Solid-Phase Total Synthesis of Trunkamide A[1]

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Marine organisms are a rich source of novel, biologically active compounds. Herein, the solid-phase total synthesis of trunkamide A, currently in preclinical trials, is presented. Trunkamide A contains a thiazoline heterocycle and two residues of Ser and Thr with the hydroxy function modified as reverse prenyl (rPr). Cornerstones of the synthesis are as follows: (i) solid-phase peptide chain elongation using a quasi-orthogonal protecting scheme with *tert*-butyl and fluorenyl based groups, on a chlorotrityl resin; (ii) concourse of HOAt-based coupling reagents; and (iii) cyclizations in solution. Furthermore, the following synthetic steps are discussed: (i) preparation of the reverse prenyl derivatives of Ser and Thr; (ii) introduction of precursor of thiazoline as a protected amino thionoacid derivative; and (iii) formation of the thiazoline ring with DAST. All these features make this strategy particularly suitable for the large-scale synthesis of trunkamide A and other peptides containing the same motifs.

Introduction

Marine organisms are increasingly recognized as a useful source of compounds of biomedical interest with novel pharmaceutical properties.[2] Thus, for instance, new candidates for cancer therapy include the following compounds: ecteinascidine-743, which was isolated from the tunicate *Ecteinascidia turbinata*,[3] aplidine, also isolated from a tunicate (*Aplidium albicans*),[4] and kahalalide F, isolated from the Sacoglossan mollusc *Elysia rufescens* and the green alga *Bryopsis* sp.[5] These compounds are presently in phase III, II, and I clinical trials, respectively.

In 1996, Bowden and co-workers[6] isolated trunkamide A from the colonial ascidian *Lissoclinum* sp., which was obtained from the Great Barrier Reef, Australia. Trunkamida A has been initially selected by the National Cancer Institute (NCI) for further testing due to a good COMPARE correlation analysis and specificity against UO-31 renal cell line, that is an multidrug resistant (MDR) line. This could suggest a potential novel mechanism of action and a possible non-MDR activity, as well. Trunkamide A is a cyclic heptapeptide that incorporates motifs that are common to other members of the Patellin family, which are also isolated from ascidians. Thus, trunkamide A contains hydroxy side-chain amino acids with the hydroxy function modified as dimethylallyl ethers [reverse prenyl, (rPr)] and a thiazoline heterocycle. Initially, Bowden and co-workers assigned the L-configuration to the seven stereogenic carbons present in the macrocyclic ring (structure **1a**). More recently, and when the work described here was close to completion, Wipf and Uto[7] were the first to demonstrate that the initial assignment was erroneous and, later, that the stereocenter exocyclic to the thiazoline ring has a D-configuration (structure **1b**).[8] Furthermore, during the preparation of this manuscript, McKeever and Pattenden have published a solution synthesis of trunkamide A.[9]

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(1) Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC−IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983. The following additional abbreviations are used: ACH, α-cyano-4-hydroxycinnamic acid; CI, chemical ionization; Cl-TrtCl-resin, 2-chlorotrityl chloride resin; DAST, (diethylamino)sulfur trifluoride; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DHB, 2,5-dihydroxybenzoic acid; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; ES-MS, electrospray mass spectrometry; EtOAc, ethyl acetate; FAB, fast atom bombardment; Fmoc, 9-fluorenylmethoxycarbonyl; Fmoc-Phe(S)-NBt, 1-(*N*-Fmoc-D-thionophenylalanyl)-6-nitrobenzotriazole; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HPLC, high performance liquid chromatography; HOBt, 1-hydroxybenzotriazole; HRMS, high-resolution mass spectrometry; rPr, 1,1-dimethylallyl, reverse prenyl; MALDI-TOF, matrix assisted laser desorption ionitzation-time-of-flight; MeOH, methanol; NMM, *N*-methylmorpholine; NMR, nuclear magnetic resonance; PyAOP, 7-azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; SPS, solid-phase synthesis; Tce, trichloroethanol; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl; Tzn, thiazoline; UV, ultraviolet. Amino acid symbols denote the L-configuration unless stated otherwise. All solvent ratios are volume/volume unless stated otherwise.

(2) See, for example: (a) Reviews on marine natural products: Faulkner, J. *Nat. Prod. Rep.* covered yearly since 1977. (b) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115−2134. (c) Carte, B. K. *Bioscience* **1996**, *46*, 271−286. (d) Riguera, R. *J. Mar. Biotechnol.* **1997**, *5*, 187−193.

(3) (a) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512−4515. (b) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1991**, *56*, 1676.

(4) (a) Rinehart, K. L.; Bertelloni, L. *Chem. Abstr.* **1991**, *115*, 248086q. (b) Schmitz, F. J.; Yasumumoto, T. *J. Nat. Prod.* **1991**, *54*, 1469−1490.

(5) Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 5825−5826.

(6) Carroll, A. R.; Coll, J. C.; Bourne, D. J.; McLeod, J. K.; Zabriskie, T. M.; Ireland, C. M.; Bowden, B. F. *Aust. J. Chem.* **1996**, *49*, 659−667.

(7) Wipf, P.; Uto, Y. *Tetrahedron Lett.* **1999**, *40*, 5165−5169.

(8) Wipf, P.; Uto, Y. *J. Org. Chem.* **2000**, *65*, 1037−1049.

(9) McKeever, B.; Pattenden, G. *Tetrahedron Lett.* **2001**, *42*, 2573−2577.

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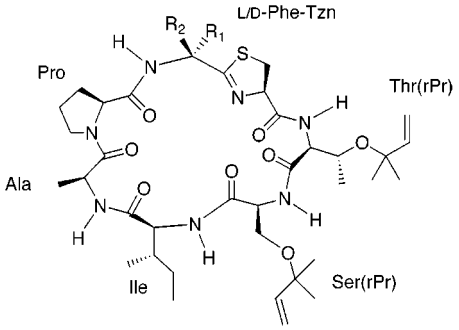
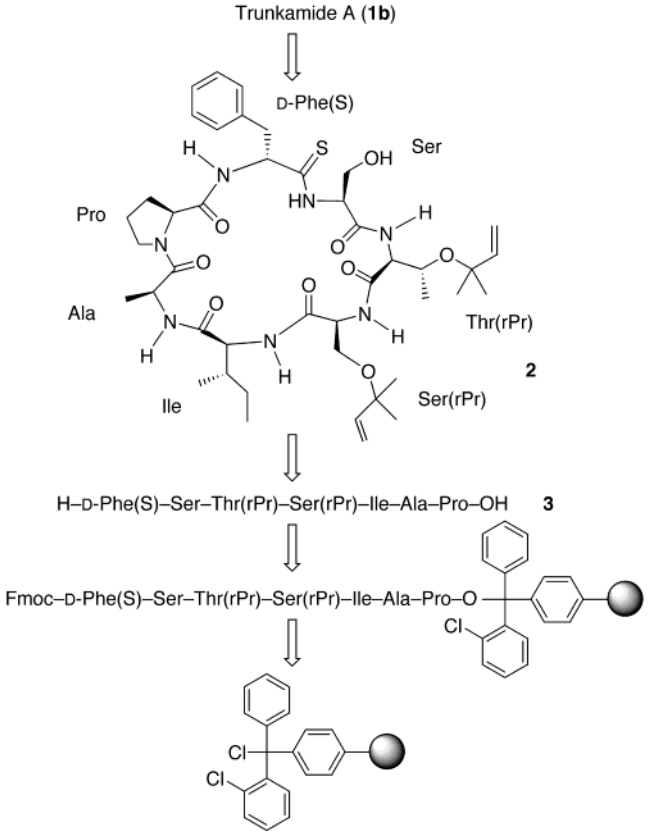


R_1 = H; R_2 = CH_2–C_6H_5 (**1a**)
R_1 = CH_2–C_6H_5; R_2 = H (**1b**)

Results and Discussion

With the aim of preparing a sufficient quantity of trunkamide A (**1b**) to carry out a complete study of its biological activity, as well as a conformational study, an efficient synthesis was developed. The retrosynthetic analysis (Scheme 1) shows the cornerstones of this approach: (i) the use of the solid-phase strategy for the elongation of the peptide chain,[10] where the molecule under construction is bound to an insoluble support during all synthetic operations. The use of this technique means that excess reagents and soluble byproducts can be removed simply by washing the molecule-resin with suitable solvents. Large excesses of the soluble reagents can, therefore, be used in order to drive the reactions to completion in relatively short periods of time, thus avoiding racemization (where applicable) and other secondary reactions; (ii) the use of a fluorenylmethyloxycarbonyl (Fmoc) base strategy,[10] because the high acid lability of the reverse prenyl ether prevents the use of acid-labile protecting groups; (iii) the use of the super-acid-labile chlorotrityl chloride resin (ClTrt-Cl-resin),[11] which allows the cleavage of the peptide under extremely mild acidic conditions, such as mixtures of hexafluoro2-propanol (HFIP) and CH_2Cl_2;[12] (iv) macrocyclization through the formation of the peptide bond between the amino function of the thiophenylalanyl residue and the carboxylic acid function of the Pro in the penultimate step. The choice of this cyclization point was based on a number of desirable features. For example, the benzyl group in the α-postion with respect to the amino function is rather unhindered in comparison with *β*-branched residues and/or reverse prenyl-protected hydroxy functions. Likewise, the carboxylic acid function forms part of the Pro and therefore cannot give an oxazolone during the activation step, which is one of the mechanisms through which the epimerization occurs.[13] Furthermore, the protected amino thionoacid is incorporated in the last step through a very mild activation process, which allows the presence of the free hydroxyl function of the Ser; and (v) formation of the thiazoline ring in the last step through the exposure of a *β*-hydroxy thiopeptide to an activating reagent of the hydroxy function. If the thiazoline ring is formed in an earlier step of the synthetic process, the exposure of the ring to further treatments could cause the epimerization of the two stereocenters of the Phe-Tzn moiety.[14]

Scheme 1



To fulfill the requirements of Scheme 1, Fmoc-Ser/Thr-(rPr)-OH and an activated species of the Fmoc-D-thiophenylalanine acid [Fmoc-D-Phe(S)-OH] were synthesized. The reverse-prenylated derivatives were prepared according to Scheme 2 from the commercially available Fmoc-Ser/Thr(*t*Bu)-OH (**8**). Thus, the following steps were undertaken: (i) orthogonal protection of the carboxyl group under the form of its trichloroethyl ester by reaction with the appropriate alcohol, DCC, and DMAP; (ii) removal of the *tert*-butyl groups with TFA-H_2O (19:1); (iii) formation of the 1,1-dimethylpropionyl ether by reaction with the corresponding trichloroacetimidate, which was prepared by a slightly modified version of the protocol described by Armstrong and co-workers;[15] (iv) partial reduction of the triple bond to the double bond by catalytic hydrogenation in the presence of Pd/C and quinoline;[16] and (v) removal of the trichloroethyl ester with Zn and NH_4OAc. The preparation of this propionyl

(10) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton, FL, 1997.

(11) Barlos, K.; Gatos, D.; Schäfer, W. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 590−593.

(12) Bollhagen, R.; Schmiedberger, M.; Barlos, K.; Grell, E. *J. Chem. Soc., Chem. Commun.* **1994**, 2559−2560.

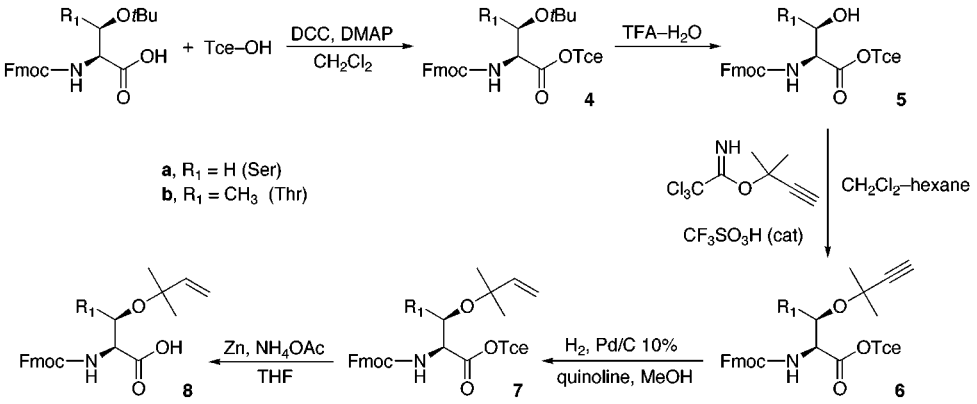
(13) Albericio, F.; Carpino. L. A. In *Methods in Enzymology;* Fields, G. B., Ed.; Academic Press: New York, 1997; pp 104−126.

(14) Alternatively, the synthesis of the linear sequence of the ^{t}Bu analogue of Trunkamide A was attempted in the solid phase by incorporation of the Fmoc-Phe-Tzn-OH (the configuration of the stereocenter of Phe corresponded to that originally described by Bowden, ref 6), which was synthesized according to a published method (Lee, J.; Griffin, J. H.; Nicas, T. I. *J. Org. Chem.* **1996**, *61*, 3983−3986), onto the H−Thr(^{t}Bu)-Ser(^{t}Bu)-Ile-Ala-Pro-ClTrt-resin. The analysis by HPLC of the cleaved material showed four major peaks with a mass of (M + 18). This could indicate the opening of the thiazoline ring due to the acid treatment and the formation of the four diastereomers due to the epimerization of the two stereocenters during the coupling reaction and/or cleavage from the solid support.

(15) Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M. *Tetrahedron Lett.* **1988**, *29*, 2483−2486.

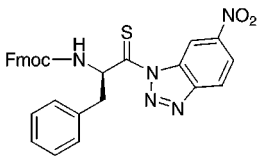
(16) The use of the propionyl derivative was also reported by Wipf and Venkatraman for the preparation of reverse prenylamines. Wipf, P.; Venkatraman, S. *J. Org. Chem.* **1996**, *61*, 6517−6522.

Scheme 2



ether and its subsequent reduction to the allyl derivative avoids the lack of regioselectivity encountered with the use of 1,1-dimethylallyl derivatives.[17] This five-step scheme allowed the preparation of these derivatives with an overall, nonoptimized yield of 28% for Ser and 25% for Thr.

For the activation of the thio acid, the 1-(*N*-Fmoc-D-thionophenylalanyl)-6-nitrobenzotriazole [Fmoc-D-Phe(S)-NBt] (**9**) derivative was used.[18] This kind of derivative, which had been previously used only in the Boc form in solution, is perfectly stable, can be stored, and is compatible with the solid-phase approach, as demonstrated for first time in the present work.



Fmoc-D-Phe(S)-NBt (**9**)

The linear sequence was synthesized on the ClTrt-Cl-resin.[11] The partial incorporation of Fmoc-Pro-OH (0.5 equiv) was performed in the presence of DIEA (2 equiv).[19,20] The incorporation of the first six protected amino acids was carried out using DIPCDI as the coupling reagent. The reverse-prenylated derivatives were incorporated with an excess of 1.7 equiv. The last Ser unit was incorporated with an unprotected hydroxy side chain. Finally, the Fmoc-D-Phe(S)-NBt coupled very smoothly to give a negative ninhydrin test after 90 min.[21]

Cleavage of the reverse-prenylated peptide was carried out very smoothly with HFIP−CH_2Cl_2 (1:4) and gave a yield of 51%. HPLC analysis of the crude product showed a purity of 63%.

The macrocyclization was carried out with PyAOP/DIEA.[22] PyAOP is a phosphonium salt based on HOAt, which reduces the risk of racemization[23] and does not lead to the termination of the peptide often associated with the corresponding uronium/aminium salts.[24]

The formation of the thiazoline ring was performed in a single step by exposure of the β-hydroxy thiopeptide to DAST.[25,26] This cyclization was also attempted by the use of other methods such as Burgess[27,28] or Mitsunobu[29] reactions. In both cases, although traces of product were obtained, the quality of the product was clearly inferior to that obtained with DAST. The reaction mixture was not worked-up and the crude product was purified by semipreparative HPLC to give Trunkamide A (**1b**) (overall yield of 5%),[30] which showed an excellent purity by HPLC (Figure 1), was characterized by MS and ^{1}H NMR spectroscopy (600 MHz) (Table 2). The resulting data confirmed the configuration proposed by Wipf.[8]

In conclusion, a total solid-phase synthesis of trunkamide A (**1b**) is reported. The incorporation of the modified residues Ser, Thr, and Phe into the peptidic chain avoided further manipulation of these residues in subsequent steps of the synthesis. This process takes advantage of the benefits of the solid-phase approach,

(17) Initially, the introduction of the allyl moiety was attempted directly from 1,1-dimethylallyl derivatives. Thus, reaction of Fmoc-Ser/Thr-OTce with 1,1-dimethylallyl alcohol in the presence of acids such as citric, phosphoric, sulfuric, or trifluoroacetic led in all cases to a mixture of the target compound and its 3,3-dimethylallyl isomer. Furthermore, the reaction with 1,1-dimethylallyl trichloroacetimidate led regioselectively to the formation of the undesired isomer and the attempt at displacement of the mesyl group from the alkoxide led to the dehydroalanine derivative. Finally, the best yields were obtained by using 3,3-dimethylallyl alcohol in the presence of 85% aqueous H_3PO_4 (3.3 equiv) for 4 h at 35−40 °C in a sealed tube. The two isomers were purified by silica gel chromatography, followed by preparative reverse phase HPLC. Thus, although the two target compounds were obtained, the low yields obtained make this method unsuitable for their large scale preparation.

(18) Shalaby, M. A.; Grote, C. W.; Rapoport, H. *J. Org. Chem.* **1996**, *61*, 9045−9048.

(19) The use of ClTrt-Cl-resins of high loading (>0.5 mmol/g) for the preparation of hydrophobic peptides such as Kahalalide F usually leads to impure crude peptide. Chiva, C.; Vilaseca, M.; Giralt, E.; Albericio, F. *J. Pept. Sci.* **1999**, *5*, 131−140.

(20) Unreacted reactive sites were capped with MeOH-DIEA.

(21) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

(22) Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853−4856.

(23) Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279−2283.

(24) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678−9683.

(25) (a) Halasz, S. P.; Glemser, O. *Chem. Ber.* **1970**, *103*, 594−602. (b) Lafargue, P.; Guenot, P.; Lellouche, J. P. *Heterocycles* **1995**, *41*, 947−958.

(26) DAST has been used by Wipf and co-workers for the preparation of the thiazoline from a β-hydroxy thiopeptide in an oxazoline → thiazoline sequence strategy; refs 7 and 8.

(27) (a) Atkins, G. M.; Burgess, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 4744−4745. (b) Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26−31.

(28) Burgess' reagent has been used by Wipf and co-workers. (Wipf, P.; Venkatraman, S. *Synlett* **1997**, 1−10) and Pattenden and co-workers (Boden, C. D. J.; Pattenden, G. *Tetrahedron Lett.* **1995**, *36*, 6153−6156) for the preparation of thiazolines.

(29) Galéotti, N.; Montagne, C.; Poncet, J.; Jouin, P. *Tetrahedron Lett.* **1992**, *33*, 2807−2810.

(30) If the crude reaction mixture is washed with dilute aqueous solutions of $NaHCO_3$, conversion (40−70%) to a compound with a mass ion (M + 16) took place. An NMR study of this compound, which shows chromatographic behavior very similar to trunkamide A, only shows different chemical shifts for the proton belonging to the D-Phe-Tzn, indicating that an oxidation at the sulfur atom could have taken place.

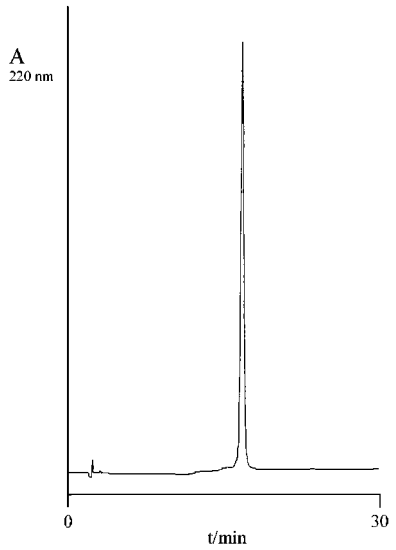


Figure 1. HPLC chromatograms of pure trunkamide A (**1b**). A reversed-phase C-18 column was used for the analysis with elution by a linear gradient over 30 min of 0.036% TFA in ACN and 0.045% TFA in H_2O from 45:55 to 70:30, flow rate 1.0 mL min^{-1}.

which allows rapid incorporation of the residues. Finally, an efficient method for the preparation of reverse-prenlylated derivatives of Ser and Thr has been developed and consists of five robust steps from commercially available compounds. All these features make this strategy particularly suitable for the large scale synthesis of trunkamide A (**1b**) and other peptides containing the same motifs.

Experimental Section

General Procedures. Cl-TrtCl-resin, protected Fmoc-amino acid derivatives, HOBt, and PyAOP were obtained from PerSeptive Biosystems (Framingham, MA), Bachem (Bubendorf, Switzerland), Albatross (Montreal, Canada), and Nova-Biochem (Läufelfingen, Switzerland). DIEA, DIPCDI, piperidine, Fmoc-Cl, NMM, isobutyl chloroformate, 4-nitro-1,2-phenylenediamine, P_4S_{10}, 2-methyl-3-butyn-2-ol, trichloroacetonitrile, TFA, DMAP, DCC, HFIP, DAST, Pd−C 10%, CF_3SO_3H, 2,2,2-trichloroethanol, and quinoline were obtained from Aldrich (Milwaukee, WI). DMF, CH_2Cl_2, $CHCl_3$, and EtOAc were obtained from SDS (Peypin, France). Acetonitrile (HPLC grade) and THF were obtained from Scharlau (Barcelona, Spain). Hexane, Et_2O, and methanol were obtained from Panreac (Moncada i Reixac, Barcelona). All commercial reagents and solvents were used as received with the exception of DMF and CH_2Cl_2, which were bubbled with nitrogen to remove volatile contaminants (DMF) and stored over activated 4A molecular sieves (Merck, Darmstadt, Germany) (DMF) or $CaCl_2$ (CH_2Cl_2). Et_2O was stored over Na.

Solution reactions were performed in round-bottomed flasks. Organic solvent extracts were dried over anhydrous $MgSO_4$, followed by solvent removal under reduced pressure and at <40 °C.

Solid-phase syntheses were carried out in polypropylene syringes (10/20 mL) fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Removal of the Fmoc group was carried out with piperidine-DMF (2:8, v/v) (2 × 1 min, 2 × 20 min). Washings between deprotection, coupling, and final deprotection steps were carried out with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) using 10 mL solvent/g resin for each wash. Peptide synthesis transformations and washes were performed at 25 °C.

HPLC columns (Nucleosil C_{18} reversed-phase column, 4.6 × 250 mm, 10 mm) were obtained from Scharlau (Barcelona, Spain). Analytical HPLC was carried out on a Shimadzu instrument comprising two solvent delivery pumps (model LC-6A), automatic injector (model SIL-6B), variable wavelength detector (model SPD-6A), system controller (model SCL-6B) and plotter (model C-R6A). UV detection was performed at 220 nm, and linear gradients of CH_3CN (+0.036% TFA) into H_2O (+0.045% TFA) were run at 1.0 mL/min flow rate from the following: (condition A) 0:1 to 1:0 over 30 min; (condition B) 1:1 to 10:0 over 30 min. Flash chromatography was carried out using silica gel 60 A C C 50−70 mm SDS (Peypin, France).

Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained using a Nicolet 510 FT-IR spectrometer. MALDI-TOF- and ES(+)-MS analyses of peptide samples were performed in a PerSeptive Biosystems Voyager DE RP, using ACH or DHB matrixes, and in a Micromass VG-quattro spectrometer. CI-MS analyses of amino acid derivatives were performed using a Hewlett-Packard HP-5988A spectrometer. ^1H NMR (500 MHz, 200 MHz) and ^{13}C NMR (50 MHz) spectroscopy was performed on a Bruker DMX-500 (11.7 T) and Varian Gemini 200 (4.7 T). Chemical shifts (d) are expressed in parts per million downfield from TMS. Coupling constants are expressed in Hertz.

***N*-Boc-D-phenylalanine 2-amino-5-nitroanilide (10).** Boc-D-Phe-OH (6 g, 22.6 mmol) was dissolved in THF (180 mL), and NMM (4.98 mL, 45.2 mmol, 2 equiv) was added at −20 °C under N_2, followed by the dropwise addition of isobutyl chloroformate (2.95 mL, 22.6 mmol, 1 equiv). The reaction mixture was stirred for 10 min, a solution of 4-nitro-1,2-phenylenediamine (3.46 g, 22.6 mmol, 1 equiv) in THF (60 mL) was added, and the mixture stirred for a further 2 h at −20 °C and 16 h at 23 °C. The precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (200 mL) and extracted with aqueous solutions of 1 M NaH_2PO_4 (2 × 100 mL), saturated brine (2 × 100 mL), saturated $NaHCO_3$ (2 × 100 mL), and saturated NaCl (2 × 100 mL), dried over Na_2SO_4, and evaporated to dryness to give the title compound (8.77 g, 97%) as a yellow solid: analytical HPLC (t_R 22.1 min, condition A; t_R 7.4 min, condition B); $[\alpha]_D$ −43 (*c* 0.01, DMSO); IR (KCl) ν_{max} 3442, 1733, 1507, 1387, 1152 cm^{-1}; CI-MS calcd for $C_{20}H_{24}N_4O_5$ 400, found 401 [$(M + H)^+$, 46], 418 [$(M + NH_4)^+$, 100]; HRMS (FAB) ES-MS calcd 401.1825, found 401.1812; ^1H NMR (300 MHz, DMSO-d_6) δ 9.37 (1H, s, NH-ar), 7.94 (1H, s, ar), 7.85 (1H, dd, J = 9.0 Hz, 1.2 Hz, ar), 7.30−7.20 (6H, m, 5H ar, NH Phe), 6.72 (1H, d, J = 9.0 Hz, ar), 6.35 (2H, s, NH_2), 4.35−4.25 (1H, m, α-CH Phe), 3.10−3.00 (1H, m, β-CH_2 Phe), 2.90−2.82 (1H, m, β-CH_2 Phe), 1.33 (9H, s, $3CH_3$ Boc); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8 (CO Phe), 156.1 (CO Boc), 150.3 (C ar), 138.2 (C ar), 135.8 (C ar), 129.7 (CH ar), 128.5 (CH ar), 126.8 (CH ar), 123.8 (CH ar), 122.8 (CH ar), 121.4 (C ar), 113.9 (CH ar), 78.8 (C Boc), 56.8 (α-C Phe), 37.4 (β-C Phe), 28.5 ($3CH_3$ Boc).

***N*-Boc-D-phenylalanine2-amino-5-nitrothioanilide(11).** P_4S_{10} (4.72 g, 10.6 mmol, 0.5 equiv) was added to a suspenssion of Na_2CO_3 (1.13 g, 10.6 mmol, 0.5 equiv) in THF (350 mL) at 23 °C under a flow of N_2. After 1 h the mixture was cooled to 0 °C and *N*-Boc-D-phenylalanine 2-amino-5-nitroanilide (**10**) (8.50 g, 21.2 mmol) was added and the mixture stirred for 30 min at 0 °C and 2.5 h at 23 °C. The mixture was filtered through Celite and the filtrate evaporated to dryness. The residue was dissolved in EtOAc−hexane (2:1, 200 mL), extracted with 5% aqueous $NaHCO_3$ (3 × 10 mL) and the aqueous phase extracted with EtOAc−hexane (2:1, 100 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated to dryness to give *N*-Boc-D-phenylalanine 2-amino-5-nitrothioanilide (8.40 g, 95%) as a yellow-brown solid: analytical HPLC (t_R 25.4 min, condition A; t_R 10.1 min, condition B); $[\alpha]_D$ − 80 (*c* 0.01, $CHCl_3$DMF, 97:3); IR (KCl) ν_{max} 3357, 1677, 1509, 1304, 1023 cm^{-1}; CI-MS calcd for $C_{20}H_{24}N_4O_4S$ 416, found 417 [$(M + H)^+$, 46], 434 [$(M + NH_4)^+$, 20]; HRMS (FAB) ES-MS calcd 417.1596, found 417.1587; ^1H NMR (300 MHz, $CDCl_3$) δ 9.53 (1H, bs, NH-ar), 7.93 (1H, dd, J = 9.2, 2.8 Hz, ar), 7.62 (1H, s, ar), 7.40−7.20 (5H, m, ar), 6.62 (1H, d, J = 9.2 Hz, ar), 5.61 (1H, d, J = 7.8 Hz, NH Phe), 4.85−4.75 (1H, m, α-CH Phe), 4.62 (2H, bs, NH_2), 3.25−3.10 (2H, m, β-CH_2 Phe), 1.33 (9H, s, $3CH_3$ Boc); ^{13}C NMR (75 MHz, $CDCl_3$)

δ 205.6 (CS Phe), 156.2 (CO Boc), 148.8 (C ar), 137.7 (C ar), 135.9 (C ar), 129.3 (CH ar), 128.8 (CH ar), 127.4 (CH ar), 125.4 (CH ar), 124.8 (CH ar), 121.9 (C ar), 114.6 (CH ar), 81.1 (C Boc), 63.1 (α-C Phe), 41.3 (*β*-C Phe), 28.1 ($3CH_3$ Boc).

***N*-Fmoc-D-phenylalanine 2-amino-5-nitrothioanilide (12).** *N*-Boc-D-phenylalanine 2-amino-5-nitrothioanilide (**11**) (8.40 g, 20.2 mmol) was dissolved in TFA−CH_2Cl_2 (45:55, 50 mL), and the solution was stirred for 20 min. The solvent was evaporated, Et_2O was added, and the solvent was evaporated again. This procedure was repeated two more times. The residue was dissolved in 10% aqueous Na_2CO_3dioxane (2:1, 250 mL), Fmoc-Cl (5.23 g, 20.2 mmol, 1 equiv) in dioxane (65 mL) was added, and the mixture was stirred for 1 h at 0 °C. The dioxane was removed by evaporation under reduced pressure and the resulting solid was dissolved in EtOAc (100 mL). The solution was cooled to 0 °C, and the pH was adjusted to <3 with 5% aqueous $KHSO_4$. The aqueous phase was extracted with EtOAc (2 × 75 mL). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated to dryness to give *N*-Fmoc-D-phenylalanine 2-amino-5-nitrothioanilide (8.80 g, 80%) as an orange-yellow solid: analytical HPLC (t_R 27.3 min, condition A; t_R 15.1 min, condition B); $[\alpha]_D$ −14.3 (*c* 0.01, DMSO); IR (KCl) ν_{max} 1702, 1509, 1322, 1031 cm^{-1}; ES-MS calcd for $C_{30}H_{26}N_4O_4S$ 538.2, found 539.4 $(M + H)^+$; HRMS (FAB) ES-MS calcd 539.1753, found 539.1761; ^1H NMR (200 MHz, $CDCl_3$) δ 9.19 (1H, bs, NH-ar), 7.82 (1H, dd, J = 9.2 Hz, 1.2 Hz, ar), 7.80−7.10 (14H, m, ar), 6.43 (1H, d, J = 9.2 Hz, ar), 5.93 (1H, bs, NH Phe), 4.95−4.85 (1H, m, α-CH Phe), 4.20−4.00 (3H, m, CH Fmoc, CH_2 Fmoc), 3.30−3.10 (2H, m, *β*-CH_2 Phe); ^{13}C NMR (50 MHz, $CDCl_3$) δ 205.1 (CS Phe), 156.5 (CO Fmoc), 148.2 (C ar), 143.3 (C ar), 141.0 (C ar), 138.2 (C ar), 135.7 (C ar), 129.3 (CH ar), 128.9 (CH ar), 127.8 (CH ar), 127.5 (CH ar), 127.0 (CH ar), 125.3 (CH ar), 124.9 (CH ar), 124.7 (CH ar), 121.0 (C ar), 120.0 (CH ar), 115.6 (CH ar), 67.0 (CH_2 Fmoc), 62.9 (α-C Phe), 46.7 (CH Fmoc), 41.7 (*β*-C Phe).

1-(*N*-Fmoc-D-thionophenylalanyl)-6-nitrobenzotriazole (9). To a solution of *N*-Fmoc-D-phenylalanine 2-amino-5-nitrothioanilide (**12**) (8.80 g, 16.4 mmol) in 95% aqueous HOAc (185 mL) was added $NaNO_2$ (1.70 g, 24.6 mmol, 1.5 equiv) portionwise during 5 min at 0 °C. The mixture was stirred for 30 min at 0 °C, and during this time a precipitate formed. Cold H_2O (200 mL) was added to the mixture, and the precipitate was filtered off, washed with cold H_2O and dissolved in CH_2Cl_2 (100 mL). The organic phase was extracted with H_2O (2 × 75 mL), and the combined aqueous phases were extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with H_2O (2 × 75 mL) and dried over Na_2SO_4, and the solvent was removed to give 1-(*N*-Fmoc-D-thionophenylalanyl)-6-nitrobenzotriazole (7.70 g, 85%) as an orange-brown solid: analytical HPLC (t_R 29.7 min, condition A; t_R 19.2 min condition B); $[\alpha]_D$ −97 (*c* 0.014, $CHCl_3$); IR (KCl) ν_{max} 3309, 1696, 1537, 1348, 1254 cm^{-1}; ES-MS calcd for $C_{30}H_{23}N_5O_4S$ 549.1, found 550.2 $(M + H)^+$; HRMS (FAB) ES-MS calcd 550.1549, found 550.1569; ^1H NMR (200 MHz, $CDCl_3$) δ 9.61 (1H, s, ar), 8.41 (1H, dd, J = 8.9 Hz, 1.2 Hz, ar), 8.25 (1H, d, J = 8.9 Hz, ar), 7.80−7.10 (13H, m, ar), 6.65−6.50 (1H, m, α-CH Phe), 5.79 (1H, d, J = 9.2 Hz, NH), 4.45−4.30 (2H, m, CH_2 Fmoc), 4.20−4.10 (1H, m, CH Fmoc), 3.41 (1H, dd, J = 13.6, 5.0 Hz, *β*-CH_2 Phe), 3.08 (1H, dd, J = 13.6, 8.0 Hz, *β*-CH_2 Phe); ^{13}C NMR (50 MHz, $CDCl_3$) δ 207.9 (CS Phe), 155.3 (CO Fmoc), 149.4 (C ar), 148.8 (C ar), 143.5 (C ar), 141.1 (C ar), 135.0 (C ar), 131.5 (C ar), 129.2 (CH ar), 128.5 (CH ar), 127.7 (CH ar), 127.3 (CH ar), 127.0 (CH ar), 124.0 (CH ar), 122.2 (CH ar), 121.4 (CH ar), 119.9 (CH ar), 112.5 (CH ar), 66.9 (CH_2 Fmoc), 62.3 (α-C Phe), 47.1 (CH Fmoc), 42.6 (*β*-C Phe).

1,1-Dimethylpropionyl 2,2,2-trichloroacetimidate (13). A suspension of either KH in paraffin oil (35%, 1.17 g, 10.2 mmol, 0.1 equiv) or NaH (60%, 0.82 g, 10.2 mmol, 0.1 equiv) was washed with hexane (3 × 4 mL) under a N_2 atmosphere. The resulting hydride was suspended in anhydrous Et_2O (10 mL), and a solution of 2-methyl-3-butyne-2-ol (10 mL, 102.2 mmol, 1 equiv) in anhydrous Et_2O (14 mL) was added dropwise. The mixture was stirred for 10 min at 23 °C while the precipitate redissolved. At −5 °C and under a N_2 atmosphere, the above solution was added dropwise during 20 min to a solution of trichloroacetonitrile (10.3 mL, 102.2 mmol, 1 equiv) in anhydrous Et_2O (20 mL), and the reaction was stirred for 2 h, during which time the tempearture increased to 23 °C. The solvent was removed, MeOH−hexane (1:19, 10 mL) was added, and the mixture was vigorously stirred for 1 min. During this time, a precipitate appeared that was filtered off and washed with cold hexane. The filtrates were combined and evaporated to dryness to give 1,1-dimethylpropionyl 2,2,2-trichloroacetimidate (15.9 g, 69%): IR (film) ν_{max} 3307, 1669, 1366, 1314, 1140, 1077 cm^{-1}; CI-MS calcd for $C_7H_8NOCl_3$ 227, found 228 $[(M + H)^+$, 100], 245 $[(M + NH_4)^+$, 19]; ^1H NMR (200 MHz, $CDCl_3$) δ 8.56 (1H, bs, NH), 2.62 (1H, s, CH), 1.82 (6H, s, $2CH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 159.6 (CN), 92.0 (CCl_3), 83.7 (C), 75.2 (C), 73.2 (CH), 28.5 ($2CH_3$).

Fmoc-Ser-OTce (5a). Fmoc-Ser(^{t}Bu)-OH (1 g, 2.6 mmol) was dissolved in CH_2Cl_2 (7 mL). DMAP (0.15 g, 1.3 mmol, 0.5 equiv) and 2,2,2-trichloroethanol (Tce) (0.3 mL, 3.1 mmol, 1.2 equiv) were added, followed by a solution of DCC (0.63 g, 3.1 mmol, 1.2 equiv) in CH_2Cl_2 (2.5 mL): (all additions were performed under a N_2 atmosphere at 0 °C). The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was cooled to 0 °C, filtered and the filtrate was concentrated in vacuo. The resulting crude material was dissolved in EtOAc (7 mL) and washed with 10% aqueous citric acid (2 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo to give Fmoc-Ser(^{t}Bu)-OTce (**4a**) (1.34 g), which was used without further purification.

Fmoc-Ser(^{t}Bu)-OTce (**4a**) was dissolved in TFA−H_2O (19:1, 10 mL) and the solution was stirred for 5 h. The reaction mixture was concentrated in vacuo and the residue purified by flash chromatography (EtOAc−hexane, 3:7) to give Fmoc-Ser-OTce (**5a**) (0.83 g, 1.8 mmol, 69% overall yield for the two steps): analytical HPLC (t_R 12.2 min, condition B); $[\alpha]_D$ −5.6 (*c* 0.01, $CHCl_3$, 23 °C); IR (film) 3407, 1769, 1692, 1516, 1209, 1082 cm^{-1}; CI-MS calcd for $C_{20}H_{18}O_5NCl_3$ 457, found 475 $[(M + NH_4^+$, 100]; HRMS (FAB) ES-MS calcd 458.0329, found 458.0332; ^1H NMR (200 MHz, $CDCl_3$) δ 7.70−7.20 (8H, m, Ar), 6.05 (1H, d, J = 8.0 Hz, NH), 4.85 (1H, d, J = 12.0 Hz, CH_2 Tce), 4.67 (1H, d, J = 12.2 Hz, CH_2 Tce), 4.60−4.54 (1H, m, α-CH Ser), 4.45−4.30 (2H, m, CH_2 Fmoc), 4.20−4.15 (1H, m, CH Fmoc), 4.10−3.85 (2H, m, *β*-CH_2 Ser), 3.50 (1H, bs, OH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.0 (CO Ser), 156.2 (CO Fmoc), 143.5 (C Ar), 141.1 (C Ar), 127.6 (CH Ar), 126.9 (CH Ar), 124.9 (CH Ar), 119.8 (CH Ar), 94.2 (CCl_3 Tce), 74.4 (CH_2 Tce), 67.2 (CH_2 Fmoc), 62.6 (*β*-CH_2 Ser), 55.9 (α-CH Ser), 46.9 (CH Fmoc).

Fmoc-Ser(rPr)-OH (8a). Fmoc-Ser-OTce (**5a**) (0.83 g, 1.8 mmol) was dissolved in CH_2Cl_2−hexane (2:1, 4 mL), and 1,1-dimethylpropionyl trichloroacetimidate (**13**) (0.41 g, 1.8 mmol, 1 equiv) and CF_3SO_3H (40 μL) were added under a N_2 atmosphere. The reaction mixture was stirred for 4 days, and the same quantities of the trichloroacetimidate and CF_3SO_3H were added every 24 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo, dissolved in EtOAc (10 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), H_2O (2 × 10 mL), and brine (2 × 10 mL). The organic solution was dried ($MgSO_4$) and concentrated in vacuo to give the 1,1-dimethylpropionyl ether of Fmoc-Ser-OTce (**6a**) (0.95 g), which was used without further purification.

The 1,1-dimethylpropionyl ether of Fmoc-Ser-OTce (**6a**) was dissolved in MeOH (20 mL) and 10% Pd/C (38 mg, 4% of the crude weight) and quinoline (0.44 mL, 0.45 mL per g of crude) were added under a N_2 atmosphere. The atmosphere was changed from N_2 to H_2 and the mixture was stirred for 2 h. The reaction mixture was concentrated in vacuo and the residue purified by flash chromatography ($CHCl_3$−hexane, 8:2) to give Fmoc-Ser(rPr)-OTce (**7a**) (0.38 g, 0.72 mmol): analytical HPLC (t_R 20.7 min, condition B); $[\alpha]_D$ −26.4° (*c* 0.05, $CHCl_3$, 23 °C); IR (film) 3442, 2979, 1782, 1506, 1451 cm^{-1}; CI-MS calcd for $C_{25}H_{26}O_5NCl_3$ 525, found 526 $[(M + H)^+$, 34], 543 $[(M + NH_4)^+$, 100]; HRMS (FAB) ES-MS calcd 526.0955, found 526.0940; ^1H NMR (200 MHz, $CDCl_3$) δ 7.80−7.22 (8H, m, Ar), 5.82−5.65 (2H, m, CH ^{i}Pr, NH), 5.20−5.10 (2H, m, CH_2 ^{i}Pr), 4.87 (1H, d, J = 10.8 Hz, CH_2 Tce), 4.74 (1H, d, J = 10.8 Hz,

Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) Data for 2

residue	NH	H_α	H_β	other
Ala	5.90 (bs)	4.65 (qd, J = 7.0, 7.0 Hz)	1.23 (3H, d, J = 7.0 Hz)	
Ile	7.19 (d, J = 7.0 Hz)	4.24−4.18 (m)	2.22−2.16 (m)	1.42−1.34, 1.12−1.06 (2H, 2m, γCH_2); 0.92 (3H, d, J = 7.0 Hz, γCH_3); 0.89 (3H, t, J = 7.5 Hz, δCH_3)
Ser(rPr)	7.98 (bs)	4.73 (ABX, J = 8.0, 8.0, 3.5 Hz)	3.62−3.58 (1H, m); 3.42−3.37 (1H, m)	5.83−5.74 (1H, m, CH ^{i}Pr); 5.17−5.09 (2H, m, CH_2 rPr); 1.29 (6H, s, $2CH_3$ rPr)
Thr(rPr)	6.78 (bs)	4.36−4.44 (m)	4.35−4.32 (m)	5.83−5.74 (1H, m, CH rPr); 5.17−5.09 (2H, m, CH_2 rPr); 1.29 (6H, s, $2CH_3$ rPr); 1.11 (3H, d, J = 6.5 Hz, γCH_3)
Ser	8.83 (bs)	5.66−5.60 (m)	3.99 (1H, dd, J = 12.5, 4.0 Hz); 3.62−3.58 (1H, m)	
D-Phe(S)	6.56 (bs)	5.08−5.00 (m)	3.54−3.46 (1H, m); 3.02 (1H, dd, J = 14.0, 9.0 Hz)	7.29−7.23 (5H, m, ar)
Pro		4.18−4.14 (m)	3.84−3.77 (1H, m, δCH_2); 3.54−3.46 (1H, m, δCH_2); 2.10−2.06, 2.02−1.96, 1.92−1.76 (4H, 3m, βCH_2, γCH_2)	

CH_2 Tce), 4.70−4.60 (1H, m, α-CH Ser), 4.50−4.35 (2H, m, CH_2 Fmoc), 4.32−4.25 (1H, m, CH Fmoc), 3.90 (1H, dd, J = 8.0, 2.8 Hz, β-CH_2 Ser), 3.60 (1H, dd, J = 8.0, 2.8 Hz, β-CH_2 Ser), 1.26 (6H, s, 2 CH_3 ^{i}Pr); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.1 (CO Ser), 155.2 (CO Fmoc), 143.2 (C Ar), 142.6 (CH ^{i}Pr), 141.1 (C Ar), 127.6 (CH Ar), 126.9 (CH Ar), 125.0 (CH Ar), 119.9 (CH Ar), 114.5 (CH_2 ^{i}Pr), 94.0 (CCl_3 Tce), 75.6 (C ^{i}Pr), 74.5 (CH_2 Tce), 67.2 (CH_2 Fmoc), 62.5 (β-CH_2 Ser), 54.4 (α-CH Ser), 47.0 (CH Fmoc), 25.6 (CH_3 ^{i}Pr), 25.3 (CH_3 ^{i}Pr).

Fmoc-Ser(rPr)-OTce(**7a**) (0.38 g, 0.72 mmol) was dissolved in THF (6 mL), and Zn dust (1.56 g, 24 mmol, 33.1 equiv) and 1 M NH_4OAc (1.35 mL, 1.3 mmol, 1.87 equiv) were added under a N_2 atmosphere. The reaction mixture was stirred for 14 h, filtered, and concentrated in vacuo. The crude material was dissolved in EtOAc (10 mL), washed with 5% aqueous $KHSO_4$ (2 × 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo to give Fmoc-Ser(rPr)-OH (**8a**) (0.28 g, 0.71 mmol, 40% overall yield for three steps): analytical HPLC (t_R 11 min, condition B); $[\alpha]_D$ + 11 (*c* 0.009, $CHCl_3$, 23 °C); IR (film) 2977, 1725, 1510, 1451, 1209 cm^{-1}; CI-MS calcd for $C_{23}H_{25}O_5N$ 395, found 396 [$(M + H)^+$, 18], 413 [$(M + NH_4)^+$, 59]; HRMS (FAB) ES-MS calcd 396.1811, found 396.1814; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80−7.20 (8H, m, Ar), 5.80−5.60 (2H, m, NH, CH ^{i}Pr), 5.11 (1H, d, J = 19.4 Hz, CH_2 ^{i}Pr), 5.00 (1H, d, J = 11.6 Hz, CH_2 ^{i}Pr), 4.45−4.05 (4H, m, α-CH Ser, CH_2 Fmoc, CH Fmoc), 3.72 (1H, dd, J = 9.4, 2.6 Hz, β-CH_2 Ser), 3.48 (1H, dd, J = 9.4, 2.6 Hz, β-CH_2 Ser), 1.24 (6H, s, 2 CH_3 ^{i}Pr); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0 (CO Ser), 156.0 (CO Fmoc), 143.6 (C Ar), 142.7 (CH ^{i}Pr), 141.1 (C Ar), 127.5 (CH Ar), 126.9 (CH Ar), 125.0 (CH Ar), 119.8 (CH Ar), 114.4 (CH_2 ^{i}Pr), 75.7 (C ^{i}Pr), 67.2 (CH_2 Fmoc), 62.6 (β-CH_2 Ser), 54.3 (α-CH Ser), 47.0 (CH Fmoc), 25.5 (2 CH_3 ^{i}Pr).

Fmoc-Thr-OTce (5b). According to the procedure used for the synthesis of Fmoc-Ser-OTce (**5a**), Fmoc-Thr(*t*Bu)-OH (1 g, 2.5 mmol) provided Fmoc-Thr-OTce (**5b**) (0.81 g, 1.7 mmol, 68% overall yield for two steps): analytical HPLC (t_R 14 min, condition B); CI-MS calcd for $C_{21}H_{20}O_5NCl_3$ 472, found 473 [$(M + H)^+$, 76]; HRMS (FAB) ES-MS calcd 472.0485, found 472.0486; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80−7.20 (8H, m, Ar), 5.80 (1H, d, J = 8.8 Hz, NH), 4.90 (1H, dd, J = 12 Hz, CH_2 Tce), 4.70 (1H, dd, J = 12 Hz, CH_2 Tce), 4.57−4.40 (4H, m, α-CH Thr, CH_2 Fmoc, β-CH Thr), 4.25−4.18 (1H, m, CH Fmoc), 2.41 (1H, bs, OH), 1.27 (3H, d, J = 7 Hz, γ-CH_3 Thr); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.4 (CO Thr), 156.7 (CO Fmoc), 143.4 (C Ar), 141.0 (C Ar), 127.6 (CH Ar), 126.9 (CH Ar), 124.9 (CH Ar), 119.8 (CH Ar), 94.3 (Cl_3 Tce), 74.3 (CH_2 Tce), 67.5 (β-CH Thr), 67.2 (CH_2 Fmoc), 59.2 (α-CH Thr), 46.9 (CH Fmoc), 20.0 (γ-CH_3 Thr).

Fmoc-Thr(rPr)-OH (8b). According to the procedure used for the synthesis of Fmoc-Ser(rPr)-OTce (**7a**), Fmoc-Thr-OTce (**5b**) (0.81 g, 1.7 mmol) provided Fmoc-Thr(rPr)-OTce (**7b**) (0.34 g, 0.63 mmol): analytical HPLC (t_R 21.5 min, condition B); $[\alpha]_D$ −6.9 (*c* 0.024, $CHCl_3$, 23 °C); IR (film) 2929, 1732, 1507, 1261, 1092 cm^{-1}; ES-MS calcd for $C_{26}H_{28}O_5NCl_3$ 539.1, found *m*/*z* 540.4 $[M + H]^+$; HRMS (FAB) ES-MS calcd 540.1111, found 540.1112; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.80−7.30 (8H, m, Ar), 5.81−5.70 (1H, m, CH ^{i}Pr), 5.66 (1H, d, J = 9.5 Hz, NH), 5.2−5.12 (2H, m, CH_2 ^{i}Pr), 4.88 (1H, dd, J = 12 Hz, CH_2 Tce), 4.62 (1H, dd, J = 12 Hz, CH_2 Tce), 4.45−4.40 (3H, m, α-CH Thr, CH_2 Fmoc), 4.35−4.20 (2H, m, CH Fmoc, β-CH Thr), 1.30−1.27 (9H, m, γ-CH_3 Thr, 2 CH_3 ^{i}Pr); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.8 (CO Thr), 156.6 (CO Fmoc), 143.7 (C Ar), 143.5 (CH ^{i}Pr), 141.3 (C Ar), 127.7 (CH Ar), 127.0 (CH Ar), 125.2 (CH Ar), 120.0 (CH Ar), 114.2 (CH_2 ^{i}Pr), 94.3 (CCl_3 Tce), 76.0 (C ^{i}Pr). 75.0 (CH_2 Tce), 68.0 (β-CH Thr), 67.3 (CH_2 Fmoc), 59.8 (α-CH Thr), 47.2 (CH Fmoc), 26.6 (CH_3 ^{i}Pr), 26.1 (CH_3 ^{i}Pr), 20.7 (γ-CH_3 Thr).

According to the procedure used for the synthesis of Fmoc-Ser(rPr)-OH (**8a**), Fmoc-Thr(rPr)-OTce (**7b**) (0.34 g, 0.63 mmol) provided Fmoc-Thr(rPr)-OH (**8b**) (0.24 g, 0.6 mmol, 35% overall yield for three steps): analytical HPLC (t_R 13.4 min, condition B); ES-MS calcd for $C_{24}H_{27}O_5N$ 409.2, found *m*/*z* 410.7 $[M + H]^+$; HRMS (FAB) ES-MS calcd 410.1967, found 410.1974; $[\alpha]_D$ 18.3° (*c* 0.009, $CHCl_3$, 23 °C); IR (film) 2979, 1726, 1506, 1451, 1211 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80−7.20 (8H, m, Ar), 5.90−5.70 (2H, m, CH ^{i}Pr, NH), 5.20 (1H, d, J = 10.2 Hz, CH_2 ^{i}Pr), 5.13 (1H, d, J = 3.2 Hz, CH_2 ^{i}Pr), 4.40−4.10 (5H, m, α-CH Thr, β-CH Thr, CH_2 Fmoc, CH Fmoc), 1.31 (3H, d, J = 3.0 Hz, γ-CH_3 Thr), 1.25 (6H, s, 2 CH_3 ^{i}Pr); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6 (CO Thr), 156.3 (CO Fmoc), 143.5 (C Ar), 142.4 (CH ^{i}Pr), 141.1 (C Ar), 127.6 (CH Ar), 126.1 (CH Ar), 125.0 (CH Ar), 119.0 (CH Ar), 114.9 (CH_2 ^{i}Pr), 77.1 (C ^{i}Pr), 67.7 (β-CH Thr), 67.2 (CH_2 Fmoc), 58.9 (α-CH Thr), 47.0 (CH Fmoc), 26.6 (CH_3 ^{i}Pr), 25.6 (CH_3 ^{i}Pr), 19.1 (γ-CH_3 Thr).

H-D-Phe(S)-Ser-Thr(rPr)-Ser(rPr)-Ile-Ala-Pro-OH (3). Cl-TrtCl-resin (1 g, 1.6 mmol/g) was placed in a 20 mL polypropylene syringe fitted with a polyethylene filter disk. The resin was then washed with CH_2Cl_2 (5 × 1 min) and a solution of Fmoc-Pro-OH (0.27 g, 0.8 mmol, 0.5 equiv) and DIEA (0.28 mL, 1.6 mmol, 2 equiv) in CH_2Cl_2 (2.5 mL) was added. The reaction mixture was stirred for 1 h. The reaction was terminated by addition of MeOH (0.8 mL) and stirred for a further 10 min. The Fmoc-Pro-O-TrtCl-resin (0.8 mmol) was subjected to the following washings with CH_2Cl_2 (3 × 0.5 min), DMF (3 × 0.5 min), piperidine−DMF (1:4, 2 × 1, 2 × 20 min), DMF (5 × 1 min), 2-propanol (2 × 1 min), DMF (5 × 1 min), MeOH (2 × 1 min), and CH_2Cl_2 (3 × 1 min) and then dried under vacuum.

Fmoc-Ala-OH (1.24 g, 4 mmol, 5 equiv), Fmoc-Ile-OH (1.4 g, 4 mmol, 5 equiv), Fmoc-Ser(rPr)-OH (0.54 g, 1.4 mmol, 1.7 equiv), Fmoc-Thr(rPr)-OH (0.56 g, 1.4 mmol, 1.7 equiv), and Fmoc-Ser-OH (1.05 g, 3.2 mmol, 4 equiv) were added sequentially to the H-Pro-O-TrtCl-resin obtained above using DIPCDI (0.62 mL, 4 mmol, 5 equiv, for Ala and Ile; 0.21 mL, 1.4 mmol, 1.7 equiv, for Ser(rPr) and Thr(rPr); 0.49 mL, 3.2 mmol, 4 equiv, for Ser) and HOBt (0.54 g, 4 mmol, 5 equiv, for Ala and

Table 2. ^{1}H NMR (600 MHz, $CDCl_3$−DMSO-d_6 7:3) Data for 1b

residue	NH	H_α	H_β	other
Ala	7.29 (d, J = 5.5 Hz)	4.32 (qd, J = 7.4, 7.4 Hz)	1.09 (3H, d, J = 8.4 Hz)	-
Ile	6.31 (d, J = 9.9 Hz)	4.40 (dd, J = 9.9, 3.3 Hz)	2.26−2.20 (m)	1.16, 1.04 (2H, 2m, γ-CH_2); 0.88−0.82 (6H, m, γ-CH_3, δ-CH_3).
Ser(rPr)	7.53 (d, J = 8.1 Hz)	4.43 (ABX, J = 8.1, 8.1, 2.6 Hz)	3.78 (1H, dd, J = 10.5, 2.6 Hz); 3.38 (1H, dd, 10.5, 2.6 Hz)	5.65 (1H, dd, J = 17.6, 11.0 Hz, CH ^{i}Pr); 5.08 (1H, d, J = 11.0 Hz, CH_2 ^{i}Pr); 5.04 (1H, d, J = 17.6 Hz, CH_2 ^{i}Pr); 1.20 (6H, s, $2CH_3$ ^{i}Pr)
Thr(rPr)	7.82 (d,J = 7.3 Hz)	4.55 (dd, J = 7.3, 4.8 Hz)	3.85−3.80 (m)	5.83 (1H, dd, J = 17.6, 11.0 Hz, CH ^{i}Pr); 5.17 (1H, d, J = 17.6 Hz, CH_2 ^{i}Pr); 5.13 (1H, d, J = 11.0 Hz, CH_2 ^{i}Pr); 1.40 (3H, s, CH_3 ^{i}Pr); 1.30 (3H, s, CH_3 ^{i}Pr); 0.95 (3H, d, J = 8.1 Hz, γ-CH_3)
Tzn	-	5.00−4.91 (m)	3.67 (1H, dd, J = 12.3, 12.3 Hz); 3.54 (1H, dd, J = 12.3, 12.3 Hz)	
D-Phe	8.42 (d, J = 8.8 Hz)	5.00−4.91 (m)	3.10 (1H, dd, J = 16.6, 4.6 Hz); 2.72 (1H, dd, J = 16.6, 12.5 Hz)	7.20−7.10 (5H, m, Ar)
Pro	-	4.26 (t, J = 9.4 Hz)	3.45−3.40 (1H, m, δ-CH_2); 3.30−3.25 (1H, m, δ-CH_2); 1.80−1.61 (3H, m, β-CH_2, γ-CH_2); 1.07 (1H, m, β-CH_2)	

Ile; 0.18 g, 1.4 mmol, 1.7 equiv, for Ser(rPr) and Thr(rPr); 0.43 g, 3.2 mmol, 4 equiv, for Ser) in DMF (7 mL, for Ala, Ile and Ser) or CH_2Cl_2 [4 mL, for Ser(rPr) and Thr(rPr)]. Finally, a solution of Fmoc-D-Phe(S)-NBt (1.32 g, 2.4 mmol, 3 equiv) in CH_2Cl_2 (7 mL) was added to the peptide resin. In all cases, the ninhydrin test was negative after 90 min of coupling. Removal of the Fmoc group and washings were carried out as described in General Procedures.

The peptide was cleaved from the resin using HFIP−CH_2Cl_2 (1:4, 4 × 3 min). The combined filtrates were evaporated to dryness under reduced pressure to give 0.34 g of the title compound with a purity of >63% as determined by HPLC (t_R 21.2 min, condition A): ES-MS calcd for $C_{43}H_{67}N_7O_{10}S$ 873.5, found m/z 874.6 $[M + H]^+$.

Cyclo[D-Phe(S)-Ser-Thr(rPr)-Ser(rPr)-Ile-Ala-Pro] (2). The crude linear peptide (0.34 g, 0.39 mmol) was dissolved in CH_2Cl_2−DMF (9:1, 150 mL), and PyAOP (0.41 g, 0.78 mmol, 2 equiv) and DIEA (0.27 mL, 1.55 mmol, 4 equiv) were added. The mixture was stirred for 1 h, and then the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$−MeOH, 9.8:0.2) to give the title product (100 mg, 0.12 mmol, 15% yield): analytical HPLC (t_R 14.7 min, condition B); ES-MS calcd for $C_{43}H_{65}N_7O_9S$ 855.5, found 856.4 $[M + H]^+$; HRMS (FAB) ES-MS calcd 856.4642, found 856.4637; ^{1}H NMR (500 MHz, $CDCl_3$) shown in Table 1.

Trunkamide A. Cyclo[D-Phe-Tzn-Thr(rPr)-Ser(rPr)-Ile-Ala-Pro] (1b). The cyclic peptide was dissolved in CH_2Cl_2 (1 mL), and a solution of DAST (17 μL, 0.13 mmol, 1.1 equiv) in CH_2Cl_2 (0.1 mL) was added dropwise at −20 °C under a N_2 atmosphere. After 30 min, a further 1.1 equiv of DAST was added under the same conditions and the reaction was stirred for an additional 30 min. The solvent was removed by evaporation under reduced pressure. The crude product was purified by HPLC (Vydac C_{18} reversed-phase column, 15−20 μm, 250 × 10 mm), linear gradient from 55% to 70% of acetonitrile in water in 30 min, 3 mL/min, detection at 220 nm, to give the title product (35 mg, 0.04 mmol, 33% yield): analytical HPLC (t_R 16.2 min, condition B); ES-MS calcd for $C_{43}H_{63}N_7O_8S$ 837, found m/z 838 $[M + H]^+$, 860 $[M + Na]^+$; HRMS (FAB) ES-MS calcd 838.4537, found 838.4556; ^{1}H NMR (600 MHz, $CDCl_3$−DMSO-d_6, 7:3) shown in Table 2.

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