next step, activation of the linker derivatives **4** for attachment to the resin support.[8] In addition, the 2,4-dichlorophenyl ester was found to be stable to silica gel chromatography conditions.

Attachment of the preformed Fmoc–amino acid–SAC linker derivatives **4** to amino-functionalized supports was carried out using 1.2–1.5 equiv of the linker ester in DMF in the presence of equimolar HOBt/DIEA[9] for 5 h. After acetylation to cover the unreacted amino groups, the resulting Fmoc–amino acid–SAC resin was subjected to spectrophotometric analysis for quantitation of coupling[10] (Table 1). Because use of DMAP as catalyst for esterification is known to cause racemization of α-amino acids,[8] resins prepared via **4** were subjected to analysis of optical purity by the method of Kinoshita.[11] The analysis of Fmoc–amino acid–resin derivatives showed less than 0.1% D-isomer content consistent with little or no racemization occurring under conditions used to prepare the reported ester derivatives **4**.

Table 1. Preparation of Fmoc-Amino Acid SAC Derivatives **4** and Attachment to Aminomethylpolystyrene

Fmoc-AA-SAC derivatives		yield[a] (%)	substitution (mmol/g)
4a	Pro	75.0	0.30
4b	Trp	81.0	0.29
4c	Gly	79.0	0.29
4d	Phe	79.0	0.28

[a] Isolated yield of derivatives **4**.

The stability of the SAC-anchored amino acid residues was tested under the following conditions: 0.3 M HOBt/DIEA in DMF solution for 12 h, 0.3 M HOBt in DMF solution for 12 h, and 0.3 M Fmoc-Gly-OH in DMF solution for 12 h. In no case was loss of the amino acid from the resin detected as determined by subsequent spectrophotometric quantitation of the Fmoc group. These results clearly indicated that the Fmoc–amino acid–SAC linkage was stable under typical peptide synthesis conditions.

Prevention of Tryptophan Alkylation. Previously high yield syntheses of tryptophan-containing peptide C-terminal amides were demonstrated using SAL linker.[4] Conceptually SAC linker was designed similarly to SAL linker to take advantage of the β-elimination propensity of organosilicon compounds[12,13] which serves to quench resin-bound linker derived carbocations generated under cleavage conditions. For this reason it was expected that use of SAC linker would prevent tryptophan alkylation by resin derived carbocations and thereby improve yields. To verify this hypothesis, a model study using Fmoc–Trp–linker resin derivatives was performed by treating Fmoc-Trp-linker-resin derivatives with TFA/phenol/thioanisole (95:2.5:2.5). As shown in Figure 1, SAC linker gave quantitative cleavage at 15 min. On the other hand, even after 2 h Fmoc-Trp-HMPA-resin and Fmoc-Trp-(Boc)-HMPA-resin derivatives[14,15] only gave 55% and 65% cleavage yields, respectively. Importantly, the cleavage yield for SAC linker stayed constant over time which suggested that the styrene derivative formed under cleavage conditions from the linker was stable to protonation, presumably, due to the electron withdrawing *p*-carboxyl group. Apparently no protonation of the linker derived styrene derivative had occurred during cleavage with TFA, otherwise the yield would decrease with time because of tryptophan alkylation by the resulting carbocations. Demonstration of this advantage (lack of tryptophan alkylation) of using SAC linker was provided by the synthesis of a C-terminal tryptophan dodecapeptide derived from the carboxyl terminus of penicillinase, H-Leu-Ala-Glu-Leu-Gly-Ala-Ser-Leu-Leu-Lys-His-Trp-OH (**5**). A low yield synthesis (37% of the crude product) of this peptide was previously reported by Sheppard et al.[2b] using the Fmoc/*t*-Bu strategy and the conventional HMPA[16] linker and was later verified in this laboratory. These results are

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(15) The low cleavage yield suggested that alkylation of a tryptophan residue by resin-bound carbocations had occurred, presumably because of partial loss of Boc protection of the indole under our cleavage conditions. In a separate experiment, 10 mg of Fmoc-Trp(Boc)-OH was treated with 200 μL of TFA/phenol/thioanisole (95:2.5:2.5) solution for 30 min, the same mixture used for the resin cleavage study and in addition to Fmoc-Trp(CO_2H)-OH, Fmoc-Trp-OH was also detected (TLC). Moreover, when Fmoc-Trp(Boc)-OH was used for preparation of peptide H-Cys-Lys-Gly-Pro-Phe-Asn-Val-Ser-Trp-Gln-Gln-Gln-Arg-OH, the crude peptide obtained after cleavage with TFA/phenol/DTT (90:5:5) solution for 90 min, showed an identical HPLC profile and MS/FAB (1577, calcd based on the free peptide, found M + H$^+$ = 1578.2) before and after lyophilization which indicated complete removal of the Boc group under cleavage conditions.
(16) Sheppard, R. C.; Williams, B. J. *Int. J. Pept. Protein Res.* **1982**, *20*, 451.

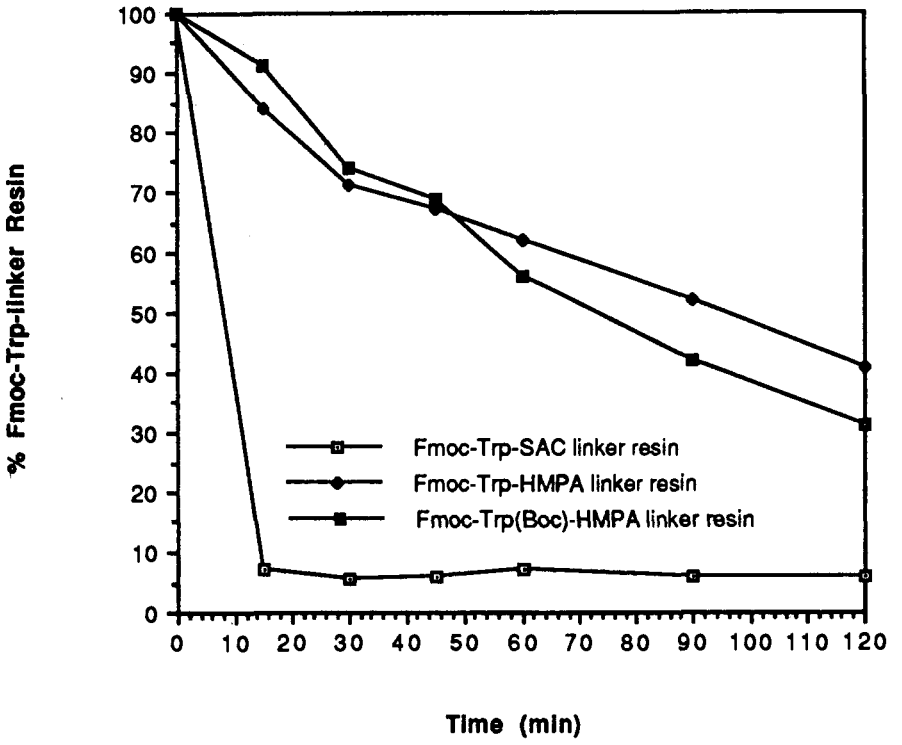
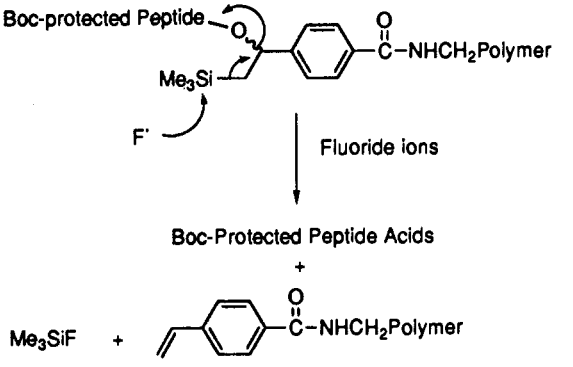


Figure 1. Time course for the cleavage of Fmoc-Trp-linker-resin derivatives. Peptidyl resin samples (200 mg) were treated with 2 mL of TFA/phenol/thioanisole (90:2.5:2.5) solution. An aliquot of the resin (10–15 mg) was removed at various times, washed, dried, and subjected to UV analysis.

attributable to C-terminal tryptophan alkylation by linker derived carbocations. When the dodecapeptide **5** was synthesized using Fmoc-Trp-SAC-polystyrene, the crude peptide, after ether precipitation and drying in vacuo, was obtained as a tri-TFA salt in a dramatically improved 92% yield and was determined to be 70% pure by HPLC. After preparative HPLC purification the pure peptide was isolated in 31% yield, and its identity was established by MS/FAB and amino acid analysis.

Generation of Protected Peptide Fragments Using Either Fluoridolysis or Dilute Acid. Earlier Sieber[17] and Carpino et al.[18] reported use of the trimethylsilylethyl group for carboxyl and nitrogen protection with the deprotection of the trimethylsilylethyl moiety being accomplished by treatment with fluoride ions. Later this strategy was developed into fluoridolyzable linkage agents by Barany[19] and Ramage[20] for the preparation of protected peptide fragments. Since SAC linker has a similar structural arrangement, specifically an α-substituted trimethylsilylethyl alcohol, it was not unreasonable to assume that protected peptide fragments could be obtained using SAC linker with cleavage by fluoride ions (Scheme 2). In order to support this notion, Boc-Trp-Val-SAC-amide was prepared for further study. As expected, upon treatment of the dipeptide–SAC–amide with 2 equiv of TBAF in solvents such as DMF, THF, and CH_2Cl_2 the desired Boc-protected dipeptide acid was obtained in less than 5 min by TLC. However, as noted by Sieber[17] and Ueki et al.[21] that owing to its strongly basic character, TBAF causes side reactions such as formation of succinimide from sequences containing aspartyl residues with β-ester protection. Base-catalyzed succinimide formation followed by α-β rearrangement

Scheme 2



is a serious side observed in Boc/Bzyl chemistry.[22] This is less of a problem when Fmoc/*t*-Bu chemistry is employed presumably due to the better stability of side chain *tert*-butyl protection as opposed to benzyl protection. In some cases, however, loss of *tert*-butyl alcohol from aspartic acid β-*tert*-butyl ester has been observed.[17,23] This side reaction not only reduces yield but also causes purification difficulties. To minimize the side reactions associated with the strongly basic character of TBAF, selectively reducing the basicity but not the nucleophilicity toward the silicon atom might be beneficial. In order to reduce the basicity, TBAF was buffered with weak acids such as HOAc, R_3N·HCl, or alcohol (MeOH, TFE). In these cases, however, both the basicity and the nucleophilicity were reduced and consequently yielded unsatisfactory results (slow and incomplete cleavage). In addition to TBAF, other fluoride ion sources were also explored such as $LiBF_4$, KBF_4, KF/HMPA, $PhCH_2NMe_3F$, and $PhCH_2NMe_3HF_2$. Among the fluoride sources tested only benzyltrimethyl ammonium fluoride ($PhCH_2NMe_3F$, BTAF) and benzyltrimethylammonium hydrogen difluoride ($PhCH_2NMe_3HF_2$, BTAHF) gave satisfactory results. Owing to the limited solubility of BTAF

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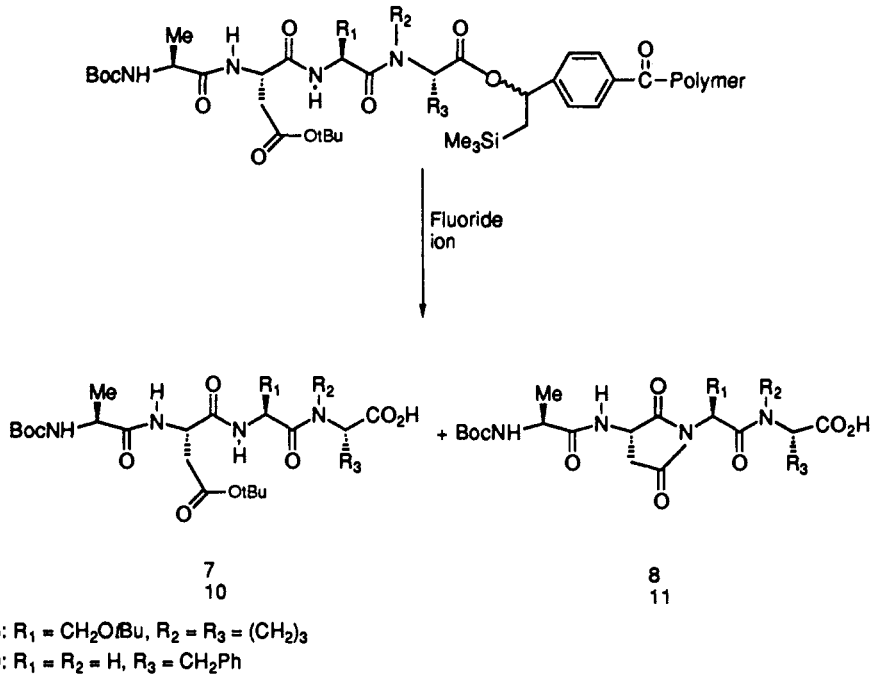
Scheme 3



7
10

8
11

6: R_1 = CH_2OtBu, R_2 = R_3 = $(CH_2)_3$
9: R_1 = R_2 = H, R_3 = CH_2Ph

Table 2. Cleavage Studies of Tetrapeptide **6** and **9** by Either Fluoridolysis or 1% TFA in CH_2Cl_2

cleavage reagents	7[a] (%)	8 (%)	10 (%)	11 (%)
TBAF	72.1	27.9	14.1	85.9
BTMDF	100		91.2	8.8
1% TFA	100			

[a] This percentage is based on HPLC integration and normalized using **7** + **8** or **10** + **11**.

in DMF, we focused our attention on BTAHF and TBAF. Even though BTAHF itself is buffered with HF, it still cleaved the SAC linker efficiently in a dipeptide based solution study. In order to evaluate the usefulness of these quaternary ammonium fluorides, two model tetrapeptides, Boc-Ala-Asp(OtBu)-Ser(tBu)-Pro-SAC-resin (**6**) and Boc-Ala-Asp(OtBu)-Gly-Phe-SAC-resin (**9**) derivatives were prepared. These model peptides allowed us to study not only the deblocking conditions but also the side reactions associated with cyclization of the aspartyl residue to form succinimide under alkaline conditions (Scheme 3). Results are summarized in Table 2. In the case of TBAF, a significant amount of succinimide **8** was formed which indicated the susceptibility of aspartyl residue to form succinimide under the fluoridolytic conditions even with *β-tert*-butyl ester, whereas with BTAHF only the desired peptide **7** was obtained (Figure 2). In a more stringent test using Boc-Ala-Asp(OtBu)-Gly-Phe-SAC-resin derivative **9**, succinimide formation was as high as 80% for TBAF and 7% for BTAHF, respectively.[24] These results are in agreement with literature in that the Asp-Gly sequence is more prone to succinimide formation under alkaline conditions.[25] Attempting to optimize the TBAF cleavage conditions by shortening cleavage time and/or using fewer equivalents of TBAF failed to suppress succinimide formation, and only resulted in low cleavage yield. Although use of BTAHF has been shown to minimize the formation of succinimide, the low cleavage yield

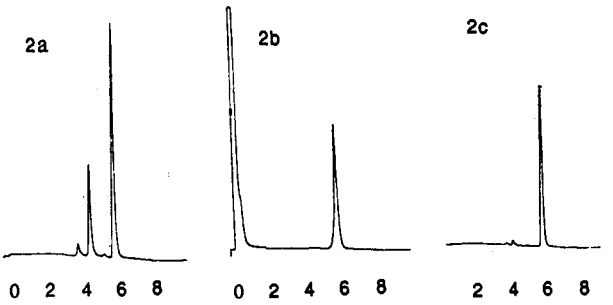


Figure 2. HPLC chromatograms of the crude protected tetrapeptide Boc-Ala-Asp(O*t*Bu)-Ser(*t*Bu)-Pro-OH (**7**). HPLC conditions: YMC ODS-AQ column, 4 mm × 50 mm, 3 μm particle, f = 2 mL/min, 220 nm, eluent A, 0.1% (w/v) TFA in H_2O; B, 0.1% (w/v) TFA in CH_3CN containing 5% A, gradient 6–64% B in 6 min, linear; (a) obtained by TBAF treatment, (b) obtained by BATHF treatment, and (c) obtained by 1% TFA/CH_2Cl_2 treatment.

observed in resin-bound peptides remains to be improved and is currently under investigation.[26]

In addition to fluoridolysis, it was found that treatment of Fmoc (**12**) or Boc-Ala-Asp(OtBu)-Ser(tBu)-Pro-SAC resin derivatives (**6**) with 1% TFA/CH_2Cl_2 solution gave the protected tetrapeptides in high purity and 80–90% isolated yields. This is particularly useful for the preparation of Fmoc-protected peptide fragments which were otherwise not possible to obtain by using the fluoridolysis methodology already discussed.[27]

In order to assess the utility of the SAC linker for the preparation of protected peptide acids, a cyclic heptapeptide cyclo-($^{\alpha}NH_2$-Orn-Phe-Leu-Leu-Arg-Asn-Pro) (**15**) was synthesized using Fmoc-Pro-SAC-polystyrene (Scheme 4). The intermediate Boc-protected linear peptide acid **14** was obtained by treatment of peptide resin **13** with 2 equiv of TBAF in DMF. The crude protected peptide acid **14** was used directly and cyclized using DPPA in DMF. After removal of side-chain protecting groups

(24) In some cases, in addition to the desired compound and succinimide byproduct, a third component Boc-Ala-Asp-Gly-Phe-OH (presumably a mixture of α and β peptides) was also observed.

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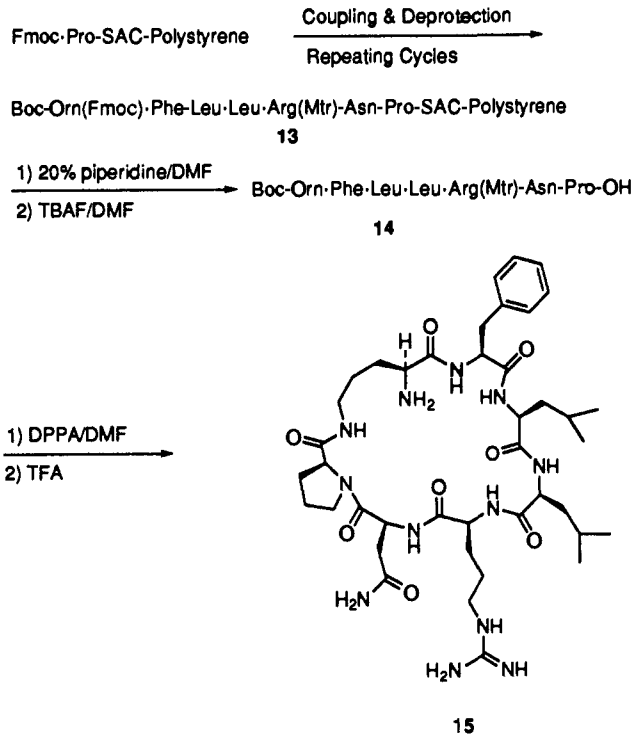
(26) An aliquot of peptide resin, after cleavage by fluoride ion, was hydrolyzed and analyzed by amino acid analysis to confirm the cleavage yield. According to amino acid analysis, there was 40% of peptide remaining on the resin after treatment with BTMHF thus confirming the low isolated yield was attributable to incomplete cleavage. In the case of TBAF there was only 2% of peptide remaining on the resin.

(27) Under the fluoridolysis conditions rapid delocking of Fmoc group was observed as determined by a ninhydrin test.

Scheme 4



with TFA and preparative HPLC purification, pure cyclic peptide was obtained in 11% yield (9 mg). The identity of the cyclic peptide was established by MS/FAB and amino acid analysis.

Suppression of Diketopiperazine Formation. In the fragment condensation strategy, proline and glycine are often chosen for the C-terminal residues because of their low tendency to racemize during activation and coupling. The preparation of C-terminal proline peptides, however, poses another problem—diketopiperazine formation.[2] Cyclization at the free amino dipeptide stage resulting in diketopiperazine formation is a serious side reaction observed in the Fmoc/*t*-Bu strategy and causes low or even no yields for synthesis in some sequences due to premature loss of the peptide from the solid support.[2,28] Although shorter deprotection time (5 min for 1:1 piperidine/DMF[28] and 2 min for TBAF[29]) and alternatively coupling the third and second amino acids as an Fmoc–dipeptide have been recommended, not until recently has the development of tertiary alcohol based linkers been successfully applied to the C-terminal proline problem.[30] That work indicated that steric hindrance plays a critical role in suppressing the DKP formation, i.e., the tertiary alcohol based linker forms less DKP than the primary alcohol based-linkers. Since SAC linker is derived from a secondary alcohol and also has the bulky trimethylsilyl group in the β-position, it was reasoned that it may be useful in suppressing DKP formation. Thus it was anticipated that less DKP formation would occur using the SAC linker compared to the conventional HMPA linker. To test the validity of this hypothesis, several resin-bound Fmoc-dipeptide linker derivatives, notorious for DKP formation, such as Tyr-Pro, Pro-Pro, and Pro-Gly from both SAC linker and HMPA linker were prepared. In the case of SAC linker, the results clearly showed that, except for the Pro-Pro sequence, even after 30 min the dipeptides remained on the resin support during treatment with 20% piperidine/DMF (Figure 3). In contrast, the HMPA linker retained very little (2–3%) of Tyr-Pro or Pro-Pro at 5 min (Pro-Gly sequence was not tested). Encouraged by

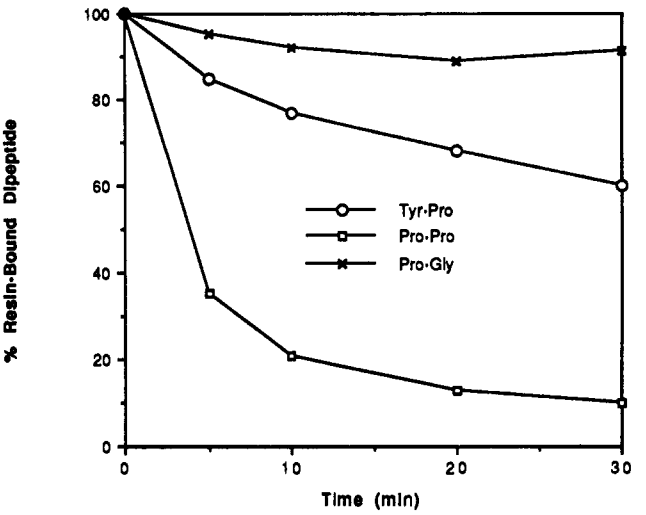


Figure 3. Time course for the DKP formation of dipeptide-SAC-linker-resin derivatives. Fmoc-dipeptide-SAC-linker-resin derivatives (200 mg) were treated with 2 mL of 20% piperidine/DMF solution. An aliquot of the resin (20 mg) was removed at various times, washed, dried, and subjected to amino acid analysis.

these model studies, a C-terminal proline undecapeptide H-Cys-Thr-His-Leu-Pro-Glu-Thr-Lys-Pro-Ser-Pro-OH (**12**) was synthesized using Fmoc-Pro-SAC-resin. After cleavage with TFA/Et_3SiH[31]/phenol/DTT (95:2:2:1), the crude peptide was isolated as a tri-TFA salt in 88% yield. After preparative HPLC purification, the pure peptide was obtained in 40% yield, and its identity was confirmed by MS/FAB and amino acid analysis.

Conclusion

A novel and versatile silicon-derived linker SAC was prepared in high yield. Two major side reactions, diketopiperazine (DKP) formation and tryptophan alkylation by resin-derived carbocations, are successfully minimized using SAC linker. Furthermore, SAC linker was shown to be a useful tool for the preparation of protected peptide fragments by either fluoridolysis or 1% TFA in CH_2Cl_2.

Experimental Section

General Methods. Normal workup from an organic solvent involved drying over $MgSO_4$ and rotary evaporation. Melting points were obtained using a micro hot plate apparatus and are uncorrected. NMR spectra were recorded on a General Electric QE-300 spectrometer. Chemical shift are recorded in δ units (ppm) using tetramethylsilane as an internal standard or standardized to the residual protons in deuterated solvents. FAB mass spectra were recorded on a JEOL-SX spectrometer and obtained from the in-house facility. Hydrolysis of peptides for amino acid composition analysis was performed according to the procedure of Liu and Boykins.[32] Amino acid analysis was carried out on a Shimadzu LC-600 system using the Pico Tag method.[33] TLC was performed on precoated silica gel 60 F254 plates. Compounds on TLC plates were visualized with UV light, iodine, and 2% ninhydrin in EtOH. Analytical HPLC was performed on the Shimadzu LC-600 system using a YMC ODS-AQ column, 4 mm × 50mm, 3 μm particle (Morris Plains, NJ). Peaks were observed with a photodiode array detector. Solvents used for HPLC elution were as follows: A, 0.1% (w/v) TFA in H_2O; B, 0.1% (w/v) TFA in acetonitrile containing 5% A. The column was eluted with a linear gradient of 6–64% solvent B in A over the course of 6 min at a flow rate of 2.0 mL/min. For preparative HPLC, the work was performed on a Shimadzu LC-8A system using a Waters (Milford, MA) PrepPak (C_{18}, 25 × 100mm, 100 Å pore) column. Sep-Pak cartridges (C_{18}) used

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for desalting were obtained from Millipore (Milford, MA). Acid washed glass beads (150–212 microns) used in continuous flow solid phase synthesis were obtained from Sigma (St. Louis, MO).

DIEA and piperidine were purchased from Fluka (Buchs, Switzerland) and used as supplied. 4-Bromobenzaldehyde, 2,4-dichlorophenol, and trimethylsilylmethylmagnesium chloride were purchased from Aldrich (Milwaukee, WI) and used without further purification. BOP reagent was purchased from Milligen/Biosearch (Burlington, MA) and used without further purification. THF was distilled from sodium/benzophenone prior to use. Aminomethylpolystyrene resin was purchased from Peninsula Laboratories with a substitution level of 0.72 mmol of amino function/g. Sequencing grade DMF was purchased from Fisher Scientific (Fair Lawn, NJ) and used without purification. All other solvents were analytical reagent grade or better and used as supplied.

1-Bromo-4-[1-Hydroxy-2-(trimethylsilyl)ethyl]benzene (1). To 100 mL (0.1 mol) of a trimethylsilylmethylmagnesium chloride solution (1 M in ether) at 0 °C was added 14.8 g (80 mmol) of the 4-bromobenzaldehyde in 80 mL of THF dropwise. After addition was complete, the resulting solution was stirred at 0 °C for 2 h and quenched by addition of saturated aqueous NH_4Cl. The organic layer was collected and washed with NH_4Cl twice and then brine. After removal of solvent, the residual liquid was purified by column chromatography (200 g of silica) with elution by 7% acetone in hexane to give 18.6 g (86%) of the product which was solidified on standing: mp 31–2 °C; ^1H NMR ($CDCl_3$) δ –0.082 (s, 9H), 1.09–1.32 (m, J = 7.5 Hz, 2H), 2.38 (s, 1H), 4.73 (t, J = 7.5 Hz, 1H), 7.16–7.19 (d, J = 8.4 Hz, 2H), 7.42–7.45 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) –1.1, 28.3, 72.1, 121.1, 127.5, 131.4, 145.4. Anal. Calcd for $C_{11}H_{17}BrOSi$: C, 48.35; H, 6.27; Br, 29.24. Found: C, 48.51; H, 6.20; Br, 29.17. Generally, the crude product was obtained in high purity (>95%) and used without further purification.

4-[1-Hydroxy-2-(trimethylsilyl)ethyl]benzoic Acid. (2) A solution of 21 g (77 mmol) of crude **1** in 100 mL of dry THF was cooled to –78 °C. To the solution was added 3 equiv of *n*-BuLi (92 mL, 2.5 M in hexane) over the course of 30 min. The reaction mixture was stirred at –78 °C for 1 h and then poured onto 400 g of finely crushed dry-ice in one portion. The resulting mixture was stirred (overhead stirrer) for 1 h below 0 °C and quenched by slow addition of 200 mL of H_2O. (Note: When reaction was carried out above 0 °C, a significant amount of styrene 4-carboxylic acid was observed due to Peterson olefination.[12]) The organic layer was extracted with 5% Na_2CO_3 (50 mL × 2). The combined aqueous solution, after extraction with ether, was acidified with 5% $KHSO_4$ to pH 2. The product, which had precipitated, was extracted twice with EtOAc. The EtOAc solution was washed with brine and dried. After removal of solvent, the solid was trituated with hexane to give 14.1 g (70%) of the desired compound as white crystals: mp 132–133 °C; ^1H NMR ($CDCl_3$) δ –0.03 (s, 9H), 1.18–1.38 (m, J = 7.6 Hz, 2H), 4.98 (t, J = 7.5 Hz, 1H), 7.45–7.48 (dd, J = 8.3 Hz, 2H), 8.07–8.10 (dd, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) –1.1, 28.4, 71.5, 125.6, 129.3, 139.9, 152.1, 168.7. Anal. Calcd for $C_{12}H_{18}O_3Si$: C, 60.47; H, 7.61. Found: C, 60.50; H, 7.55.

2,4-(Dichlorophenyl)-4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoate (3). To a solution of 4.9 g (20 mmol) of **2** and 4.1 g (25 mmol) of 2,4-dichlorophenol in 20 mL of THF at 0 °C was added 4.6 g (22 mmol) of DCC. The resulting mixture was stirred at 0 °C for 2 h and then at room temperature for 3 h. DCU was removed by filtration, and the filtrate evaporated to dryness to give a brown residue which was dissolved in 15 mL of ether-hexane (2:1) solution and kept at 4 °C overnight. Insoluble material was removed by filtration, and the filtrate evaporated to dryness. Trituration with hexane gave 4.6 g (60%) of the ester as white crystals: mp 77–78 °C; ^1H NMR ($CDCl_3$) δ 0.03 (s, 9H), 1.15–1.38 (m, J = 7.6 Hz, 2H), 1.83 (d, 1H), 4.95–5.05 (m, 1H), 7.20–7.39 (m, 3H), 7.50–7.55 (dd, J = 8.4 Hz, 2H), 8.18–8.25 (dd, J = 8.4 Hz, 7H); ^{13}C NMR ($CDCl_3$) –1.0, 28.6, 72.2, 124.6, 125.9, 127.3, 127.9, 130.0, 130.6, 131.8, 145.8, 153.2, 163.8. Anal. Calcd for $C_{18}H_{20}Cl_2O_3Si$: C, 56.40; H, 5.26; Cl, 18.50. Found: C, 56.28; H, 5.26; Cl, 18.15.

General Procedure for the Preparation of 2,4-(Dichlorophenyl)-4-[1-[[(N$^\alpha$Fmoc-amino)acyl]oxy]-2-(trimethylsilyl)ethyl]benzoate (Fmoc-AA-SAC Linker Derivatives 4). To a solution of compound **3** (1.94 g, 5 mmol) and 5.5 mmol of Fmoc-amino acid in 15 mL of THF at 0 °C was added DCC (1.1 g, 5.25 mmol) in one portion followed by DMAP (30.5 mg, 0.25 mmol). The resulting mixture was stirred at 4 °C overnight and then at room temperature for an additional 2 h. DCU was removed by filtration and washed thoroughly with 30 mL of EtOAc. The organic solution was washed with 10% aqueous $NaHCO_3$ (30 mL × 2), 2% aqueous $KHSO_4$ (30 mL), and brine. After removal of solvent, the residue was purified by silica gel column chromatography with elution by 25% EtOAc in hexane, and the mixture of diastereomers were collected in one fraction.

2,4-(Dichlorophenyl)-4-[1-[(N$^\alpha$Fmoc-prolyl)oxy]-2-(trimethylsilyl)ethyl]benzoate (Fmoc-Pro-SAC Linker 4a). The title compound was isolated in 75% (2.7 g) as a foam after silica gel chromatography. Anal. Calcd for $C_{38}H_{37}Cl_2NO_6Si$: C, 64.95; H, 5.31; N, 1.99; Cl, 10.09. Found: C, 64.73; H, 5.40; N, 1.93; Cl, 9.87.

2,4-(Dichlorophenyl)-4-[1-[(N$^\alpha$Fmoc-tryptophanyl)oxy]-2-(trimethylsilyl)ethyl]benzoate (Fmoc-Trp-SAC Linker 4b). The title compound was isolated in 81% (3.2 g) as a foam after silica gel chromatography. Anal. Calcd for $C_{44}H_{40}Cl_2N_2O_6Si$: C, 66.74; H, 5.09, N, 3.54; Cl, 8.96. Found: C, 66.76; H, 5.01, N, 3.55; Cl, 8.77.

2,4-(Dichlorophenyl)-4-[1-[(N$^\alpha$Fmoc-glycyl)oxy]-2-(trimethylsilyl)ethyl]benzoate (Fmoc-Gly-SAC Linker 4c). The title compound was isolated in 79% (2.6 g) as a foam after silica gel chromatography. Anal. Calcd for $C_{35}H_{33}Cl_2NO_6Si$: C, 63.40; H, 5.01; N, 2.11; Cl, 10.70. Found: C, 63.56; H, 5.01, N; 2.33; Cl, 10.65.

2,4-(Dichlorophenyl)-4-[1-[(N$^\alpha$Fmoc-phenylalaninyl)oxy]-2-(trimethylsilyl)ethyl]benzoate (Fmoc-Phe-SAC Linker 4d). The title compound was isolated in 79% (3.0 g) as a foam after silica gel chromatography. Anal. Calcd for $C_{42}H_{39}Cl_2NO_6Si$: C, 67.01; H, 5.22; N, 1.86; Cl, 9.42. Found: C, 66.96; H, 5.42; N, 1.80; Cl, 9.67.

General Procedure for Attachment of the Preformed Fmoc-AA-SAC Linker Derivatives 4 onto Amino Functionalized Supports. (Aminomethyl)polystyrene resin (0.72 mmol amino function/g, 5 g) was washed with DMF, 5% DIEA/DMF, and DMF. To the resin was added 1.5 equiv of the Fmoc-amino acid-SAC linker derivative **4** in 10 mL of DMF containing equimolar HOBt/DIEA.[9] The resulting mixture was left standing at room temperature for 5 h with occasional stirring, drained, and washed with DMF. Unreacted amino groups were capped by reaction with acetic anhydride and pyridine (500 μL each) in 9 mL of DMF for 15 min, after which the ninhydrin test[34] of a resin sample was negative. The reagent was drained and the resin washed with DMF, MeOH, and ether. After being dried in vacuo, the peptide resin was subjected to Fmoc quantitation by UV analysis[10] and found to have a substitution level of about 0.3 mmol of Fmoc /g (Table 1).

General Procedure for the Cleavage of Boc-Protected-Peptide-SAC-Resin by Fluoridolysis. The peptide resin derivatives (150 mg, 45 μmol) in a 20-mL syringe fitted with a polypropylene frit were treated with 10 mL of DMF containing 2 equiv of quaternary ammonium fluorides for 20 min. After filtration, DMF solution was quenched with 2.5 mL of MeOH, and this process was repeated one more time. The combined DMF solution was evaporated to dryness with the aid of a vacuum pump at room temperature. The resultant residue was dissolved in 10 mL of 20% aqueous DMF, desalted on a C_{18} Sep-Pak with washing by H_2O containing 0.1% TFA, and then eluted with 60% acetonitrile/H_2O containing 0.1% TFA. The fractions containing peptide were collected, evaporated, and analyzed by HPLC.

Synthesis of Boc-Ala-Asp(OtBu)-Ser(tBu)-Pro-OH (7). (a) Treatment with TBAF. The crude Boc-Ala-Asp(OtBu)-Ser(tBu)-Pro-OH **7** was obtained as an oily solid (18 mg, 60%). HPLC analysis showed the crude peptide containing two peaks with retention time 4.6 and 5.9 min, respectively, and in 12:88 ratio based on integration (Figure 2a). The peak (4.6 min) was isolated in 6% yield (1.5 mg) and identified to be the succinimide **8**, 74 mass units less than the title compound. ^1H NMR spectra of compound **8** was rather complex, but a serine *tert*-butyl ether resonance at 1.15 ppm was clearly observed as two singlets with 1:1 ratio presumably due to at least two conformers present: MS/FAB (M + H$^+$) 527 (M – *t*-BuOH), calcd 526 (M); amino acid analysis, Asp 1.00 (1), Ala 0.97 (1), Ser 0.84 (1), Pro 0.95 (1). The desired product (5.9 min) was isolated in 44% yield (12 mg): MS/FAB (M + H$^+$) 601, calcd 600 (M); amino acid analysis, Ala 1.00 (1), Asp 0.98 (1), Ser 0.87 (1), Pro 1.03 (1), ^1H NMR ($CDCl_3$) δ 1.12 (s, 9H, Me_3C, Ser), 1.39 (d, 3H, CH_3, Ala), 1.43 (s, 9H, Me_3C), 1.46 (s, 9H, Me_3C), 2.01 (m, 3H, Pro), 2.38 (m, 1H, Pro), 2.65 (dd, 1H, Asp), 2.91 (dd, 1H, Asp), 3.54 (m, 2H, Ser), 3.63 (m, 1H, Pro), 3.91 (1H, m, Pro), 4.18 (m, 1H, α-CH, Ala), 4.56 (m, 1H, α-CH, Pro), 4.71 (m, 1H, α-CH, Asp), 4.88 (m, 1H, α-CH, Ser), 5.05 (Bs, 1H, NH), 7.49 (m, 2H, NH). Anal. Calcd for $C_{28}H_{48}N_4O_{10}$: C, 55.98; H, 8.05; N, 9.33. Found: C, 56.32; H, 8.00; N, 9.21.

(b) Treatment with BTAHF. The crude Boc-Ala-Asp(O*t*Bu)-Ser(*t*Bu)-Pro-OH (**7**) was obtained in 37% yield (10 mg) with 100% purity

(34) Kaiser, E; Colescott, R. L.; Bossinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595.

(35) Although removal of Fmoc group and cleavage of peptide resin can be carried out simultaneously using TBAF, deblocking the Fmoc group first followed by cleavage from the resin was preferred to avoid dealing with insoluble dibenzofulvene derived polymers.

which coeluted with sample 7 prepared using TBAF (retention time 5.9 min, Figure 2b): amino acid analysis, Ala 1.03 (1), Asp 0.97 (1) Ser 0.85 (1), Pro 1.07 (1).

Synthesis of Boc-Ala-Asp(OtBu)-Gly-Phe-OH (10). (a) Treatment with TBAF. The crude Boc-Ala-Asp(O*t*Bu)-Gly-Phe-OH (**10**) was obtained as an oil (13.2 mg, 54%). HPLC analysis showed the crude peptide containing two major peaks with retention time 5.85 and 6.73 min, respectively, and in 19:81 ratio based on integration. The first peak (5.85 min) was isolated in 40% yield (10 mg) and found to be the succinimide **11**, 74 mass units less than the title compound: MS/FAB ($M + H^+$) 491; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, CH_3, Ala), 1.44 (s, 9H, Me_3C), 2.72 (d, 1H, CH, Asp), 3.03–3.18 (m, 3H, CH_2 + CH, Phe + Asp), 4.0–4.6 (m, 4H), 4.67 (bs, 1H), 5.61–6.1 (bs, 1H), 7.18–7.33 (m, 5H, aromatic, Phe), 7.6 (bs, 1H, NH), 8.0–8.3 (m, 1H, NH). The desired product (6.73 min) was obtained in 10% yield (2.4 mg): ^1H NMR ($CDCl_3$) δ 1.33 (d, 3H, CH_3, Ala), 1.43 (s, 18H, 2 Me_3C), 2.69–2.87 (m, 2H, CH_2, Asp), 3.02–3.26 (m, 2H, CH_2, Phe), 3.84–3.93 (m, 2H, CH_2, Gly), 4.08 (m, 1H, α-CH, Ala), 4.67–4.73 (m, 2H, 2 α-CH), 5.27 (bs, 1H, NH), 7.19 –7.30 (m, 6H, aromatic + NH), 7.64 (bs, 2H, 2 NH), MS/FAB ($M + H^+$) 565, calcd 564 (M); amino acid analysis: Ala 1.00 (1), Asp 1.07 (1), Gly 1.03 (1), Phe 0.94 (1).

(b) Treatment with BTAHF. The crude Boc-Ala-Asp(O*t*Bu)-Ser-(*t*Bu)-Pro-OH (**10**) was obtained as a white solid (8.3 mg, 34%). HPLC analysis showed the crude peptide containing two major peaks with retention time 5.85 and 6.73 min, respectively and in 9:91 ratio based on integration. The first peak (5.85 min) was isolated in 4% yield (0.9 mg) and identified to be the succinimide **11**, 74 mass unit less than proposed structure. MS/FAB ($M + H^+$) 491. The desired product (6.73 min) was isolated in 26% yield (6.5 mg): MS/FAB ($M + H^+$) 565, calcd 564 (M); amino acid analysis, Ala 1.00 (1), Asp 0.99 (1), Gly 1.05 (1), Phe 0.97 (1).

General Procedure for Cleavage of Protected Peptide-SAC-Resins Derivatives Using 1% TFA/CH_2Cl_2. Peptide resin (150 mg, 45 umol) was treated with 5 mL of 1% TFA/CH_2Cl_2 solution for 15 min twice, collected, and neutralized with DMF. The combined solution was concentrated to 1–2 mL and diluted with 2 mL of H_2O. The resulting solution was desalted on a C18 Sep-Pak with elution by H_2O containing 0.1% TFA and then 60% acetonitrile/H_2O containing 0.1% TFA. The fractions containing the peptide were collected, lyophilized, and analyzed by HPLC.

Boc-Ala-Asp(O*t*Bu)-Ser(*t*Bu)-Pro-OH (7) was obtained, after lyophilization from dioxane, in 95% yield (28.2 mg) as a white powder which coeluted with an authentic sample (retention time 5.9 min, Figure 2c): MS/FAB ($M + H^+$) 601, calcd 600 (M); amino acid analysis, Asp 1.00 (1), Ala 0.99 (1), Ser 0.87 (1), Pro 0.95 (1).

Fmoc-Ala-Asp(O*t*Bu)-Ser(*t*Bu)-Pro-OH (12) was obtained, after lyophilization from dioxane, in 81% yield (28 mg) as a white solid: ^1H NMR (CDCl3) δ 1.05 (s, 9H, Me_3C), 1.38 (d, 3H, CH_3), 1.42 (s, 9H, Me_3C), 2.01 (m, 3H, Pro), 2.38 (m, 1H, Pro), 2.62 (dd, 1H, Asp), 2.95 (dd, 1H, Asp), 3.4–3.95 (m, 4,H), 4.2–4.4 (m, 4H), 4.6–4.95 (m, 3H), 5.4 (d, 1H), 7.31–7.80 (m, 8H); MS/FAB ($M + H^+$) 723, ($M - H^-$) 721, calcd 722 (M). Anal. Calcd for $C_{38}H_{50}N_4O_{10}$: C, 63.14; H, 6.97; N, 7.75. Found: C, 63.0; H, 7.04; N, 7.84.

Cyclo($^{\alpha}NH_2$-Orn-Phe-Leu-Leu-Arg-Asn-Pro) (15). As an intermediate, Boc-protected peptide acid **14** was synthesized manually using Fmoc-Pro-SAC-(aminomethyl)polystyrene (0.1 mmol, 330 mg; 0.3 mmol Fmoc/g substitution level). The peptide chain was assembled using 4 equiv of Fmoc-amino acids/BOP/DIEA, except for asparagine where the corresponding Pfp ester was used in the presence of equimolar HOBt. In general, coupling reactions required 30–45 min to reach a negative ninhydrin test end point. For deprotection 20% piperidine in DMF was used (10 min). Arginine was protected by the Mtr group and ornithine was incorporated as Boc-Orn(Fmoc)-OH. After completion of the synthesis, the side-chain Fmoc-protecting group was removed with 20% piperidine in DMF[35] and washed the resin with DMF. The resulting resin was treated twice with 10 mL of DMF solution containing 2 equiv of TBAF for 20 min. The combined DMF solution was evaporated to dryness. The resultant residue was dissolved in 10 mL (1:4) DMF/H_2O solution and desalted on a C18 Sep-Pak as described in the General Procedure for Fluoridolysis. After concentration and lyophilization, the protected peptide acid **14** was obtained in 43% (51 mg) yield. HPLC analysis showed the desired peptide to be the major product (retention time 6.13 min, 89% pure, MS/FAB ($M + H^+$) 1186, calcd 1185 (M)) which was used for the cyclization without further purification. To the protected peptide acid **14** in 20 mL of DMF containing DIEA (2.2 equiv) was added DDPA (1.9 equiv) in 2 mL of DMF dropwise at 4 °C. After stirring overnight, DMF solution was concentrated to 2–3 mL, diluted with 5 mL of H_2O, and desalted on a C18 Sep Pak with elution by 70% acetonitrile/H_2O containing 0.1% TFA. The fractions containing peptide were combined and evaporated to dryness to give an oil which was treated with 5 mL of TFA/phenol/Et_3SiH (95:3:2) solution for 2 h. After evaporation and ether precipitation, the crude peptide was isolated and purified by preparative HPLC to give 9 mg of the cyclic peptide as a white powder (retention time 4.62 min): MS/FAB ($M + H^+$) 855.7, calcd (M) 854.8; amino acid analysis, Asx 0.97 (1), Arg 1.05 (1), Leu 2.11 (2), Phe 0.93 (1), Pro 1.02 (1), Orn 1.10 (1).

H-Leu-Ala-Glu-Leu-Gly-Ala-Ser-Leu-Leu-Lys-His-Trp-OH (5). This peptide was assembled automatically on a continuous flow peptide synthesizer, using Fmoc-Trp-SAC-resin with 0.29 mmol Fmoc-Trp/g substitution level (0.2 mmol, 690 mg) mixed with 2.7 g of glass beads. Coupling reactions were carried out using 4 equiv of Fmoc-amino acids/BOP/DIEA for 60 min. After cleavage of the peptide resin with 20 mL of TFA/thioanisole/phenol (95:2.5:2.5) solution for 60 min, the crude peptide was isolated in 92% yield (270 mg) as a tri-TFA salt with approximately 70% purity. After preparative HPLC purification, 98 mg (31% based on quantitative amino acid analysis) of the homogenous peptide was obtained as a white solid: MS/FAB ($M+ H^+$) 1337.5, calcd (M) 1336.8; amino acid analysis, Asp 0.95 (1), Gly 1.10 (1), His 1.08 (1), Ala 2.02 (2), Leu 3.75 (4), Lys 1.11 (1), Ser 0.76 (1), Trp 0.57 (1).

H-Cys-Thr-His-Leu-Pro-Glu-Thr-Lys-Pro-Ser-Pro-OH (15). This peptide was assembled automatically on a continuous flow peptide synthesizer, using Fmoc-Pro-SAC-resin with 0.30 mmol Fmoc-Pro/g substitution level (0.2 mmol, 670 mg) mixed with 2.7 g of glass beads. Coupling reactions were carried out using 4 equiv of Fmoc-amino acids/BOP/DIEA for 60 min. After cleavage of the peptide resin with 20 mL of TFA/phenol/Et_3SiH/DTT (95:2:2:1) solution for 2 h, 273 mg of the crude peptide was obtained as a tri-TFA salt with approximately 88% purity. After preparative HPLC purification, there was obtained 161 mg (40% yield based on quantitative amino acid analysis) of the homogenous peptide as a white solid: MS/FAB ($M + H^+$) 1209.7, calcd (M) 1209; amino acid analysis, Glu 1.00 (1), His 0.90 (1), Leu 1.00 (1), Pro 3.07 (3), Lys 1.03 (1), Ser 0.95 (1), Thr 1.64 (2).