Preparation and Use of the 4-[1-[*N*-(9-Fluorenylmethyloxycarbonyl)-amino]-2-(trimethylsilyl)ethyl]phenoxyacetic Acid Linkage Agent for Solid-Phase Synthesis of C-Terminal Peptide Amides: Improved Yields of Tryptophan-Containing Peptides[1,2]

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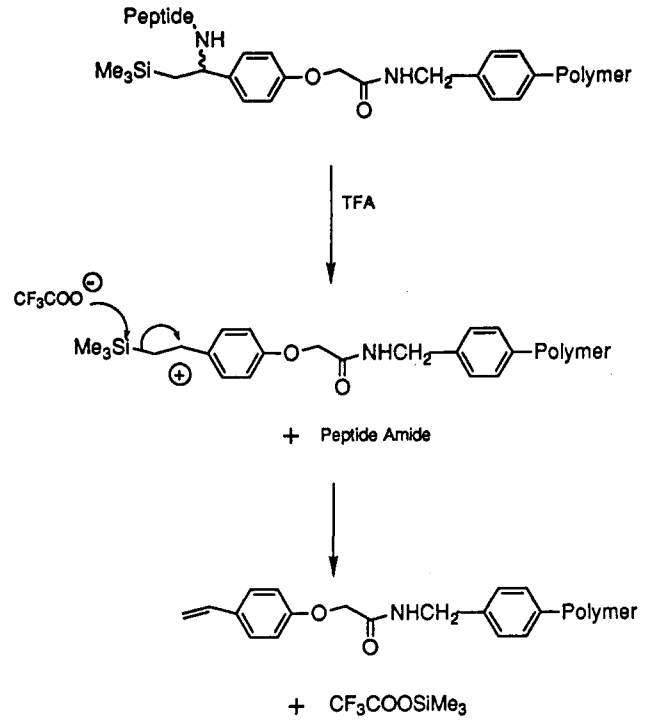
Introduction

In the past decade, a variety of linkage agents have been designed and prepared for the production of peptide C-terminal amides using the Fmoc/*tert*-butyl strategy for solid phase peptide synthesis.[3] Many of the linkage agents have common properties and all have the tendency to produce stable carbocations during their cleavage with TFA. The side reactions caused by such stable carbocations have been well documented.[3l,4] In order to suppress such problems, the use of scavengers or scavenger combinations during TFA cleavage is common but not always completely satisfactory.[3l,5]

Scheme I



In this account we would like to describe an approach utilizing organosilicon chemistry to minimize the problems arising from linkage agent derived carbocations. The use of organosilicon compounds as reagents and as intermediates in organic synthesis has become a field of considerable interest.[6] Because of the unique properties of trialkylsilyl moieties, they are commonly used in synthesis for oxygen and nitrogen protection and can also be cleaved with fluoride ion under mild conditions.[7] Silicon-derived fluoridolyzable linkage agents for the synthesis of protected peptide segments have also been reported.[8] In addition, the trialkylsilyl moieties are known to stabilize β-carbocations by hyperconjugation.[9] Silyl-linkage agent 1 ("SAL" linker, silyl amide linker) was synthesized with the intention of taking advantage of organosilicon chemistry. Specifically, linkage agent 1 was designed to undergo deblocking by a β-elimination mechanism under acidic conditions as illustrated in Scheme I. Although Scheme I depicts a nonconcerted mechanism, we could not exclude the possibility of a concerted mechanism. In either case, the β-elimination process would neutralize the transient carbocation to form a stable styrene derivative. Through such neighboring group participation, the carbocation should be rapidly quenched. In this sense, use of the trimethylsilyl moiety was expected to avoid the need for a scavenger. Such a possibility was anticipated to be

(1) Dedicated to Professor Louis A. Carpino on the happy occasion of his 65th birthday.

(2) 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 follows: AAA = amino acid analysis; BOP = benzotriazolyl *N*-oxytris(dimethylamino)phosphonium hexafluorophosphate; n-BuLi = n-butyllithium; Cbz = benzyloxycarbonyl; DCC = *N*,*N*′-dicyclohexylcarbodiimide; DIEA = diisopropylethylamine; DMF = *N*,*N*-dimethylformamide; EDT = 1,2-ethanedithio; EtOAc = ethyl acetate; MS/FAB = mass spectrometry/fast atom bombardment; Fmoc = 9-fluorenylmethyloxycarbonyl; HOAc = acetic acid; HOBt = 1-hydroxybenzotriazole; HPLC = high-performance liquid chromatography; Linker AM = 2-(4-Fmoc-aminomethyl(2,4-dimethoxyphenyl)phenoxy)acetic acid; OSu = *N*-oxysuccinimidyl; PAL = 5-(4-(9-Fmoc-aminomethyl-3,5-dimethoxy)phenoxy)valeric acid; TBAF = tetrabutylammonium fluoride; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin-layer chromatography.

(3) (a) Rich, D. H.; Gurwara, S. K. *Tetrahedron Lett.* **1975**, 301. (b) Colombo, R. *J. Chem. Soc., Chem. Commun.* **1981**, 1012. (c) Penke, B.; Rivier, J. *J. Org. Chem.* **1987**, *52*, 1197. (d) Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 2107. (e) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787. (f) Breipohl, G.; Knolle, J.; Stuber, W. *Tetrahedron Lett.* **1987**, *28*, 5651. (g) Funakoshi, S.; Murayama, E.; Guo, L.; Fujii, N.; Yajima, H. *Collect. Czech. Chem. Commun.* **1988**, *53*, 2791. (h) Stuber, W.; Knolle, J.; Breipohl, G. *Int. J. Peptide Protein Res.* **1989**, *34*, 215. (i) Breipohl, G.; Knolle, J.; Stuber, W. *Int. J. Peptide Protein Res.* **1989**, *34*, 262. (j) Soucek, M. In *Peptides 1990*; Proceedings of the Twenty-First European Peptide Symposium; Giralt, E., Andreu, D., Eds.; Escom Science Publishers: Leiden, 1991; pp 129. (k) Shao, J.; Li, Y.-H.; Voelter, W. *Int. J. Peptide Protein Res.* **1990**, *36*, 182. (l) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730 and references cited therein. (m) Penke, B.; Nyerges, L. *Pept. Res.* **1991**, *4*, 203.

(4) (a) Riniker, B.; Kamber, B. In *Peptides 1988*; Proceedings for the 20th European Peptide Symposium; Jung, G., Bayer, E., Eds.; Walter de Gruyter: Berlin, New York, 1989; pp 115–117. (b) Atherton, E.; Sheppard, R. C. *Tetrahedron* **1988**, *44*, 843. (c) Gesellchen, P. D.; Rothenberger, R. B.; Dorman, D. E.; Paschal, J. W.; Elzey, T. K.; Campell, C. S. In *Peptides; Chemistry, Structure and Biology*; Proceedings for the Eleventh American Peptide Symposium; Rivier, J. E.; Marshall, G. R., Eds.; Escom Science Publisher: Leiden, The Netherlands, 1990; pp 957–959.

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(8) (a) Mullen, D. G.; Barany, G. *J. Org. Chem.* **1988**, *53*, 5240 and references cited therein. (b) Ramage, R.; Barron, C. A.; Bielecki, S.; Holden, R.; Thomas, D. W. *Tetrahedron* **1992**, *48*, 499 and references cited therein.

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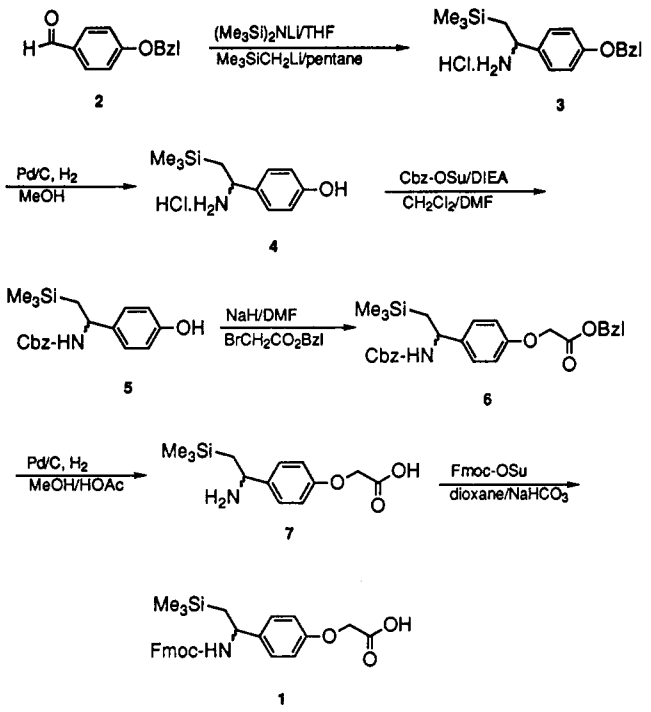
Scheme II



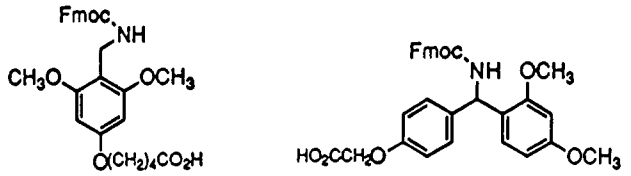
particularly advantageous in the case of peptides with C-terminal tryptophan. C-Terminal tryptophan-containing peptides can be obtained only in poor yields using conventional linkage agents which give rise to stable carbocations. These low yields are attributed to irreversible alkylation of the tryptophan indole moiety by such carbocations.[3l,4] Using SAL linker 1, improved yields of C-terminal tryptophan amides were thus expected.

Results and Discussion

The linkage agent 1 was prepared in six steps in overall yields ranging from 17 to 21% (Scheme II). 4-(1-Amino-2-(trimethylsilyl)ethyl)phenyl benzyl ether (3) was synthesized in 40–50% yield according to a literature procedure[10] by treatment of 4-(benzyloxy)benzaldehyde with lithium bis(trimethylsilyl)amide, followed by addition of [(trimethylsilyl)methyl]lithium. The benzyl group was removed by hydrogenolysis to give 4-(1-amino-2-(trimethylsilyl)ethyl)phenol (4), isolated as the hydrochloride salt in 95% yield. Selective protection of the amino group was accomplished to furnish the Cbz-protected 4-(1-amino-2-(trimethylsilyl)ethyl)phenol (5) in 85% yield. The phenolic function of 5 was alkylated with benzyl bromoacetate to provide the benzyl acetate intermediate 6, which without purification was hydrogenated to remove both protecting groups simultaneously to give the free amino acid 7 in 70% yield.[11] Finally, the protected linkage agent 1 was obtained by reaction of amino acid 7 with Fmoc-OSu in 75% yield. The Fmoc-protected SAL linker resin support was produced by coupling of 0.5 equiv of linkage agent 1 using a BOP/DIEA protocol[12] in DMF onto high loading (aminomethyl)polystyrene resin support (a substitution level of 3.38 mmol of amino function/g). After acetylation to cover the unreacted amino groups, the resulting linker resin support was subjected to spectrophotometric analysis and found to have a substitution level of 0.6 mmol of Fmoc/g (see Experimental Section for details).

In order to assess the stability of the linkage agent, the resin-bound Fmoc-protected SAL linker was treated with 0.2 M Fmoc-Gly-OH in DMF solution for 4 h, 0.3 M HOBt/DIEA in DMF solution for 3 h, and 0.3 M HOBt in DMF solution for 12 h, respectively. In all cases the Fmoc substitution level remained unchanged, thus confirming the stability of the linkage agent under typical synthesis conditions used for coupling of amino acids.

In pursuing this work Fmoc-valine was chosen as the test amino acid for linkage to resins due to its bulky properties that would presumably make it more difficult to couple and cleave. Fmoc-Val-OH was coupled to the amino group of deprotected resin-bound 1 using BOP/DIEA in DMF. In order to evaluate relative reactivity, the Fmoc-Val-linker-resin derivatives of 1, as well as 8 and 9 (two of the most widely used commercially available



linkage agents also known as "PAL"[3l] and "Linker AM",[3l,13] respectively) were prepared and treated with TFA containing 5% phenol by weight, a reagent commonly used for deprotection and cleavage of peptides from resins after synthesis by the Fmoc strategy. After treatment with TFA solution at room temperature for 15 min, the silicon-based linkage agent 1 gave greater than 97% cleavage whereas the linkage agents 8 and 9 gave only 75% and 60% cleavage, respectively.[14] The extent of cleavage was assessed by UV analysis using the fluorene chromophore of the dibenzofulvene–piperidine adduct at 301 nm (ϵ = 7800 M^{-1} cm^{-1}). Although incidental to the present study, these results are in agreement with previous observations that linkage agent 8 is more acid labile than 9.[13] In a separate experiment, an Fmoc-methionine resin derivative of linkage agent 1 was prepared and subjected to the same cleavage conditions. The rate of cleavage was comparable to that of the Fmoc-valine derivative. No complications were observed due to the presence of the side-chain thioether moiety.[15]

These encouraging preliminary results led to examination of our hypothesis that less cation alkylation of C-terminal tryptophan would occur. The Fmoc-Val-Trp-linker resin derivatives were chosen for this model study

(10) (a) Hart, D. J.; Kanai, K.-I.; Thomas, D. G.; Yang, T.-K. *J. Org. Chem.* **1983**, *48*, 289. (b) Colvin, E. W.; McGarry, D.; Nugent, M. J. *Tetrahedron* **1988**, *44*, 4157.

(11) In order to reduce the number of synthetic steps, reaction of the Fmoc-derivative of compound 4 with 2,4-dichlorophenyl bromoacetate under various conditions was attempted but found to be unsuccessful.

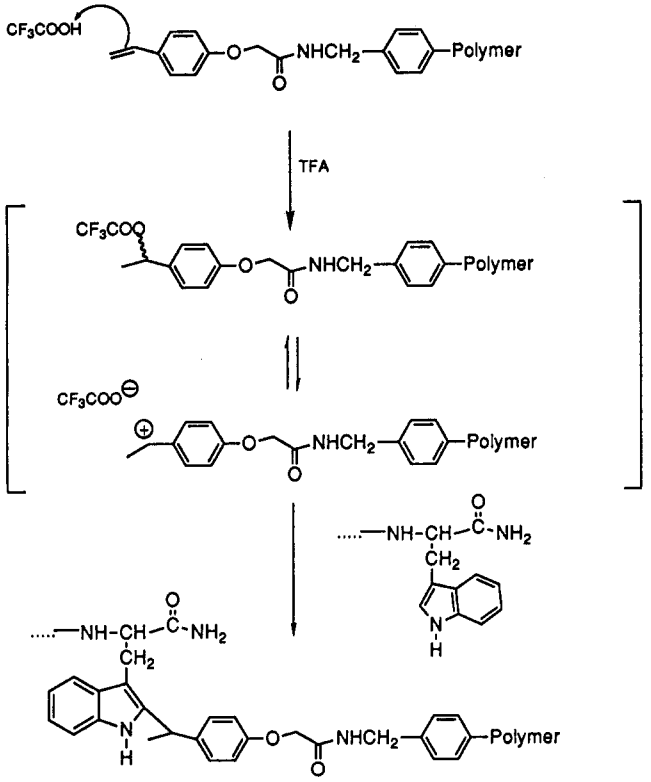
(12) Castro, B.; Dormoy, J.-R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.

(13) Bernatowicz, M. S.; Daniels, S. B.; Köster, H. *Tetrahedron Lett.* **1989**, *30*, 4645.

(14) Cleavage of the SAL linker by fluoride ion was examined: treatment of 100 mg of Bz-Ala-Gly-SAL-linker (aminomethyl)polystyrene resin (0.6 mmol/g) with 1 mL of 0.2 M tetrabutylammonium fluoride (TBAF) in DMF for 2 h failed to release protected peptide amide from the resin support. After TBAF treatment, the resin was washed, dried, and cleaved with TFA to give Bz-Ala-Gly-NH_2 in greater than 95% yield indicating that the SAL linkage was unaffected by TBAF treatment.

(15) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 223–233.

Scheme III



because C-terminal tryptophan can easily be alkylated by resin-bound carbocations derived from the linkage agents during cleavage with TFA.[3l,4] The bulkiness of the valine moiety would add another dimension of complexity to this dipeptide model. When the dipeptide derivative of linkage agent 1 was treated with a TFA solution containing 5% phenol, the extent of cleavage observed was only 45% after 30 min and no further cleavage was observed even after 3 h. Under the same cleavage conditions, the dipeptide derivative of linkage agents 8 and 9 gave less than 5% cleavage. Initially the results were surprising in view of the assumed mechanism depicted in Scheme I. These results, however, can be rationalized by the results of Lundt et al.,[16] who demonstrated that the reaction of isobutylene with TFA gives *tert*-butyl trifluoroacetate which is a potent *tert*-butylating agent and can undergo an aromatic substitution reaction with tryptophan to produce *tert*-butyl-substituted tryptophan derivatives. By this analogy, it is not unreasonable to assume that the styrene derivative of 1 derived from the TFA cleavage reaction of the SAL linker can be protonated by TFA to generate a carbocation or ester of trifluoroacetate, either of which can undergo an electrophilic aromatic substitution reaction with the indole moiety (Scheme III) thereby lowering cleavage yield. In order to verify this hypothesis, 0.1 mmol of 4-(benzyloxy)styrene and 0.1 mmol of Fmoc-Trp-OMe were dissolved in 1 mL of TFA/CH_2Cl_2 (3:1) solution with stirring at room temperature for 20 min. After workup and evaporation, the reaction mixture was examined by MS/FAB. The MS/FAB of the crude mixture showed not only the monoalkylated compound, $(M + H)^+$ 651, but also the dialkylated compound, $(M + H)^+$ 861. This result clearly supports the hypothesis that a carbocation can be generated from the reaction of TFA with

(16) Lundt, B. F.; Johansen, N. L.; Volund, A.; Markussen, J. *Int. J. Peptide Protein Res.* 1978, *12*, 258.

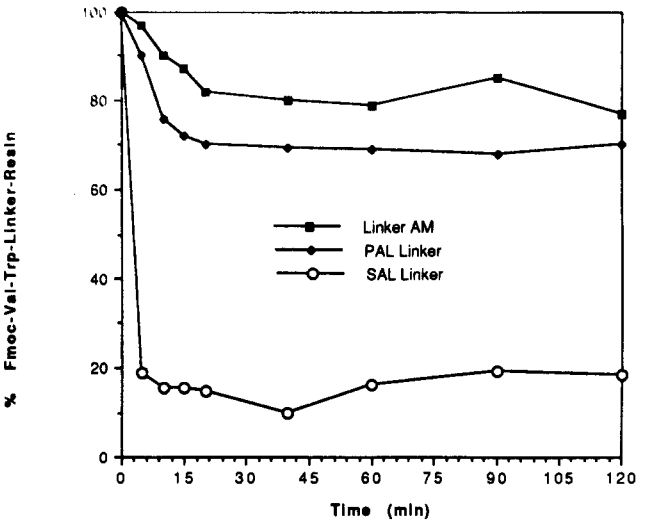


Figure 1. Time course for the cleavage of Fmoc-Val-Trp-Linker-resin derivatives. Peptidyl resin samples (200 mg) were treated with 2 mL of TFA/EDT/phenol/thioanisole (90:5:3:2) solution. An aliquot of the resin (10–15 mg) was removed at various times, washed, dried, and subjected to UV analysis.

the styrene intermediate. Subsequent alkylation of tryptophan by the carbocation reduces the cleavage yield.

In order to improve cleavage yield, scavenger combinations such as thioanisole, ethanediol, etc. were examined in an effort to quench effectively any carbocations generated. When Fmoc-Val-Trp-resin linker derivatives were treated with TFA/EDT/phenol/thioanisole (90:5:3:2) solution, the cleavage yield of the SAL derivative dramatically increased to 90% after 45 min compared to 45% yield using 5% phenol as scavenger. The PAL (8) and Linker AM (9) derivatives also gave improved yields under these conditions, but only to the extent of maximum yields of about 20–30%. The time course of these reactions was monitored spectrophotometrically,[13] the comparative data being given in Figure 1. Clearly these data indicate a great benefit in using the SAL linker for the synthesis of C-terminal tryptophan peptides and also suggest advantages in its use for the synthesis of tryptophan-containing peptide amides in general.[17]

In order to demonstrate the utility of the SAL linker two C-terminal tryptophan-containing peptide amides, RHK1 (458-465) H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-NH_2 (10) and RHK1 (458-466) H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-Trp-NH_2 (11), peptides contained in a homologous region of voltage-sensitive potassium channels,[18] were prepared using the Fmoc-SAL-linker (aminomethyl)-polystyrene support. Peptide chains were assembled manually using the Fmoc/*tert*-butyl strategy. Coupling reactions were carried out using either preformed pentafluorophenyl esters in the presence of HOBt/DIEA mixture[19] or by activation of the free Fmoc amino acids with BOP/DIEA. In both cases peptide chain elongation

(17) In the course of our study, Fmoc-Trp(Boc)-OH was introduced and more recently became commercially available. Fmoc-Trp(Boc)-OH may serve as an alternative approach to minimize tryptophan alkylation problems. For details see: White, P. In *Peptides; Chemistry and Biology*; Proceedings for the Twelfth American Peptide Symposium; Smith, J. A., Rivier, J. E., Eds.; Escom Science Publishers: Leiden, The Netherlands, 1992; pp 537–538.

(18) Tamkun, M. M.; Knoth, E. M.; Walbridge, J. A. Kroemer, H.; Roden, D. M.; Glover, D. M. *FASEB J.* 1991, *5*, 331.

(19) (a) For a review of earlier work, see: Bodanszky, M.; Bednarek, M. A. *J. Prot. Chem.* 1989, *8*, 461. (b) Carpino, L. A.; Chao, H.-G.; Beyermann, M.; Bienert, M. *J. Org. Chem.* 1991, *56*, 2635.

proceeded smoothly as judged by a quantitative ninhydrin test.[20] After completion of the synthesis the peptidyl resin samples were treated with TFA/EDT/phenol/thioanisole (90:5:3:2) solution leading to the isolation of crude products in greater than 90% yields. Purification by preparative HPLC furnished the homogeneous products in overall isolated yields of 40–43% (preparative HPLC purification was carried out according to the conditions given in the General section). The identity of these two purified peptides was further established by HPLC, AAA, and MS/FAB.

Conclusion. A novel silicon-based linkage agent, SAL linker, was synthesized and characterized. The benefits of using the SAL linker have been clearly demonstrated particularly with respect to tryptophan-containing peptides. Furthermore SAL linker is also expected to be a useful linkage agent for the generalized production of C-terminal peptide amides using the Fmoc/*tert*-butyl strategy. Development of silicon based linkage agents for the synthesis of C-terminal peptide carboxylic acids has also been completed, and that work will be the subject of a future publication.

Experimental Section

General. 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 obtained on an instrument operating at 300 MHz either using tetramethylsilane as an internal standard or standardized relative to the residual proton in the deuterated solvents. Mass spectra were obtained on either CI, FAB, or ion spray instrumentation. Hydrolysis of peptides for amino acid composition analysis was performed according to the procedure of Liu and Boykins.[21] Amino acid analysis was carried out using the Pico Tag method.[22] TLC was performed on precoated silica gel 60 F254 plates. The following solvents were used for development: A, EtOAc/hexane (3:2); B, $CHCl_3$/MeOH/HOAc (9:1:0.1); C, $CHCl_3$/MeOH/HOAc (8:2:0.1). Compounds on TLC plates were visualized with UV light, iodine, and 2% ninhydrin in EtOH. Analytical HPLC was performed using a YMC ODS-AQ column, 4 mm × 50 mm, 3-μm particle (Morris Plains, NJ), and a commercial pumping system with high-pressure mixing and 0.01-mL pump head volume. 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, a Waters (Milford, MA) PrepPak (C18, 25 × 100 mm, 100-Å pore) column was eluted with the same gradient over the course of 16 min at 20.0 mL/min (230-nm detection).

DIEA and piperidine were purchased from Fluka (Buchs, Switzerland) and were used as supplied. 4-(Benzyloxy)benzaldehyde, palladium on active carbon, benzyl 2-bromoacetate, [(trimethylsilyl)methyl]lithium, and Cbz-OSu were purchased from Aldrich (Milwaukee, WI) and used without further purification. Fmoc-OSu was purchased from Bachem California and used without further purification. Fmoc-PAL polystyrene (0.27 mmol of NH_2/g), and Fmoc-Linker AM and BOP reagent were purchased from Milligen/Biosearch (Burlington, MA) and used without further purification. THF was distilled from sodium/benzophenone prior to use. 1,1,1,3,3,3-Hexamethyldisilazane was distilled from MgO prior to use. (Aminomethyl)polystyrene resin was prepared from 1% DVB cross-linked polystyrene by modification of a reported procedure to give a substitution level of 3.38 mmole of amino function/g.[23] 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 were used as supplied.

4-[1-[*N*-(9-Fluorenylmethyloxycarbonyl)amino]-2-(trimethylsilyl)ethyl]phenoxyacetic Acid (1). To a suspension of 1.34 g (5 mmol) of (aminoethyl)phenoxyacetic acid **7** in 60 mL of dioxane/10% aqueous $NaHCO_3$ solution (1:1) was added 1.85 g (5.5 mmol) of Fmoc-OSu. The resulting mixture was stirred at room temperature for 6–7 h and extracted with either three times and the aqueous solution acidified to pH 2 with 5% aqueous $KHSO_4$. The product which had precipitated was extracted into EtOAc and the organic phase washed with saturated NaCl solution. Removal of solvent and recrystallization from hexane/EtOAc gave 1.84 g (75%) of the protected amino acid as white crystals: mp 160–161 °C; ^{1}H NMR (DMSO-d_6) δ −0.11 (s, 9 H), 0.91–1.29 (m, 2 H), 4.11–4.60 (m, 3 H), 4.51–4.69 (m, 3 H), 6.80 (d, 2 H), 7.12–7.16 (m, 8 H), 7.87 (d, 2 H), 13.01 (bs, 1 H). Anal. Calcd for $C_{28}H_{31}NO_5Si$: C, 68.68; H, 6.38; N, 2.86. Found: C, 68.30; H, 6.34; N, 3.12.

4-[1-Amino-2-(trimethylsilyl)ethyl]phenyl Benzyl Ether Hydrochloride (3). To a solution of 4.6 mL (22 mmol) of hexamethyldisilizane was added 8 mL (20 mmol, 2.5 M in hexane) of n-BuLi over 5 min at room temperature. The resulting solution was stirred for 30 min and cooled to 0 °C. To the solution was added 4.14 g (20 mmol) of 4-(benzyloxy)benzaldehyde (**2**) in 30 mL of THF dropwise. After addition was complete, the resulting solution was stirred for an additional 30 min, and 22 mL (22 mmole, 1.0 M in pentane) of [(trimethylsilyl)methyl]lithium was added over 10 min. The resulting mixture was stirred at 0 °C for 2 h and at room temperature for 30 min and quenched with saturated aqueous NH_4Cl solution. The organic layer was separated and the aqueous phase extracted with 50 mL of EtOAc. The combined organic phase was washed with 10% $NaHCO_3$ and brine. After workup there was obtained a yellow oil which was dissolved in 50 mL of ether saturated with anhydrous HCl. The ether solution was kept at 4 °C overnight, and the amine hydrochloride salt which had precipitated was collected, washed with ether, and dried. Recrystallization from H_2O yielded 1.48 g (49.5%) of the title compound: mp 216–217 °C; ^{1}H NMR (MeOH-d_4) δ −0.20 (s, 9 H), 1.35 (dd, 1 H), 1.57 (t, 1 H), 4.36 (dd, 1 H), 5.11 (s, 2 H), 7.05 (d, 2 H), 7.23–7.50 (m, 7 H). Anal. Calcd for $C_{18}H_{25}NOSi$·HCl: C, 64.35; H, 7.80; N, 4.17. Found: C, 64.08; H, 7.62; N, 4.45.

4-[1-Amino-2-(trimethylsilyl)ethyl]phenol Hydrochloride (4). A suspension of 3.00 g (10 mmol) of (aminoethyl)-phenyl benzyl ether **3** and 300 mg of 10% Pd/C in 20 mL of MeOH in a pressure bottle was charged to 50 psi with hydrogen gas. The resulting mixture was shaken overnight, the pressure was released, and catalyst was removed by filtration. After removal of solvent, the resulting white solid was washed thoroughly with ether and dried in vacuo to give 2.21 g (90%) of the (aminoethyl)phenol as the hydrochloride salt: mp 198 °C dec; ^{1}H NMR (DMSO-d_6) δ −0.24 (s, 9 H), 1.18–1.41 (m, 2 H), 4.11 (dd, 1 H), 6.81 (d, 2 H), 7.26 (d, 2 H), 7.60–8.08 (bs, 3 H), 9.71 (s, 1 H); MS/FAB (M + Na^+) 232.1144, calcd for $C_{11}H_{19}NOSiNa$ 232.1134.

4-[1-[*N*-(Benzyloxycarbonyl)amino]-2-(trimethylsilyl)-ethyl]phenol (5). To a suspension of 2.45 g (10 mmol) of (aminoethyl)phenol hydrochloride salt **4** in 40 mL of 7:1 CH_2Cl_2/DMF solution was added 1.8 mL of DIEA followed by 2.61g (10.5 mmol) of Cbz-OSu. The resulting mixture was stirred at room temperature for 5 h, 100 mL of EtOAc added, and the organic phase washed with 5% $KHSO_4$, saturated $NaHCO_3$, and brine. After removal of solvent an oil was obtained which was crystallized from $CHCl_3$/hexane to furnish 3.02 g (88%) of the desired product as white crystals: mp 132–133 °C; ^{1}H NMR ($CDCl_3$) δ −0.11 (s, 9 H), 1.10–1.37 (m, 2 H), 4.82 (m, 1 H), 4.98–5.24 (m, 3 H), 5.31 (s, 1 H), 6.75 (d, 2 H), 7.26 (d, 2 H), 7.32 (bs, 5 H); MS/FAB (M + Na^+) 366.1495, calcd for $C_{19}H_{25}NO_3SiNa$ 366.1501. Anal. Calcd for $C_{19}H_{25}NO_3Si$: C, 66.44; H, 7.34; N, 4.08. Found: C, 65.91; H, 7.33; N, 4.35.

4-[1-Amino-2-(trimethylsilyl)ethyl]phenoxyacetic Acid (7). To a suspension of 212.2 mg (8.4 mmol) of 97% NaH in 20 mL of dry DMF at 0 °C was added 2.4 g (7 mmol) of the protected

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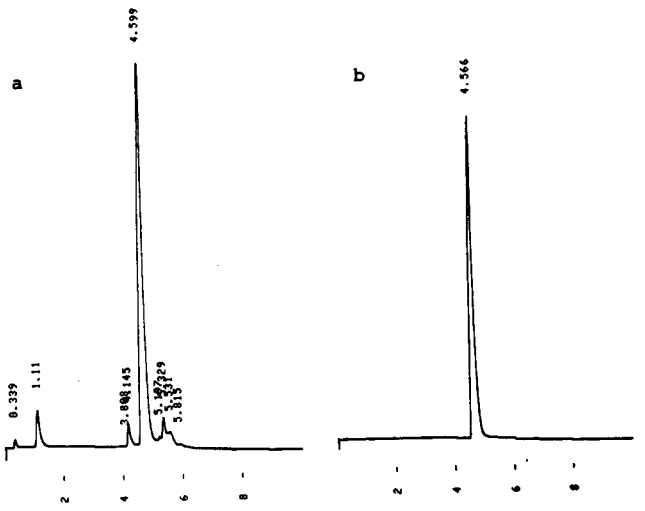

Figure 2. HPLC chromatograms of HRK1 (458-465) (10) H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-NH_2. HPLC conditions: YMC ODS-AQ column, 4 mm × 50 mm, 3-μm particle, f = 2 mL/min, 220 nm, eluent A, 0.1% (w/v) TFA in H_2O; B, 0.1% (w/v) TFA in CH_3CN containing 5% A, gradient 6–64% B in 6 min, linear: (a) crude; (b) purified.

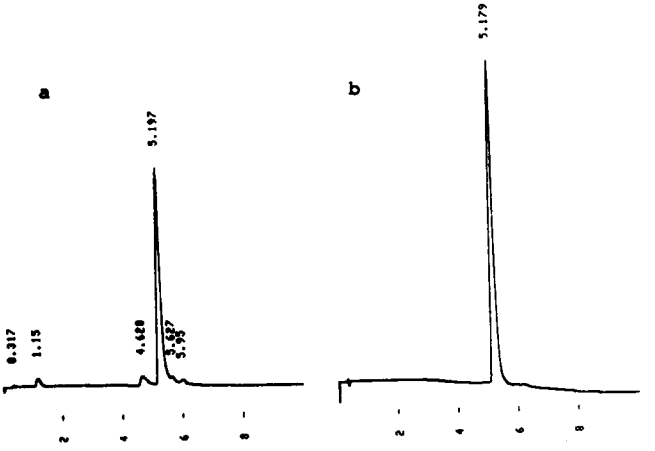

Figure 3. HPLC chromatograms of HRK1 (458-466) (11) H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-Trp-NH_2. HPLC conditions: YMC ODS-AQ column, 4 mm × 50 mm, 3-μm particle, f = 2 mL/min, 220 nm, eluent A, 0.1% (w/v) TFA in H_2O; B, 0.1% (w/v) TFA in CH_3CN containing 5% A, gradient 6–64% B in 6 min, linear: (a) crude; (b) purified.

(aminoethyl)phenol **5**. After the mixture was stirred at 0 °C for 30 min, evolution of hydrogen ceased, and 2.3 g (10 mmol) of benzyl bromoacetate in 5 mL of DMF was added. The resulting solution was stirred at room temperature for 5 h, 100 mL of ether added, and the mixture washed with aqueous NH_4Cl. After workup, the residue was dissolved in 40 mL of MeOH/HOAc (3:1) solution. To the solution at 0 °C was added 400 mg of 10% Pd/C, and the resulting mixture was hydrogenated at 50 psi overnight. The pressure was released, and the catalyst was removed by filtration and washed thoroughly with acetic acid. The filtrate was evaporated to dryness and the residue triturated with acetonitrile followed by ether to give a white solid which was collected and dried in vacuo to furnish 1.12 g (60%) of the free amino acid as a white solid: mp 195 °C dec; ^1H NMR (HOAc-d_4) δ −0.22 (s, 9 H), 1.33–1.68 (m, 2 H), 4.50 (d, 1 H), 4.73 (s, 2 H), 6.96 (d, 2 H), 7.42 (d, 2 H); MS/FAB (M + Na^+) 290.1188, calcd for $C_{13}H_{21}NO_3SiNa$ 290.1189. Anal. Calcd for $C_{13}H_{21}NO_3Si{\cdot}0.78\,H_2O$: C, 55.46; H, 8.08; N, 4.98. Found: C, 55.29; H, 7.66; N, 5.18.

Procedure for Attachment of Fmoc-Protected SAL Linker 1 to (Aminomethyl)polystyrene Resin. (Aminomethyl)-polystyrene resin (3.38 mmole amino function/g; 2 g) was washed with DMF (2 × 30 mL), 5% DIEA/DMF solution (2 × 20 mL), and DMF (3 × 20 mL). To the resin was added 3 mL of DMF solution containing 1.65 g (3.38 mmol, 0.5 equiv) of the Fmoc-SAL linker, 1.6 g (3.6 mmol) of BOP reagent, and 0.63 mL (3.6 mmol) of DIEA. After the mixture stood at room temperature for 15 min, an additional 2–4 mL of DMF was added and the resulting mixture left standing for 2–3 h, drained, and washed with DMF (3 × 25 mL). Unreacted amino groups were capped by reaction with 2 mL of acetic anhydride and 2 mL of pyridine in a minimum amount of DMF at room temperature for 30 min, after which the ninhydrin test of a resin sample was negative. The reagent was drained and the resin washed with DMF (3 × 25 mL), MeOH (2 × 20 mL), and ether (3 × 20 mL). After being dried in vacuo, the resin was subjected to UV analysis and found to have a substitution level of 0.6 mmol of Fmoc/g. The degree of substitution was determined on a weighted sample of resin (2–5 mg), which was treated with 20% piperidine in DMF for 15 min, and the solution was subjected to UV analysis using the fluorene chromophore of the dibenzofulvene–piperidine adduct at 301 nm (ϵ = 7800 M^{-1} cm^{-1}).

RHK1 458-465 (H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-NH_2) (10). The synthesis was carried out manually using 0.2 mmol (317 mg; 0.63 mmol Fmoc/g substitution level) of the Fmoc-SAL-(aminomethyl)polystyrene support. The peptide chain was assembled using 4 equiv of preformed pentafluorophenyl esters, except for Ser where the corresponding Dhbt ester was used, in the presence of equimolar HOBt/DIEA mixture. In general, the coupling reactions required 25–45 min to reach a negative ninhydrin test end point. For deprotection 20% piperidine in DMF was used (10 min). After completion of the synthesis the resin sample was treated with 15 mL of TFA/EDT/phenol/thioanisole solution (90:5:3:2) at room temperature for 1 h. The solution was collected by filtration and the resin washed thoroughly with 5 mL of the cleavage solution. The combined mixture was allowed to stand for an additional 30 min, after which 200 mL of anhydrous ether was added and the resulting mixture kept at 4 °C for 2 h. The peptide which had precipitated was collected, washed thoroughly with ether, and dried in vacuo to furnish 210 mg of the crude product as a TFA salt. The crude peptide was analyzed by reversed-phase HPLC which showed the purity to be greater than 90%. The crude product was further purified by preparative HPLC to furnish 95 mg (43%; calculated as mono TFA salt) of the homogenous peptide as a white solid: MS/FAB (M + H^+) 921, calcd 920 (M); amino acid analysis, Asp 1.10 (1), Ala 0.93 (1), Pro 1.05 (1), Ile 0.96 (1), Phe 0.96 (1), Ser 1.24 (2). For the HPLC traces of the crude and purified peptides, see Figure 2.

RHK1 458-466 (H-Ser-Ser-Ile-Pro-Asp-Ala-Phe-Trp-Trp-NH2) (11). The synthesis was carried out manually according to the protocol as previously described for RHK1 (458-465) using 0.2 mmol (317 mg; 0.63 mmol substitution level) of the Fmoc-SAL-(aminomethyl)polystyrene support, except the coupling reactions were conducted using the free Fmoc amino acids/BOP/DIEA. After cleavage of the peptidyl resin sample with TFA/EDT/phenol/thioanisole (90:5:3:2) solution, 240 mg of the crude product was obtained as the mono TFA salt. The crude peptide was analyzed by reversed-phase HPLC which showed the purity to be greater than 88%. After preparative HPLC purification there was obtained 97 mg (40%) of the homogenous peptide as a white solid: MS/FAB (M + H^+) 1107.4, calcd 1106.4 (M); amino acid analysis, Asp 1.00 (1), Ala 0.94 (1), Pro 1.15 (1), Ile 1.03 (1), Phe 0.87 (1), Ser 1.20 (2). For the HPLC traces of the crude and purified peptides, see Figure 3.

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