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A Convergent Solution-Phase Synthesis of the Macrocycle Ac-Phe-[Orn-Pro-D-Cha-Trp-Arg], a Potent New Antiinflammatory Drug

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Relatively few cyclic peptides have reached the pharmaceutical marketplace during the past decade, most produced through fermentation rather than made synthetically. Generally, this class of compounds is synthesized for research purposes on milligram scales by solid-phase methods, but if the potential of macrocyclic peptidomimetics is to be realized, low-cost larger scale solution-phase syntheses need to be devised and optimized to provide sufficient quantities for preclinical, clinical, and commercial uses. Here, we describe a cheap, medium-scale, solution-phase synthesis of the first reported highly potent, selective, and orally active antagonist of the human C5a receptor. This compound, Ac-Phe[Orn-Pro-D-Cha-Trp-Arg], known as 3D53, is a macrocyclic peptidomimetic of the human plasma protein C5a and displays excellent antiinflammatory activity in numerous animal models of human disease. In a convergent approach, two tripeptide fragments Ac-Phe-Orn-(Boc)-Pro-OH and H-D-Cha-Trp(For)-Arg-OEt were first prepared by high-yielding solution-phase couplings using a mixed anhydride method before coupling them to give a linear hexapeptide which, after deprotection, was obtained in 38% overall yield from the commercially available amino acids. Cyclization in solution using BOP reagent gave the antagonist in 33% yield (13% overall) after HPLC purification. Significant features of the synthesis were that the Arg side chain was left unprotected throughout, the component Boc-D-Cha-OH was obtained very efficiently via hydrogenation of D-Phe with PtO_2 in TFA/water, the tripeptides were coupled at the Pro-Cha junction to minimize racemization via the oxazolone pathway, and the entire synthesis was carried out without purification of any intermediates. The target cyclic product was purified (>97%) by reversed-phase HPLC. This convergent synthesis with minimal use of protecting groups allowed batches of 50−100 g to be prepared efficiently in high yield using standard laboratory equipment. This type of procedure should be useful for making even larger quantities of this and other macrocyclic peptidomimetic drugs.

Introduction

An important part of the human immune system is a set of blood proteins termed complement. One of these proteins, known as C5a, regulates many types of human immune and other cells by binding to a specific receptor on the cell surface, triggering cellular immune responses, and release of numerous inflammatory mediators.[1] However, overexpression or underregulation of C5a is implicated in the pathogenesis of many immunoinflammatory conditions, such as rheumatoid and osteoarthritis, Alzheimer's disease, cystic fibrosis, tissue graft rejection, ischaemic heart disease, psoriasis, gingivitis, atherosclerosis, lung injury, fibrosis, systemic lupus erythematosus, reperfusion injury, and major systemic disturbances such as septic and anaphylactic shock, burns, and major trauma or infection that leads to adult respiratory distress syndrome.[2] Medical conditions that arise from excessive complement activation are known to affect hundreds of millions of people and represent annual multibillion dollar pharmaceutical market opportunities in the USA alone.[3]

We recently recognized the importance of a turn conformation in the recognition of the C-terminus of C5a by its G protein-coupled receptor. From the structure[4] of a truncated hexapeptide derivative, we derived a cyclic antagonist **1** to stabilize the putative turn structure which we believed to be involved in receptor binding.[5] Compound **1**, featuring an $i \rightarrow i + 4$ side chain (ornithine-δNH_2) to main chain (arginine-CO_2H) amide bond linkage, was originally created as a molecular probe to

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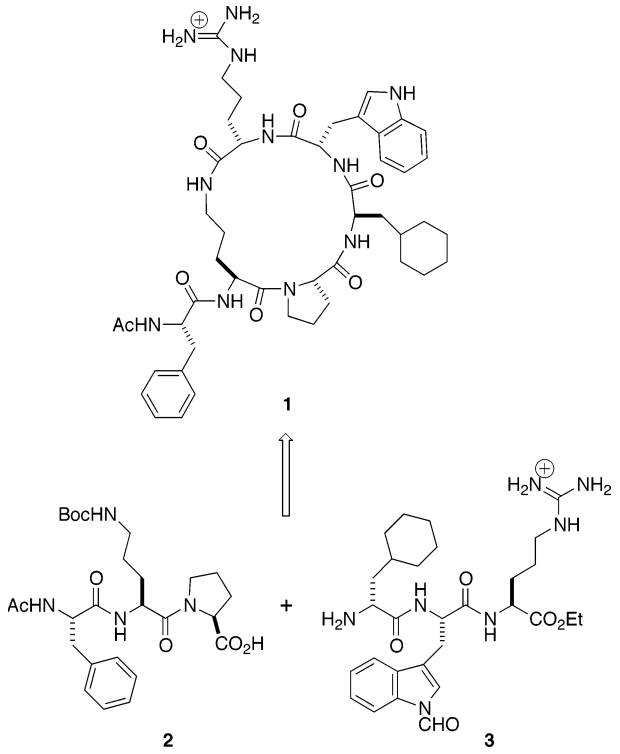
identify key features needed for construction of a nonpeptidic drug candidate. This macrocyclic compound proved to be the first potent, selective, and orally active antagonist of the human C5a receptor with potent inhibition in vitro[6−9] of C5a binding to human cells and C5a-mediated activation of neutrophils and macrophages, chemotaxis, and cytokine release from polymorphonuclear leukocytes. Since it also showed potent inhibition in vivo in many rat models of human disease, including neutropenia/sepsis,[10] arthritis,[11] immune-complex dermal inflammation,[12] arthus and endotoxic shock,[13] and ischemia-reperfusion injury,[14,15] it was decided to more extensively evaluate this compound for efficacy in vivo. We anticipated requiring much larger quantities (50−100 g) of **1** than could be obtained inexpensively and rapidly by solid-phase approaches.



A number of other cyclic peptides have entered the marketplace as drugs, including cyclosporin (immunosupressant),[16] caspofungan (fungicidal),[17] eptifibatide (antithrombotic),[18] dalfopristin and quinupristine (antibacterials),[18] atosiban (tocolytic),[19] lepirudin (anticoagulant),[20] lanreotide and octreotide (acromegaly).[21] Although most cyclic peptides synthesized for research purposes are made on a small scale using conventional Merrifield-based solid-phase peptide synthesis methods, larger quantities needed for preclinical and clinical investigations need to be obtained more cheaply. Until now, this has usually been through fermentation, but sometimes via solution-phase syntheses. Relatively few large-scale solution syntheses of cyclic peptides have been previously reported in the literature,[22,23] most using a mixed-anhydride method that appears optimal for large-scale peptide couplings in solution. The procedure is efficient and inexpensive and gives high yields with low racemization at each step. The one report[23] that dealt with an arginine-containing peptide used the tosyl protecting group for the arginine side chain. This required the use of trifluoromethanesulfonic acid, a very corrosive agent, in the final deprotection step.

Our original synthesis[4,5] of **1** involved conventional assembly of the linear hexapeptide in small quantities by solid-phase peptide synthesis using Fmoc protocols on Arg(Pmc)-Wang resin,[24,25] followed by cyclization in solution using BOP. To scale-up the synthesis via solution phase, we needed a plan to realize high yields from inexpensive reagents, to minimize purification steps, and to avoid racemization. We decided that the synthesis of **1** would be most efficient via a convergent approach, involving synthesis and coupling of the component tripeptides Ac-Phe-Orn(Boc)-Pro-OH **2** and H-D-Cha-Trp-(For)-Arg-OEt **3** to give the linear hexapeptide, which could then be cyclized. We envisaged that coupling of the two tripeptides by activation at the central peptidylproline residue would be less prone to racemization via the oxazolone pathway than at other sites in the peptide sequence.[26] We chose a mixed anhydride method, using the inexpensive ethyl chloroformate as coupling reagent where possible, and attempted a continuous synthesis without purification of intermediates by chromatography. Due to the low nucleophilicity of the positively charged guanidino group and the likelihood that it would not be acylated during peptide couplings, we decided to leave the arginine side chain unprotected throughout the synthesis. This strategy has the benefit of avoiding the use of hazardous triflic acid and minimizes deprotection

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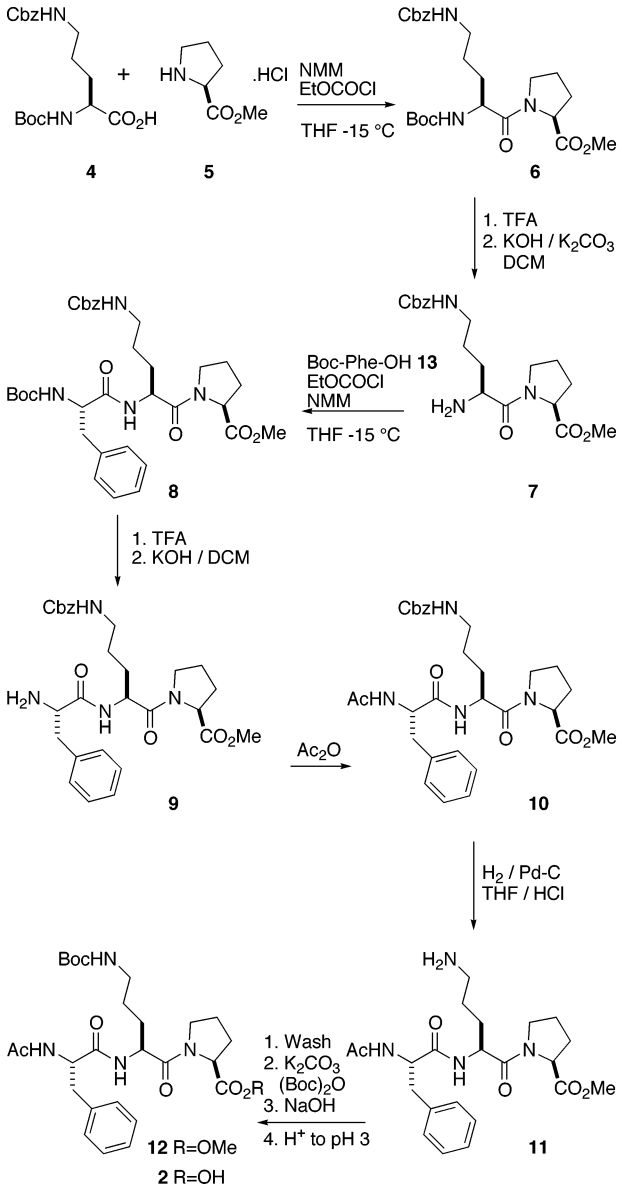
SCHEME 1



steps in the procedure. We now report a solution-phase synthesis of **1** that uses cheap reagents, no purification of intermediates, and delivers reasonable yields (12.5% overall yield from commercially available amino acids) of the required product in 50−100 g quantities and in high purity. This method is suitable for the synthesis of **1** and derivatives in medium to large scale.

Results and Discussion

Synthesis of Tripeptide Fragments 2 and 3. Tripeptide Ac-Phe-Orn(Boc)-Pro-OH **2** was prepared as shown in Scheme 1. Boc-Orn(Cbz)-OH **4** was first coupled to H-Pro-OMe **5**. Following removal of the Boc protecting group with TFA, the product **7** was then coupled to Boc-Phe-OH **13** to give the tripeptide unit **8**. After subsequent removal of the Boc group, the N-terminus was acetylated with Ac_2O to give **10**. It was necessary to replace the ornithine side chain Cbz group with Boc for compatibility with subsequent steps. This was accomplished easily by hydrogenation to give the free amine **11** that was conveniently separated from neutral impurities by extraction into an aqueous phase as the hydrochloride salt and washing with ether. After basification, the amine **11** was treated with Boc_2O to give **12**. The C-terminal methyl ester of **12** was hydrolyzed with NaOH, and after acidification and extraction, the required tripeptide **2** was obtained as a colorless brittle glass (90% overall yield) that was easily ground to a powder that stored well.

The second tripeptide H-D-Cha-Trp(For)-Arg-OEt.HSO_4 **3** presented several challenges due to side-chain functionality. The C-terminal arginine residue traditionally requires protection of the side chain guanidine group, as a sulfonamide (e.g. tosyl) or perhaps by nitration, to prevent unwanted acylation during the several subsequent coupling reactions resulting in a complex mixture. We were concerned however that such protecting groups may prove difficult to remove from the final product under mild conditions. For example, the use of trifluoromethane sulfonic acid or HF was not considered practical for removal of the tosyl group due to the difficulty of handling and the often protracted hydrogenations needed to cleave nitroarginine might not proceed to completion or may effect reduction of the tryptophan indole nucleus. For these reasons, we chose to develop conditions that would allow side chain unprotected H-Arg-OEt·2HCl **17** to be employed. It was reasoned that the much greater basicity of the guanidine group (pK_a 13) verses amino groups (pK_a 9) should permit the desired regioselective couplings. Boc-Trp(For)-OH **16** was considered to be a suitably protected tryptophan derivative for the initial coupling reaction. The coupling of **16** and **17** was best achieved via the mixed anhydride method with ethyl chloroformate using DMF as solvent. The reaction works well if the ethyl ester of arginine (**17**) is totally dissolved in the DMF solvent before addition to the mixed anhydride of **16**; otherwise, substantial acylation does occur at the guanidino side chain. Pouring the reaction mixture into a solution of 10% $KHSO_4$ causes precipitation of the dipeptide **18** as a gel which can be filtered, washed, and air-dried. To quicken the drying process, the wet gel can also be azeotroped with 1-butanol on a rotary evaporator to remove water, precipitated from the oil formed by the addition of ether, and then air-dried. The reaction was performed several times on 25−50 g batches of Boc-Trp(For)-OH with yields typically in the 70−80% range and of >93% purity.

D-Cyclohexylalanine **14** was a reasonably expensive amino acid if sourced commercially, and we sought to develop improved conditions for its preparation from D-phenylalanine **13** by hydrogenation. The use of a solvent consisting of TFA/water 1:1 was found to be the single key factor in improving the efficiency of the PtO_2 catalyzed hydrogenation of the aromatic ring over reported literature procedures.[27] The improvement was largely due to the much greater solubility of the amino acids (**13** and **14**) in this solvent mixture which enabled concentrations to be kept much higher and avoided poisoning of the catalyst due to precipitation. There is however literature precedence to suggest that TFA increases the overall rate of hydrogenation as compared

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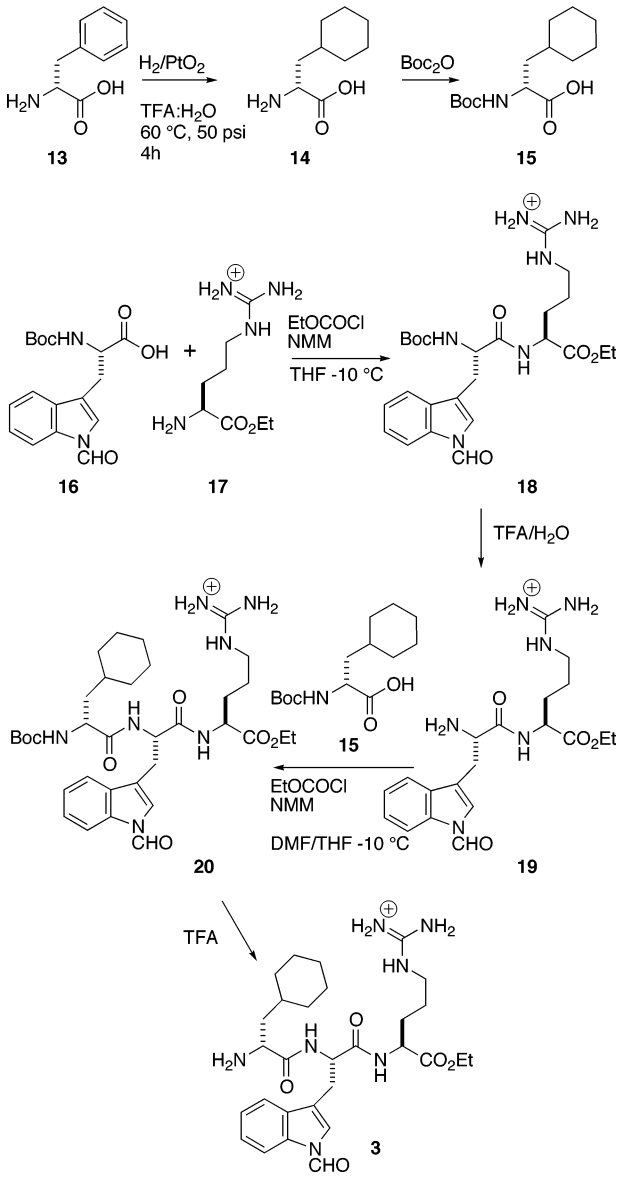
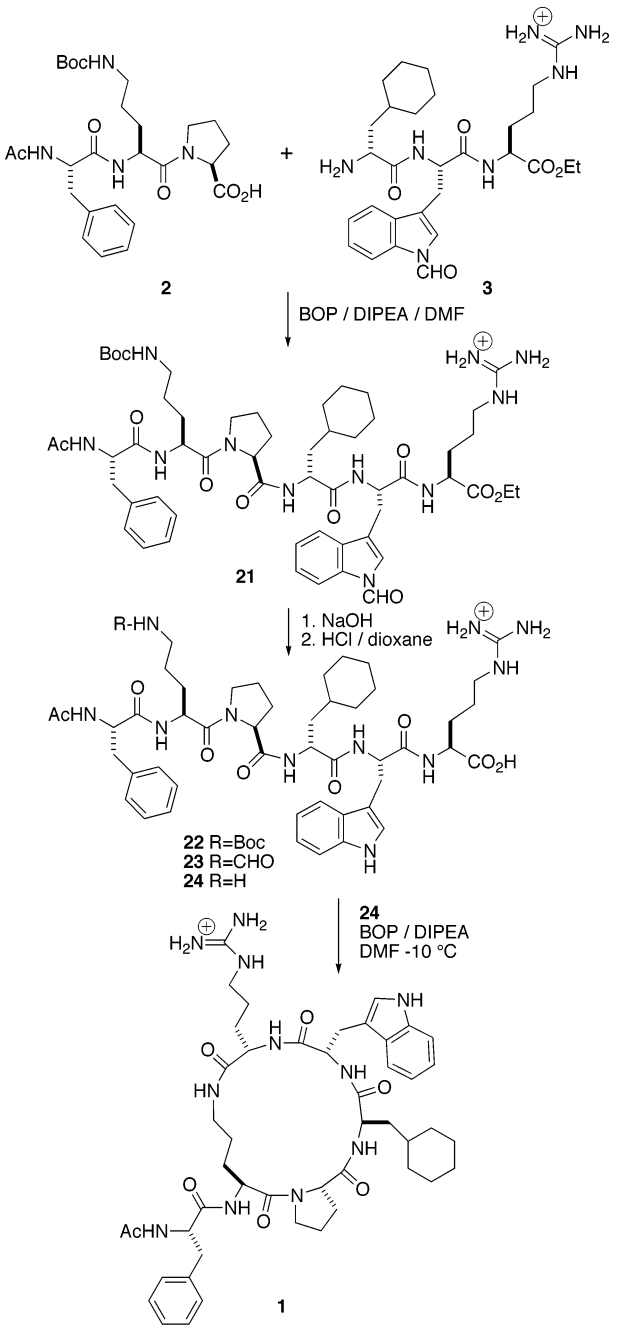
SCHEME 2



to HCl, sulfuric acid, or acetic acid.[28] The same charge of catalyst was used for 5 × 20 g batches of **13** giving complete conversion to **14** within 4 h with minimal loss of catalytic activity before recycling. D-Cyclohexylalanine **14** was converted to the Boc-derivative **15** by standard procedures[29] before coupling to H-Trp(For)-Arg-OEt **19** (Scheme 2). The coupling of dipeptide **18** and Boc-D-cyclohexylalanine **15** was achieved by the mixed anhydride method. The tripeptide product **20** could again be purified by simply pouring the reaction mixture into a solution of 10% $KHSO_4$, filtering, washing, and either air-drying or azeotroping with butanol using the same conditions as described for the dipeptide **18**. This procedure gave tripeptide **20** with typical yields of 80% and >90% purity.

Assembly of Linear Hexapeptide from Fragments 2 and 3. Coupling of tripeptides **2** and **3** (Scheme 3) was

SCHEME 3



found to be inefficient using the mixed anhydride method. There remained approximately 20% of unchanged tripeptide **2** together with the ethyl carbamate of tripeptide **3**, which was difficult to separate from the desired product **21**. An attempt to use the more hindered analogue, isobutyl chloroformate, did not improve the conversion significantly. Ultimately, the coupling was achieved cleanly using BOP reagent. The fully protected hexapeptide **21** was found to be very hygroscopic and difficult to handle and, thus, was never isolated but deprotected to product **22** which dries to a nonhygroscopic powder after ether precipitation. The coupling and partial deprotection with NaOH was repeated three times giving product **22** in 80% yield and of >94% purity.

Deprotection of both the C-terminal ethyl ester and the Boc group on the ornithine side chain of **21** is required

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prior to the final cyclization to **1**, and surprisingly, the order in which these steps were carried out was absolutely critical for success. Hydrolysis of the C-terminal ethyl ester with concomitant loss of the formyl group from tryptophan using NaOH must precede the removal of the Boc group, otherwise transfer of the formyl group from tryptophan to the ornithine side chain occurs (~50%) giving a byproduct **23** that cannot be cyclized. As the molecular weight of these isomeric formylated peptides is the same, the structure of the byproduct **23** was confirmed by NMR spectroscopy where clear correlations were observed between the formyl proton and the ornithine δ $-CH_2$ in both the HMBC and NOESY spectra. Removal of the Boc group from the linear hexapeptide **22** was accompanied by a considerable amount (~10%) of *tert*-butylation of the tryptophan residue, as a consequence of the loss of the formyl protecting group, if TFA was used even in the presence of scavengers such as water and triisopropylsilane. Efficient deprotection was achieved using a 1:1 mixture of concentrated HCl/dioxane which gave only 3.6% of the *tert*-butylated product as shown by rpHPLC. As it was not possible to retain the protection on the indole ring, the side chains of both Trp and Arg were not protected for the final cyclization (**24** to **1**, Scheme 3).

Cyclization of the Hexapeptide. The cyclization requires the activation and coupling of a peptidyl-Arg residue (unlike a Boc-Arg residue) and is expected to proceed with some degree of racemization. Considerable effort was directed toward minimizing the amount of racemization, because separation of diastereomers of **1** was known to be difficult. The coupling reagents BOP and DPPA are reportedly superior to all others in terms of suppressing racemization when cyclizing or coupling peptide fragments, although there is clear evidence that some racemization can take place.[30,31] Table 1 summarizes cyclization conditions used here and outcomes for the preparation of **1**. Clearly, the use of BOP in combination with DIPEA, especially at low temperatures, can limit racemization to as little as 4% as determined by rpHPLC. Interestingly, the use of DBU as base caused extensive epimerization in which the D-Arg containing macrocycle predominated by 60%, suggesting that it is a thermodynamically more stable diastereomer. DPPA caused at least 10% racemization, and the reaction was also 10 times slower than observed with BOP.

The cyclization of the linear hexapeptide **24** was carried out in DMF (10^{-1}M) at low temperature (−10 °C) using DIPEA as the base and monitored by analytical rpHPLC. The reaction appeared to be strikingly clean. No trace of linear hexapeptide remained after 2 h, and the crude product, after extraction and precipitation with diethyl ether, always appeared to be greater than 90% pure as indicated by gradient rpHPLC (0−90% MeCN, λ 214 nm). Although there were no low molecular weight byproducts arising from the unprotected side chains of Arg and Trp, there was evidence for considerable amounts of polymeric material that did not elute from the rpHPLC column even with 100% MeCN. To quantify this, solutions of the analytically pure cyclic peptide **1** and the crude product were accurately prepared (5 mg/mL in 50% MeCN) and analyzed by rpHPLC under the same conditions. Although both chromatograms displayed essentially only 1 peak, the integrated peak area for the crude product was only 40−60% that of the pure product, suggesting a maximum yield of only 60% w/w could be expected after purification and this indeed was found to be the case.

TABLE 1. Effect of Cyclization Conditions on Racemization

vial[a]	base[b]	*T* (°C)	diastereomer ratio of **1**[c] (%)
1	DIPEA (4 μL, 2 equiv)	rt	88.9
2	DIPEA (10 μL, 5 equiv)	rt	92.9
3	DIPEA (4 μL, 2 equiv)	0	93.5
4	DIPEA (10 μL, 5 equiv)	0	95.6
5	DIPEA (4 μL, 2 equiv)	−10	95.9
6	pyridine (5 μL, 5 equiv)	rt	81.2 (28% conversion)
7	NMM (6 μL, 5 equiv)	rt	80.8
8	$NaHCO_3$ (5 mg, 5 equiv)	rt	88.8
9	K_2CO_3 (8 mg, 5 equiv)	rt	89.2
10	DBU (8 μL, 5 equiv)	rt	40.6
11	no base	rt	81.4 (5% conversion)
12	dimethylaniline (5 equiv)	rt	82.9 (18% conversion)
13	TMEDA (2.5 equiv)	rt	89.4
14	no base after 24 h	rt	82.7 (12% conversion)

[a] Linear hexapeptide **24** (100 mg) and BOP reagent (50 mg, 1 equiv) were dissolved in DMF (1 mL), and then 10 aliquots of 100 μL were taken in separate tubes. [b] The bases in the amounts indicated in the table were added at the temperature specified. [c] After 1 h, 900 μL of 80%A/20% B was added with shaking to each tube, and then after brief centrifugation, 5 μL was injected into the HPLC with linear gradient 70% A/30% B to 55% A/45% B over 30 min. Retention times: linear **24**, 16.2 min; cyclic **1**, 26.5 min; diastereomer, 28.1 min.

Previous studies have suggested that linear peptides of similar sequence to **24**, and certainly the cyclic product **1**, adopt turn conformations in solution that are favored by the presence of a central proline residue and stabilized by one or more intramolecular hydrogen bonds.[5] This preorganization appears to greatly assist the cyclization reaction, relative to competing polymerization, and high dilution conditions were not essential for cyclization. Comparable yields of cyclic product were obtained at concentrations of 10^{-1} M (49%) and 10^{-2} M (51%) and any benefits from further dilution were offset by the excessive solvent consumption. Purification of the final product **1** was achieved by preparative rpHPLC, after an initial adsorption step to remove polymeric material, in 33% yield and >97% purity.

Conclusions

This work describes methods that can be utilized for the medium-large scale synthesis of cyclic peptides, without purification of intermediates. The convergent synthesis described above shows that arginine side-chain protection is not required and that in this case the simple precipitation of intermediates provides products of sufficient purity to carry out subsequent reactions efficiently. The tripeptides were coupled at the Pro-Cha junction to minimize racemization via the oxazolone pathway. Adequate quantities of the final product (64 g) could be purified by rp-HPLC to >97% purity in an overall yield of 12.5% from the commercially available amino acids.

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This work will be of value for the synthesis of other cyclic peptidomimetic drugs that will be discovered in the future.

Experimental Section

General Considerations. All amino acid derivatives were purchased from Novabiochem and used as received. DMF, TFA, and diisopropylethylamine (DIPEA) were "peptide grade" obtained from Auspep Pty Ltd., Australia. THF was HPLC grade (less than 0.01% H_2O). Other chemicals were obtained from Aldrich and used as received. ^{1}H NMR spectra (500 MHz, δ) were recorded at 298 K in DMSO-d_6 and were referenced to the residual solvent peak δ 2.50 ppm. Assignments and chemical shifts for overlapped signals were determined from TOCSY and DQ-COSY spectra in each case. Standard analytical HPLC conditions were used for all compounds: 30% B to 100% B linear gradient over 20 min followed by a further 10 min at 100% B where solvent B was 90% MeCN, 10% H_2O + 0.1% TFA. Solvent A was H_2O + 0.1% TFA. Column was Phenomenex Luna C18, 5 μm, 100 Å, 250 × 4.6 mm, flow rate 1 mL/min. Mass spectra were obtained using electrospray ionization (ESI-MS) from solutions in 70% MeCN 30% H_2O + 0.1% formic acid.

Boc-Orn(Z)-Pro-OMe, 6. A solution of Boc-L-ornithine(Z)-OH (100 g, 273 mmol) in dry THF (1 L) and *N*-methylmorpholine (36 mL, 327 mmol, 1.2 equiv) was stirred under argon and cooled to −15 °C. Ethyl chloroformate (32 mL, 335 mmol, 1.2 equiv) was then added while the temperature was maintained at −15 °C. Stirring was continued for 30 min, and then *N*-methylmorpholine (50 mL, 455 mmol, 1.6 equiv) was added followed by a solution of H-pro-OMe·HCl (63 g, 380 mmol, 1.4 equiv) in DMF (100 mL). The mixture was allowed to warm to rt with stirring for a further 6 h. The precipitate of *N*-methylmorpholine hydrochloride was filtered off and washed with THF and the combined THF solution evaporated to dryness. The oil residue was redissolved in ether/DCM 3:1 (1.2 L), washed with 2 M HCl (2 × 300 mL), brine (300 mL), saturated $NaHCO_3$ (300 mL), and brine (300 mL), and dried over $MgSO_4$. The solvent was evaporated giving the protected dipeptide as a clear, colorless oil (131 g > 100%). The product contains some *N*-ethoxycarbonyl-Pro-OMe but this is easily removed at a later stage. This procedure gives the best yield based on the ornithine derivative which is the most expensive ingredient: HRMS 478.2556 MH^+ calcd for $C_{24}H_{36}N_3O_7^+$ 478.2548; ^{1}H NMR (500 MHz, DMSO-d_6) 7.39−7.28 (m, 5H), 7.24 (t, J = 5.5 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 5.01 (s, 2H), 4.31 (dd, J = 8.6, 5.0 Hz, 1H), 4.15 (m, 1H), 3.66 (m, 1H), 3.58 (s, 3H), 3.52 (m, 1H), 3.06−2.93 (m, 2H), 2.17 (m, 1H), 1.90 (m, 2H), 1,80 (m, 1H), 1.59 (m, 1H), 1.64−1.40 m, 3H), 1.36 (s, 9H). Resonances at δ 4.23 4.03, 3.93, 3.38, 1.18, and 1.09 correspond to *N*-ethoxycarbonyl-Pro-OMe, (separated later). Analytical rpHPLC t_R = 18.8 min.

H-Orn(Z)-Pro-OMe, 7. The crude oily product from the above procedure (131 g containing 273 mmol of Boc-Orn(Z)-Pro-OMe) was treated with neat trifluoroacetic acid (250 mL) with swirling. CO_2 was evolved, and the mixture became warm. No attempt was made to cool the mixture as it does not mix well if cold. After about 15 min, the TFA was evaporated on a rotary evaporator at 40 °C/20 mbar. The residue was dissolved in DCM (1.2 L), cooled to 0 °C, and carefully neutralized with KOH (59 g, in water/ice 500 mL) and finally with 10% K_2CO_3 solution. The DCM layer was washed with 10% K_2CO_3 (200 mL), and the aqueous layers were back-extracted with DCM (200 mL). The combined DCM layers were dried over $MgSO_4$ and concentrated to about 500 mL. The solution was kept cool and used immediately for the next step to avoid possible diketopiperazine formation: HRMS 378.2026 MH^+ calcd for $C_{19}H_{28}N_3O_5^+$ 378.2024.

Boc-Phe-Orn(Z)-Pro-OMe, 8. A solution of Boc-Phe-OH (75 g, 283 mmol) and *N*-methylmorpholine (32 mL, 291 mmol) in THF (1 L) was stirred under argon and cooled to −15 °C. Ethyl chloroformate (26 mL, 272 mmol) was added, and stirring at −15 °C was continued for 30 min. The solution prepared above containing H-Orn(Z)-Pro-OMe (273 mmol) in DCM was added, and the temperature was maintained at 0 °C for 15 min then stirred at room temperature for a further 4 h. The precipitate of *N*-methylmorpholine hydrochloride was filtered off and washed with THF, and the combined fractions were evaporated. The oil residue was redissolved in ether/DCM 3:1 (1.2 L), washed with 2 M HC1 (2 × 300 mL), brine (300 mL), saturated $NaHCO_3$ (300 mL), brine (300 mL), and dried over $MgSO_4$. The solvent was evaporated giving the protected tripeptide as a clear, colorless oil (171 g, >100%): HRMS 625.3253 MH^+, calcd for $C_{33}H_{45}N_4O_8^+$ 625.3232; ^{1}H NMR (500 MHz, DMSO-d_6) multiple minor conformations were observed, data refers to the major conformer: 8.03 (d, J = 8.0 Hz, 1H), 7.39−7.12 (m, 10H), 7.27 (1H), 6.89 (d, J = 8.7 Hz), 5.01 (s, 2H), 4.53 (m, 1H), 4.29 (dd, J = 8.6, 5.2 Hz, 1H), 4.18 (m, 1H), 3.65 (m, 1H), 3.60 (s, 3H), 3.54 (m, 1H), 3.09−2.97 (m, 2H), 2.94 (dd, J = 13.8, 4.0 Hz, 1H), 2.70 (dd, J = 13.8, 10.5 Hz, 1H), 2.17 (m, 1H), 1.89 (m, 1H), 1.82 (m, 2H), 1.69 (m, 1H), 1.58−1.41 (m, 3H), 1.28 (s, 9H). Resonances at δ 4.23 4.03, 3.93, 3.38, 1.18, and 1.09 correspond to *N*-ethoxycarbonyl-Pro-OMe, (separated later). Analytical rpHPLC t_R = 20.9 min.

Ac-Phe-Orn(Z)-Pro-OMe 10. The crude oily product from the above procedure (171 g, containing Boc-Phe-Orn(Z)-Pro-OMe **5**, 273 mmol) was treated with neat TFA (300 mL). CO_2 and heat were evolved. After a homogeneous solution had been obtained, the TFA was evaporated on a rotary evaporator at 40 °C/20 mbar; however, 120 g of TFA was retained and could not be evaporated. The residue was dissolved in DCM (1.2 L), cooled to 0 °C, and carefully neutralized with KOH (59 g, in water/ice 500 mL) and finally with 10% K_2CO_3 solution. The DCM layer was washed with 10% K_2CO_3 (200 mL), and the aqueous layers were back-extracted with DCM (200 mL) (free amine **9** HRMS 525.2716 MH^+, calcd for $C_{28}H_{37}N_4O_6^+$ 525.2708). Acetic anhydride (27 mL, 286 mmol) was added, and the solution was stirred at rt for 1 h. Mass spectrometry showed that complete acetylation had occurred. The solution was washed with saturated $NaHCO_3$ (200 mL), water (200 mL), and 1 M HCl (200 mL), dried over $MgSO_4$, and evaporated to give a viscous colorless clear oil (155 g >100%): HRMS 567.2814 MH^+, calcd for $C_{30}H_{39}N_4O_7^+$ 567.2813; ^{1}H NMR (500 MHz, DMSO-d_6) 8.16 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.39−7.14 (m, 10H), 7.28 (1H), 5.02 (s, 2H), 4.53 (m, 1H), 4.48 (m, 1H), 4.29 (dd, J = 8.6, 5.3 Hz), 3.65 (m, 1H), 3.60 (s, 3H), 3.52 (m, 1H), 3.02 (m, 2H), 2.96 (dd, J = 13.9, 4.4 Hz, 1H), 2.70 (dd, J = 13.9, 10.0 Hz, 1H), 2.16 (m, 1H), 1.96−1.76 (m, 3H), 1.75 (s, 3H), 1.69 (m, 1H), 1.58−1.38 m). Resonances at δ 4.23 4.03, 3.93, 3.38, 1.18 and 1.09 correspond to *N*-ethoxycarbonyl-Pro-OMe (separated later). Analytical rpHPLC t_R = 15.9 min.

Ac-Phe-Orn(Boc)-Pro-OMe, 12. The crude oily product from the above procedure (155 g, containing Ac-Phe-Orn(Z)-Pro-OMe **7** 273 mmol) was dissolved in THF (650 mL) and 2 M HCl (100 mL) and hydrogenated over 10% Pd on carbon (1 g) at 35 psi and room temperature for 3 h. The catalyst was filtered off, and water (1 L) and ether (500 mL) were added to the filtrate and shaken. The aqueous layer was washed again with ether (300 mL) to complete the removal of neutral impurities such as *N*-ethoxycarbonyl-Pro-OMe. The aqueous/THF solution containing Ac-Phe-Orn-Pro-OMe **11** (273 mmol) [HRMS 433.2456, calcd for $C_{22}H_{33}N_4O_5^+$ 433.2446] was basified with solid K_2CO_3 (30 g), a solution of di-*tert*-butyl dicarbonate (60 g, 275 mmol) in THF (200 mL) was added, and the solution was stirred vigorously for 1 h. The solution was extracted with ether/DCM 2:1 (3 × 500 mL), and the combined extracts were washed with 1 M HCl (300 mL), brine (300 mL), saturated $NaHCO_3$ (300 mL), and brine (300 mL) and dried over $MgSO_4$. Removal of solvent gave Ac-Phe-Orn(Boc)-Pro-OMe **9** as a colorless viscous gum 150 g >100%: HRMS 533.2974 MH^+, calcd for $C_{27}H_{41}N_4O_7^+$ 533.2970; ^{1}H NMR (500 MHz, DMSO-d_6) 8.14 (d, J = 7.8 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.28−

7.15 (m, 5H), 6.80 (t, J = 5.6 Hz, 1H), 4.52 (m, 1H), 4.47 (m, 1H), 4.29 (dd, J = 8.5, 5.0 Hz), 3.65 (m, 1H), 3.61 (s, 3H), 3.54 (m, 1H), 3.00−2.84 (m, 2H), 2.95 (dd, J = 13.9, 4.4 Hz, 1H), 2.69 (dd, J = 13.9, 10.0 Hz, 1H), 2.16 (m, 1H), 1.97−1.76 (m, 3H), 1.74 (s, 3H), 1.65 (m, 1H), 1.54−1.36 m), 1.38 (s, 9H). Analytical rpHPLC t_R = 15.2 min.

Ac-Phe-Orn(Boc)-Pro-OH, 2. The viscous gum from the above procedure (150 g containing Ac-Phe-Orn(Boc)-Pro-OMe 273 mmol) was dissolved in MeOH (500 mL), and then a solution of NaOH (12 g, 300 mmol) in water (100 mL) was added. The solution was stirred at rt for 2 h, and the hydrolysis was monitored periodically by mass spectrometry. The solution was diluted with water (700 mL), washed with ether (2 × 500 mL), and then acidified to pH 3 with solid citric acid (60 g). The mixture was extracted with ether/DCM 2:1 (3 × 500 mL), and then the combined extracts were washed with brine (2 × 300 mL) and dried over $MgSO_4$. Removal of solvent in vacuo gave Ac-Phe-Orn(Boc)-Pro-OH as a colorless glass (127 g, 90%). The product was crushed to a dry white powder for convenient storage. Analysis by Mass spectrometry, NMR, and HPLC showed the product to be greater than 98% pure: HRMS 519.2847 MH^+, calcd for $C_{26}H_{39}N_4O_7^+$ 519.2813; ^{1}H NMR (500 MHz, DMSO-d_6) 12.4 (br s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.29−7.13 (m, 5H), 6.77 (t, J = 5.5 Hz, 1H), 4.52 (m, 1H), 4.46 (m, 1H), 4.22 (dd, J = 8.7, 4.6 Hz), 3.62 (m, 1H), 3.53 (m, 1H), 3.00−2.85 (m, 2H), 2.96 (dd, J = 13.8, 4.3 Hz, 1H), 2.70 (dd, J = 13.8, 9.8 Hz, 1H), 2.13 (m, 1H), 1.95−1.78 (m, 3H), 1.74 (s, 3H), 1.66 (m, 1H), 1.54−1.38 m), 1.37 (s, 9H). Analytical rpHPLC t_R = 13.0 min. Anal. Calcd for $C_{26}H_{38}N_4O_7$: C, 60.2; N, 10.8. Found: C, 58.9; N, 10.2.

Boc-D-Cyclohexylalanine, 15. H-D-phenylalanine-OH (20 g, MW = 165, 121 mmol) was dissolved in a 1:1 mixture of deionized water/TFA (80 mL), and to the hydrogenator vessel was added PtO_2 (800 mg, 4% w/w). The vessel was heated to 60 °C at 50 psi for 4 h. The solution was decanted and filtered from the catalyst, and the cyclohexylalanine was precipitated out of solution by the addition of concd HCl until no more precipitation was observed. The solid was filtered off, washed three times with acetone, and air-dried. This procedure gave D-cyclohexylalanine as the HCl salt (20 g, 80% yield). The catalyst could be reused without significant reduction in reactivity for *at least* 5 further 20 g batches of H-D-phenylalanine-OH before discarding. The use of TFA is essential for quick hydrogenation times. If acetic acid is used the phenylalanine is not as soluble in the solution and was found to clog the hydrogenator lines. Total reaction time in acetic acid took 2 days in one experiment.

D-Cyclohexylalanine·HCl (36.5 g, 176 mmol) was dissolved in a 1:1 solution of water/THF (600 mL). Potassium carbonate (48.7 g, 352 mmol) was added, and the solution was cooled to 0 °C and Boc carbonate (46 g, 1.2 equiv, 211 mmol) was added over 15 min, adjusting the pH to 10−11 as the addition proceeded by adding more potassium carbonate. If the pH falls below ∼6 the unprotected amino acid precipitates out of solution as the zwitterion. When all the Boc carbonate was added, the addition of a further 100 mL of water gave a homogeneous solution. This was stirred overnight at room temperature, and the THF was removed from the basic solution by rotary evaporation. The basic aqueous layer was extracted with ethyl acetate (2 × 100 mL) to remove unreacted Boc carbonate. The water layer was acidified to pH = 2−3 by the addition of citric acid and extracted again with ethyl acetate (3 × 150 mL), and the combined organic layers were dried and evaporated. This procedure yields Boc-D-cyclohexylalanine (48 g, 100%) as a colorless oil which was pure by ^{1}H NMR and ESI-MS: MS 272.19 MH^+.

Boc-Trp(For)-Arg-OEt, 18. Boc-Trp(For)-OH (25 g, MW = 332, 75.2 mmol) was dissolved in peptide grade DMF (75 mL), and to it was added *N*-methylmorpholine (18 mL, 2 equiv). The solution was cooled to −10 °C, ethyl chloroformate (7.12 mL, 75.2 mmol) was added, and the solution was stirred for a further 10 min. To this mixed anhydride was added a solution of H-Arg-OEt·2HCl (22.5 g, MW = 274, 82.11 mmol) and *N*-methylmorpholine (18 mL, 2 equiv) in DMF (75 mL). *Importantly*, if H−Arg-OEt is not totally solubilized in the basic DMF solution before being added to the mixed anhydride, coupling will also occur at the arginine side chain giving a substantial amount of side product! The reaction was stirred for 2 h allowing it to come to room temperature. This solution was subsequently poured into 10% $KHSO_4$ (500 mL) with vigorous stirring. A gel slowly precipitates out of solution. The solution is stirred for a further 10 min and the gel filtered, washed with water several times, and air-dried to give the HSO_4^- salt of the dipeptide (32.64 g, 70% yield). The compound was >93% pure by ^{1}H NMR, ESI-MS, and rpHPLC: HRMS 517.2764 MH^+, calcd for $C_{25}H_{37}N_6O_6^+$ 517.2769; ^{1}H NMR δ 9.64 (br s, 1H), 9.26 (br s, 2H), 8.51 (d, J = 7.02 Hz), 8.24 (s, 1H), 8.23 (br s, 1H), 8.00 (br s, 1H), 7.96 (br m, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.34 (m, 2H), 7.04(d, J = 8.1 Hz, 1H), 4.32 (m, 1H), 4.27 (m, 1H), 4.08 (m, 2H), 3.09 (m, 2H), 3.06 (m, 1H), 2.95 (m, 1H), 1.76 (m 1H), 1.68 (m, 1H), 1.53 (m, 2H), 1.28 (s, 9H), 1.17(t, 3H). Analytical rpHPLC t_R = 13.2 min.

H-Trp(For)-Arg-OEt, 19. Boc-Trp(For)-Arg-OEt **18** was dissolved in a mixture of 90% TFA−10% water (5 mL/gram of dipeptide). The solution was stirred at room temperature for 15 min, the TFA was evaporated in vacuo, and the product was precipitated with diethyl ether. The ether layer was decanted from the solid, and the solid was washed with two further volumes of diethyl ether to remove as much of the TFA as possible. The solid was dried in vacuo and used directly for the next coupling.

Boc-D-Cha-Trp(For)-Arg-OEt, 20. Boc-Trp(For)-Arg-OEt **18** (42.00 g, 68.44 mmol) was deprotected as described above. The deprotected solid was dissolved in DMF (70 mL) and to the solution was added *N*-methylmorpholine (15 mL, 2 equiv). *Importantly*, if H-Trp(For)-Arg-OEt is not totally solubilized in the basic DMF solution before before being added to the mixed anhydride, coupling will also occur at the arginine side chain giving a substantial amount of side product! In a separate flask, Boc-D-Cha-OH (18.18 g, 67.33 mmol) was dissolved in DMF (70 mL) and to it was added *N*-methylmorpholine (9 mL, 1.2 equiv). The solution was cooled to −10 °C, and ethyl chloroformate (6.43 mL, 67.9 mmol) was added. The reaction mixture was stirred at −10 °C for a further 15 min, and to it was added the solution of H-Trp(For)-Arg-OEt in 1 portion. The reaction mixture was stirred for a further 2 h, after which time 10% $KHSO_4$ (1 L) was added. The precipitate was filtered off, washed several times with water, and air-dried to give the tripeptide (40.66 g, 80% yield). The product was >90% pure by ^{1}H NMR, ESI-MS, and rpHPLC: HRMS 670.3935 MH^+, calcd for $C_{34}H_{52}N_7O_7^+$ 670.3923; ^{1}H NMR δ 9.65 (br s, 1H), 9.23 (br s, 2H), 8.47 (m, 1H), 8.21 (m, 1H), 8.16 (br s, 1H), 8.00 (br s, 1H), 7.74 (d, J = 7.57 Hz, 1H), 7.57 (br s, 1H), 7.33 (m, 2H), 6.76 (m, 1H), 4.67 (br s, 1H), 4.26 (br s, 1H), 4.07 (m, 2H), 3.92 (m, 1H), 3.11 (m, 1H), 3.09 (m, 2H), 2.93 (m, 1H), 1.78 (m, 1H), 1.66 (m, 1H), 1.55 (m, 4H), 1.50 (m, 2H), 1.40 (m, 1H), 1.32 (s, 9H), 1.16 (t, J = 7 Hz), 1.12−0.91 (m, 6H), 0.681 (m, 2H); MS 670.39 MH^+. Analytical rpHPLC t_R = 17.0 min. Anal. Calcd for sulfate salt $C_{68}H_{104}N_{14}O_{18}S$: C, 56.8; N, 13.6; S, 2.2. Found: C, 57.1; N, 13.6; S, 2.1.

Ac-Phe-Orn(Boc)-Pro-D-Cha-Trp-Arg-OH, 22. Boc-D-Cha-Trp(For)-Arg-OEt·HSO_4 **21** (20.14 g, MW = 766, 26.29 mmol) was deprotected according to the same procedure used for H-Trp(For)-Arg-OEt. The solid, after ether precipitation, was dissolved in DMF (50 mL), and to it were added Ac-Phe-Orn(Boc)-Pro-OH (13.07 g, 25.28 mmol), DIPEA (4 equiv, 17.2 mL), and after complete dissolution, BOP (11.18 g, 25.28 mmol). The reaction mixture was stirred overnight, to it was added 10% $KHSO_4$ solution (500 mL), and the aqueous layer was extracted with butan-1-ol/ethyl acetate 1:2 (3 × 100 mL). The combined butan-1-ol/ethyl acetate layers were extracted with 10% $KHSO_4$ (3 × 100 mL), saturated $NaHCO_3$, and water (5 × 100 mL) and evaporated to dryness. The resultant oil was

dissolved in a 1:1 mixture of water/ethanol, to it was added NaOH (2.0 g, 50 mmol), and the solution was stirred for 1 h. The solution was poured into 10% $KHSO_4$ (1 L), and the resultant precipitate was extracted with butan-1-ol/ethyl acetate 1:2 (3 × 100 mL). The combined butanol/ethyl acetate layers were washed with water several times, and the solution was evaporated in vacuo. Trituration of the oil with ether caused the compound to precipitate as a creamy solid which was filtered off and dried in an oven at 50 °C (20 g, 72%). The product was pure (>94%) by ^{1}H NMR, ESI-MS, and rpHPLC: MS 1014 MH^+, 507 $M2H^{2+}$; HRMS 1014.5765 MH^+, calcd for $C_{52}H_{76}N_{11}O_{10}^+$ 1014.5771; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.27 (d, J = 7.10 Hz, 1H), 8.13 (d, J = 7.10 Hz, 1H), 8.04 (d, J = 7.95 Hz, 1H), 8.03 (d, J = 7.74 Hz, 1H), 7.82 (d, J = 8.81 Hz, 1H), 7.62 (d, J = 7.52 Hz, 1H), 7.52 (br s, 1H), 7.30 (d, J = 7.74 Hz, 1H), 7.27−7.14 (m, 5H), 7.11 (d, J = 1.93 Hz, 1H), 7.08 (t, J = 7.52 Hz, 1H), 6.95 (t, J = 7.52 Hz, 1H), 6.73 (br s, 1H), 4.56 (m, 1H), 4.52 (m, 1H), 4.45 (m, 1H), 4.31 (m, 1H), 4.22 (m, 1H), 3.56 (m, 2H), 3.19−3.06 (m, 3H), 2.98−2.91 (m, 2H), 2.89 (m, 2H), 2.70 (dd, 1H, J = 9.67, 13.75 Hz), 1.98 (m, 1H), 1.84 (m, 1H), 1.80−1.71 (m, 2H), 1.75 (s, 3H), 1.70−1.63 (m, 3H), 1.54 (m, 2H), 1.5−1.38 (m, 7H) 1.36 (s, 9H), 1.35 (m, 1H), 1.15 (t, 2H), 1.07−0.93 (m, 4H), 0.70 (m, 2H). Analytical rpHPLC t_R = 15.8 min.

The fully protected hexapeptide before de-esterification was hygroscopic and difficult to handle. It was found that the partially deprotected product dries to a nonhygroscopic powder. The reaction was repeated several times giving product in 80% yield.

Ac-Phe-Orn-Pro-Cha-Trp-Arg-OH, 24. Ac-Phe-Orn(Boc)-Pro-D-Cha-Trp-Arg-OH (100 g, 90 mmol) was added to a solution of concd HCl/dioxane (200 mL) and stirred at room temperature for 1 h. The solution was cooled to 0 °C, and to it was slowly added a 4 M NaOH solution (∼70 mL) until the pH of the reaction mixture was ∼7. The resultant neutral solution was extracted with butanol/ethyl acetate 1:2 (3 × 300 mL), and the combined layers were washed with water (2 × 50 mL). Evaporation of the solvent and trituration with ether gave the fully unprotected linear peptide as an off-white powder (91 g, 95%). The product is greater than 93% pure by ^{1}H NMR, ESI-MS, and rpHPLC: MS 914.53 MH^+ 457.77 $M2H^{2+}$; HRMS 914.5269 MH^+, calcd for $C_{47}H_{68}N_{11}O_8^+$ 914.5247; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.36 (d, J = 7.6 Hz, 1H), 8.26 (d, J = 8.06 Hz, 1H), 8.04 (d, J = 8.17 Hz, 1H), 8.01 (d, J = 8.28 Hz, 1H), 7.90 (d, J = 8.50 Hz, 1H), 7.66 (br s, 2H), 7.63 (d, J = 8.07 Hz, 1H), 7.54 (t, J = 5.56 Hz, 1H), 7.31 (d, J = 7.96 Hz, 1H), 7.27−7.15 (m, 5H), 7.13 (d, J = 1.85 Hz, 1H), 7.04 (t, J = 7.30 Hz, 1H), 6.96 (t, J = 7.52 Hz, 1H), 4.57 (m, 1H), 4.50 (m, 2H), 4.30 (m, 2H), 4.22 (m, 1H), 3.54 (m, 2H), 3.18−3.07 (m, 3H), 2.96−2.87 (m, 2H), 2.77−2.67 (m, 3H), 2.02 (m, 1H), 1.90−1.62 (m, 7H), 1.75 (s, 3H), 1.61−1.50 (m, 8H), 1.39 (d, 1H), 1.15 (t, 2H), 1.08−0.93 (m, 4H), 0.70 (m, 2H). Analytical rpHPLC t_R = 11.7 min, peak at rt = 13.5 is *tert*-butylated material.

Ac-Phe-[Orn-Pro-D-Cha-Trp-Arg], 1 (TFA Salt). A solution of the fully deprotected hexapeptide Ac-Phe-Orn-Pro-Cha-Trp-Arg-OH (100 g, 105 mmol) in DMF (1 L) and diisopropylethylamine (100 mL, 570 mmol, 5.5 equiv) was stirred at rt until homogeneous and then cooled to −10 °C. BOP reagent (solid 50 g, 113 mmol, 1.08 equiv) was added, and the solution was stirred at −10 to −5 °C for 2 h. The DMF was evaporated, and the residue was dissolved in 1-butanol/EtOAc 3:1 (1 L) and washed with brine (300 mL), 2 M HCl (300 mL), and water (2 × 300 mL). The solvent was evaporated in vacuo, and the residue was triturated with ether giving a pale cream solid 98 g. The solid product was filtered off, washed on the filter with ether, and dried under high vacuum. The crude product dries to a nonhygroscopic powder. Analysis of the crude cyclic peptide by analytical HPLC shows the desired product together with a minor diastereomer in the ratio 96:4. The cyclization has been done on two batches of 100 g with similar results. The crude product (98 g) was dissolved in 50% MeCN/50% water (1 L) and TFA (10 mL) with stirring and warming at approximately 40 °C for 15 min. The cloudy solution was applied to a column of reversed-phase C18 silica gel (Fluka cat. 60757) of depth 10 cm contained in a sintered glass filter funnel of diameter 10 cm, and light vacuum was applied. The column was eluted with a further 500 mL of 50% MeCN/50% water, and then the combined eluent was partially evaporated on a rotary evaporator until the product began to precipitate. A small volume of MeCN was added sufficient to redissolve the precipitate, and then the solution was applied in 50 mL aliquots to a preparative HPLC column (Vydac C18, 10−15 μm, 300 Å, 50 × 250 mm) and eluted with 38% MeCN/61.9% water/0.1% TFA at 70 mL/min with UV detection at 280 nm. The peak containing the cyclic peptide (retention time 18−23 min) was fractionated into six separate vessels that were analyzed for diastereomer content by analytical HPLC (analytical HPLC conditions: column: Vydac peptide & protein C18, 300 Å 5 μm, 4.6 × 250 mm, flow rate 1 mL/min. 70% A/30% B to 55% A/45% B over 30 min where buffer A is water + 0.1% TFA, buffer B is 90% MeCN/10% water + 0.1% TFA. Retention times: linear peptide 16.2 min, cyclic product **1** 26.5 min, minor diastereomer 28.1 min). Pure fractions were combined and lyophilized giving a white powder (33 g): MS 896.52 MH^+, 448.76 $M2H^{2+}$; HRMS 896.5105 MH^+, calcd for $C_{26}H_{39}N_4O_7^+$ 896.5141. Anal. Calcd for TFA salt $C_{49}H_{66}F_3N_{11}O_9$: C, 58.3; N, 15.3. Found: C, 56.8; N, 15.5. Analytical rpHPLC t_R = 14.3 min. See Table 2 for NMR data (Table 2).

TABLE 2. ^{1}H NMR (500 MHz, DMSO-d_6, δ) for 1

residue	NH	$^3J_{NH-CH\alpha}$	Hα	Hβ	Hγ	others
AcPhe	8.11	8.4	4.50	2.69, 2.96		1.74 (Me), 7.23
Orn	7.96	7.0	4.55	1.44, 1.64	1.22	2.76, 3.38, 7.04 (NHϵ)
Pro			4.57	1.63, 1.99	1.63	3.41, 3.62
dCha	8.17	4.6	4.01	1.15, 1.26	0.93	0.62, 0.69
Trp	8.41	7.0	4.23	2.96, 3.26		7.15, 7.39, 10.86 (NH)
Arg	7.82	8.2	4.12	1.63, 1.87	1.50	3.11, 7.59 (NHϵ)

Ac-Phe-[Orn-Pro-D-Cha-Trp-Arg], 1 (Acetate Salt). The crude cyclized product (98 g) was dissolved in 50% MeCN/50% water (1 L) and glacial acetic acid (10 mL) and applied to a 10 × 10 cm precolumn of reversed-phase C18 silica gel as described above for the TFA salt. The solution obtained was purified by preparative HPLC using a solvent consisting of 38% MeCN/62% water, sodium acetate 6 g/L, adjusted to pH 6 with glacial acetic acid at 70 mL/min. Fractions containing the pure cyclic peptide were combined and partially evaporated to remove as much MeCN as possible without causing precipitation of the product. The solution (500 mL aliquots) was applied to a preparative HPLC column (Vydac C18 300 Å, 50 × 250 mm) previously equilibrated with 1% AcOH in water and eluted with 1% AcOH in water at 70 mL/min for 30 min to complete the de-salting process. The product was quickly removed from the column by elution with 50% MeCN/49% water/1% AcOH, and the solution was lyophilized giving the acetate salt as a white powder (31 g): MS 896.52 MH^+, 448.76 $M2H^{2+}$. Analytical rpHPLC t_R =14.3min.

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Supporting Information Available: Reproductions of ^{1}H NMR spectra and HPLC chromatograms of **1** and intermediate peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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