Synthesis of the Repeating Decapeptide Unit of Mefp1 in Orthogonally Protected Form

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Mefp1 is a protein produced by the marine mussel, *Mytilus edulis*, which helps the organism to adhere to surfaces in turbulent waters. To better understand the nature of the adhesion process, we sought to synthesize homogeneous oligopeptides based on the repeating decapeptide unit of the protein. The fully protected decapeptide **10** has been synthesized from appropriately protected amino acid building blocks using a fragment condensation strategy. A key feature of the strategy is the late incorporation of the synthetically valuable dihydroxyproline residue. This synthesis of the orthogonally protected repeating decapeptide unit of Mefp1 represents an important first step toward producing useful quantities of homogeneous oligopeptides related to the protein.

Introduction

A family of adhesive proteins, isolated from the edible blue mussel, *Mytilus edulis*, is believed to be responsible for the adherance of the organism to surfaces in turbulent waters. Of the adhesive proteins, *M. edulis* foot protein 1 (Mefp1, **1**, Figure 1) was the first to be isolated and studied. Waite and Tanzer reported the isolation of Mefp1 as early as 1981[1] and proposed amino acid sequences for the commonly repeated fragments in 1985.[2] They described the "polyphenolic protein" as highly basic, with a molecular weight of around 125 000.

The protein has considerable potential as an adhesive with medical and dental applications.[3,4] For an adhesive to be useful in a physiological environment, it needs to be biocompatible (i.e., nontoxic, with a low propensity to generate an immune response). It must be able to perform in an aqueous environment and not interfere with the natural healing process. A protein that is a proven adhesive in turbulent waters is thus an attractive proposition. Previous studies of the macroscopic properties of Mefp1 have included investigations into the surface properties of films containing the protein,[5] analysis of the protein in dilute aqueous solution,[6] and the adsorption and behavior of the protein on polymer films.[7,8]

The sequence of the principal repeating decapeptide unit was thought to be decapeptide **2** (Figure 2), and a substantial body of work has been based on this original sequence, e.g., molecular modeling of oligomers of decapeptide **2** to predict their conformation[9] and an NMR study that demonstrated that decapeptide **7** ($n = 1$) adopts a bent helical conformation in solution.[10]

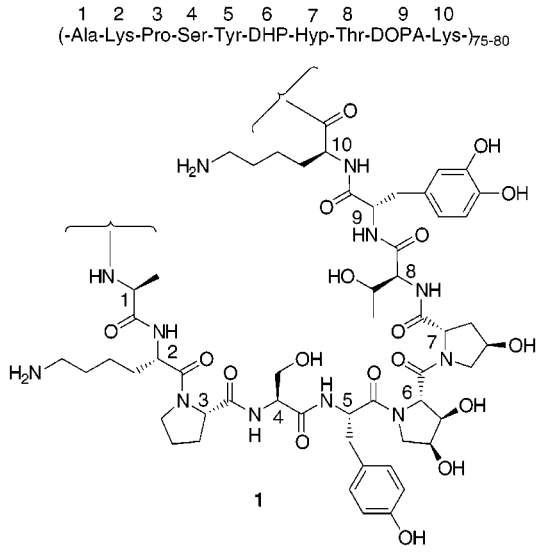

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Figure 1.

In 1994, a revised sequence for the repeating unit was unveiled. It was discovered that the residue in the sixth position is in fact L-*trans*-2,3-*cis*-3,4-dihydroxyproline (DHP), not *trans*-3-hydroxyproline.[11] It is now accepted that Mefp1 consists of the repeating unit represented by decapeptide **1**,[12] which occurs 75−80 times in nature.

A current barrier to the investigation and utilization of Mefp1 is the lack of protein. Extraction from natural sources is not efficient, although Sigma now sells the natural material under the tradename BioGlue; it fetches (U.S.) $81.55/mg.[13] While this is a tremendous step

(1) Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038−1040.
(2) Waite, J. H.; Housley, T. J.; Tanzer, M. L. *Biochemistry* **1985**, *24*, 5010−5014.
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(6) Deacon, M. P.; Davis, S. S.; Waite, J. H.; Harding, S. E. *Biochemistry* **1998**, *37*, 14108−14112.
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(11) Taylor, S. W.; Waite, J. H.; Ross, M. M.; Shabanowitz, J.; Hunt, D. F. *J. Am. Chem. Soc.* **1994**, *116*, 10803−10804.
(12) The decapeptide **1** and related compounds are drawn in such a way as to depict the conformation in which all X-Pro peptide bonds adopt the *trans* orientation. We appreciate and find interesting the possibility that other conformations may be significant.
(13) Sigma Catalogue, 1998, Sigma-Aldrich Chemical Co., p 62.

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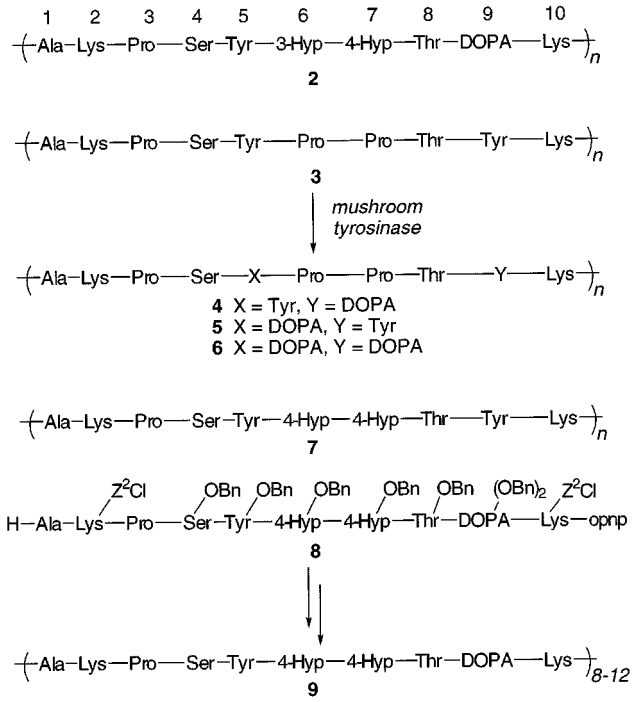

Figure 2.

forward, there is clearly a niche for useful quantities of homogeneous protein of synthetic origin.[14]

Researchers at Genex have cloned the DNA encoding Mefp1 and expressed the protein in yeast.[3] The protein produced in this manner (decapeptide **3**) contains no posttranslational hydroxylation of proline or tyrosine residues. It was possible to convert about 50% of the tyrosine residues, at both positions 5 and 9, to DOPA residues via enzymatic hydroxylation. This synthetic protein, a heterogeneous polymer represented by structures **4**−**6**, displayed promising adhesive behavior in a "wound healing model system" in animals. While this represents a valid approach to the development of adhesive substances, molecular biology fails to deliver the native protein. Moreover, it cannot be relied upon to produce homogeneous analogues in a predictable fashion. In reality, access to peptides and proteins containing uncoded amino acids, like Mefp1, is most likely to be via chemical synthesis.

Much attention has been focused on the role of DOPA and lysine in the adhesion mechanism of the protein. Indeed, polymers and copolymers of these residues have been prepared and studied by Yamamoto[15−17] and more recently by Deming.[18] Deming has put forward the hypothesis that it is the side chain functionality of these key amino acids, rather than the amino acid sequence per se, that is crucial for moisture-resistant adhesion. His group has recently demonstrated the importance of the DOPA residue: the catechol functionality is critical for moisture-resistant adhesion, while the oxidized form (the *ortho*-quinone) is largely responsible for cross-linking.[19] While the behavior of the protein is likely to be dominated by the functional groups of these amino acids, particularly under basic, oxidative conditions, we are intrigued by the high incidence of imino acids in the protein and their degree of hydroxylation. It has been suggested that the role of the hydroxyprolines is to "provide additional hydroxyl groups without losing the structure breaking characteristics of proline."[3] Not all adhesive proteins produced by marine organisms contain DOPA,[20,21] signaling that mechanisms other than quinone tanning are operative. It is also interesting that oxidative cross-linking has been observed in plant cell wall proteins, which are rich in proline and hydroxyproline.[22]

Previous Synthetic Studies. The decapeptide has been described as "a curious assortment of amino acids",[2] and as such it presents a considerable synthetic challenge. There have been two reports to date on the synthesis of decapeptide sequences related to **1**. Swerdloff et al. used a stepwise, solid-phase strategy to produce decapeptide **7** (n =1).[23] They employed the Boc group for temporary Nα-protection and the following side chain protecting groups: benzyl ethers (4-Hyp, Ser, Thr), 2-Cl-Cbz or trifluoroacetamide (Lys), and Br-Cbz (Tyr). A key feature of this synthesis was a two-step cleavage/deprotection using trifluoromethanesulfonic acid (TFMSA). Decapeptide **7**, produced in this fashion, is now marketed by Sigma.

Yamamoto used a fragment condensation approach to the synthesis of polypeptide **9** (Figure 2).[24] He also employed Boc protection for α-amino groups, accompanied by side chain protecting groups based on the benzyl group. The polymerization of **8** was described to give polymers containing 8−12 decapeptide units.

Both of these syntheses were effective in producing adhesive peptides. However, they were performed before the disclosure that the sixth residue was DHP. We wanted to revisit the synthesis of adhesive decapeptides in light of the revised structure and with some slightly different goals. Our aims were to (a) incorporate DHP, (b) produce a homogeneous decapeptide, and (c) use an orthogonal protecting group strategy that would later permit the synthesis of larger peptides in a controlled manner.

Results and Discussion

Retrosynthetic Analysis. The repetitive nature of the sequence lends itself to the fragment condensation approach. A logical target for the synthesis of adhesive peptides/proteins related to Mefp1 is a decapeptide unit. The manner in which the repeating unit is depicted in Figure 1 (running from Ala^1 to Lys^{10}) reflects the major fragment observed in tryptic digests of the protein. From the retrosynthetic standpoint, there are in fact 10 possible sites for disconnection. For example, the decapeptide could run from Ala^1 to Lys^{10} (1−2−3−4−5−6−7−8−9−10), from Lys^2 to Ala^1 (2−3−4−5−6−7−8−9−10−1), or perhaps from Pro^3 to Lys^2 (3−4−5−6−7−8−9−10−

(14) Yamamoto, H. *Biotech. Genetic Eng. Rev.* **1995**, *13*, 133−165.
(15) Yamamoto, H.; Nagai, A. *Marine Chem.* **1992**, *37*, 131−143.
(16) Yamamoto, H.; Ogawa, T.; Nishida, A. *J. Colloid Interface Sci.* **1995**, *176*, 105−110.
(17) Yamamoto, H.; Ogawa, T.; Ohkawa, K. *J. Colloid Interface Sci.* **1995**, *176*, 111−116.
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(19) Yu, M.; Hwang, J.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 5825−5826.
(20) Burzio, L. O.; Burzio, V. A.; Silva, T.; Burzio, L. A.; Pardo, J. *Curr. Opin. Biotechnol.* **1997**, *8*, 309−312.
(21) Kamino, K.; Odo, S.; Maruyama, T. *Biol. Bull.* **1996**, *190*, 403−409.
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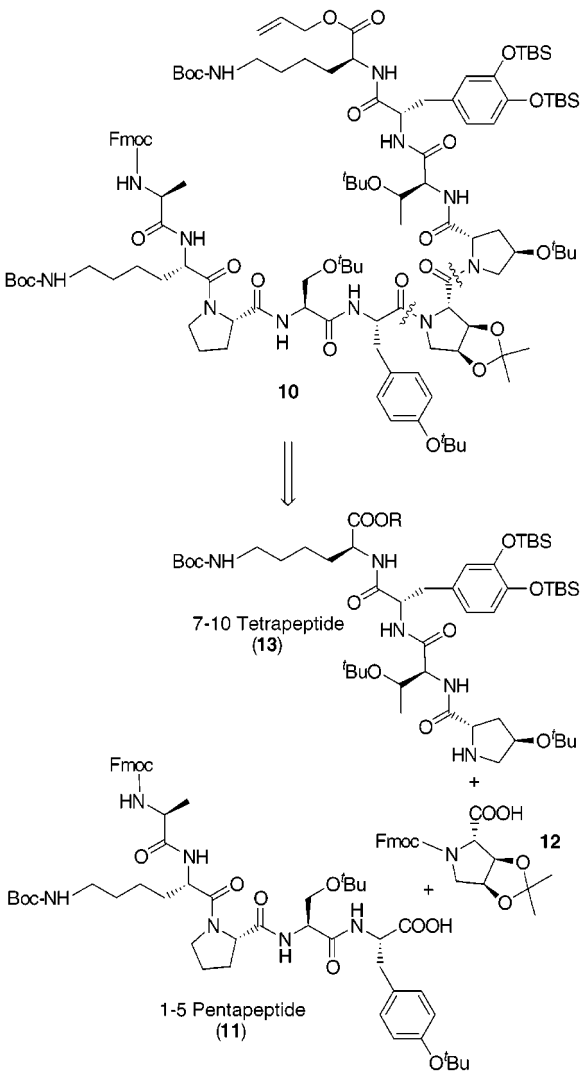
(23) Swerdloff, M. D.; Anderson, S. B.; Sedgwick, R. D.; Gabriel, M. K.; Brambilla, R. J.; Hindenlang, D. M.; Williams, J. I *Int. J. Pept. Protein Res.* **1989**, *33*, 318−317.
(24) Yamamoto, H. *J. Chem. Soc., Perkin Trans. 1* **1987**, 613−618.

Scheme 1



1−2). Despite the various possibilities, we ultimately elected decapeptide **1**, as depicted, as our target. The most difficult peptide bond constructions were anticipated to involve the proline residues as amino components. We therefore sought to bury these residues within the decapeptide framework, so that larger fragment condensations (e.g., [10 + 10] or [10 + 20]) would not involve these imino acids.

While solid-phase peptide synthesis[25] has many advantages, "conventional coupling procedures with sterically hindered or *N*-methyl amino acids often result in incomplete couplings under SPPS conditions."[26] The novelty of the amino acid collection in Mefp1 encouraged us to use solution-phase chemistry, even for assembly of the decapeptide unit. With this approach, we could carefully monitor each reaction and characterize each reaction intermediate.

We chose decapeptide **10** (Scheme 1) as our primary target. All side chain protective groups are acid labile, and we hope that it will be possible to remove them all in a single step. Interestingly, there are only two amino acids in the peptide (Ala[1] and Pro[3]) that do not contain a side chain functionality. The *N*-terminus of **10** could be liberated by treatment with a mild base to effect β-elimination of the Fmoc group.[27] The *C*-terminus ought to be freed up by treatment with Pd(0).[28] This orthogonal scheme for protection should ultimately make it possible to produce oligomers in a completely controlled fashion.

Our retrosynthetic analysis of target **10** is illustrated in Scheme 1. In planning our synthesis, careful consideration was given to the relative accessibility of the 10 amino acid building blocks. Most are readily available from commercial sources; there are two notable exceptions. While both enantiomers of DOPA are commercially available, little is known about the incorporation of this amino acid into peptides.[29] We chose to protect the catechol functionality as its bis-*tert*-butyldimethylsilyl derivative, as described by Nakonieczna et al.[30] Dihydroxyproline is not commercially available in any isomeric form. We adopted Fleet's chemistry[31] to produce a dihydroxyproline building block,[32] which is, by far, our most valuable residue. We therefore sought to incorporate the DHP residue late in the synthesis. It is worth noting that, if DHP were substituted for residue 6 in Yamamoto's synthesis,[27] it would need to be carried through five peptide coupling reactions plus associated deprotection steps. Our plan involved attachment of DHP derivative **12** to a suitably protected 7−10 tetrapeptide **13**, followed by deprotection of the amino terminus and a [5 + 5] fragment condensation to generate decapeptide **10**. We were cognisant of the fact that this would be a challenging final step (vide supra).

Synthesis of the 1−5 Pentapeptide. Our initial approach to the 1−5 pentapeptide involved a stepwise assembly, outlined in Scheme 2. The Fmoc group could be removed from the *N*-terminus of dipeptide **16**, in readiness for attachment of the third residue. Expedient purification of **17** and direct treatment with Fmoc-Pro-OH, under appropriate coupling conditions gave, at best, a 70% yield of tripeptide **18**. During the purification and coupling, amine **17** was undergoing a competitive intramolecular reaction, in which diketopiperazine **19** was formed. While tripeptide **18** was carried through to the 1−5 pentapeptide **20**, this route was abandoned because of the irreproducible ratios of **18** and **19** obtained and also because we were never able to selectively remove the methyl ester at the *C*-terminus of **20**.

Retrospectively, these problems were not surprising. To move forward we made two changes: protection of the *C*-terminal Tyr residue as an allyl ester and a [2 + 1] approach to the assembly of the tripeptide. This required an efficient synthesis of Fmoc-Pro-Ser(O^{t}Bu)-OH (**21**). *N*α-Fmoc-protected dipeptide acids (viz., **21** and **22**) were

(25) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154. (b) Merrifield, R. B. *Science* **1986**, *232*, 341−347.

(26) Angell, Y. M.; García-Echeverría, C.; Rich, D. H. *Tetrahedron Lett.* **1994**, *35*, 5981−5984.

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(28) Kunz, H.; Waldmann, H.; Unverzagt. C. *Int. J. Pept. Protein Res.* **1985**, *26*, 493.

(29) In addition to ref 24, see the following papers for the incorporation of DOPA into small peptides: (a) Bai, J. P. F. *Pharm. Res.* **1995**, *12*, 1101−1104. (b) Nakonieczna, L.; Przychodzen, W.; Chimiak, A. *Amino Acids* **1995**, *8*, 109−112. (c) Yamamoto, Y.; Nagai, A.; Harushima, Y.; Senda, T. In *Peptides 1992;* Schneider, C. H., Eberle, A. N., Eds.; ESCOM Science Publishers: B.V., 1993; pp 165−166.

(30) (a) Nakonieczna, L.; Przychodzen, W.; Chimiak, A. In *Peptides 1992*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM Science Publishers: B. V., 1993; pp 194−195. (b) Nakonieczna, L.; Przychodzen, W.; Chimiak, A. *Liebigs Ann. Chem.* **1994** 1055−1058.

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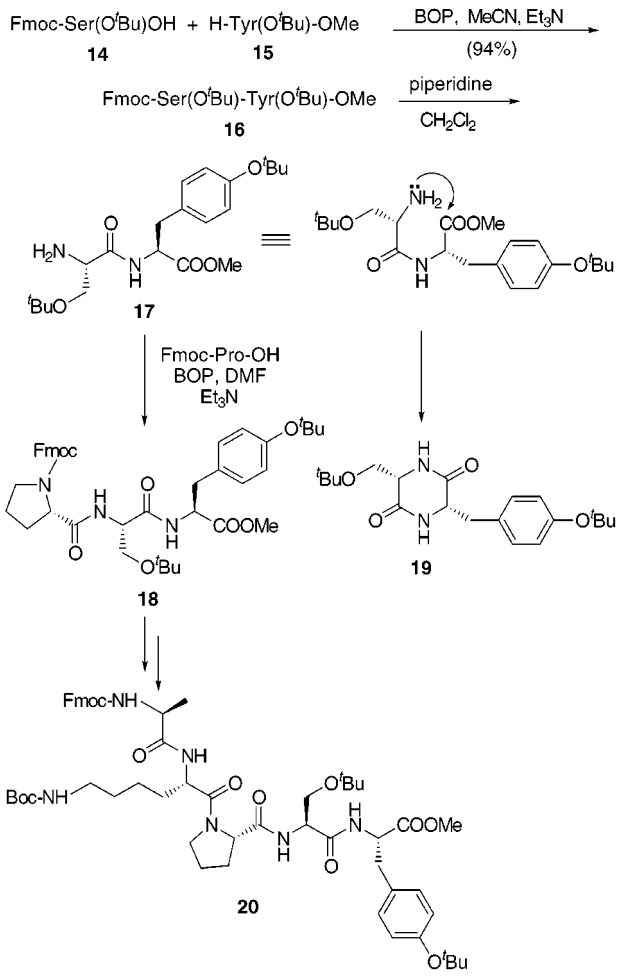
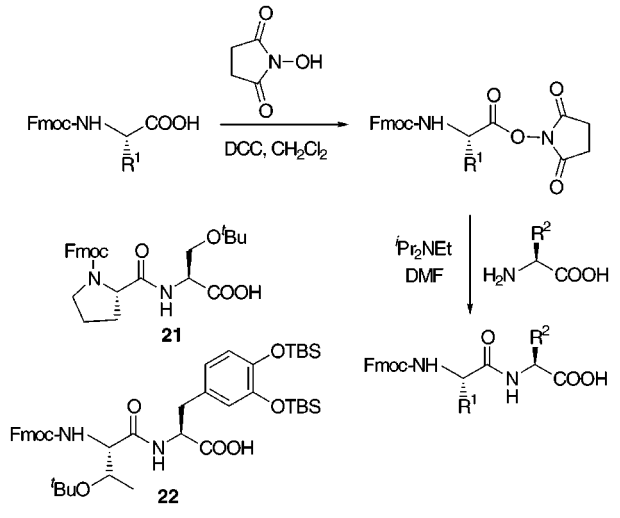
(32) Weir, C. A.; Taylor, C. M. *J. Org. Chem.* **1999**, *64*, 1554−1558.

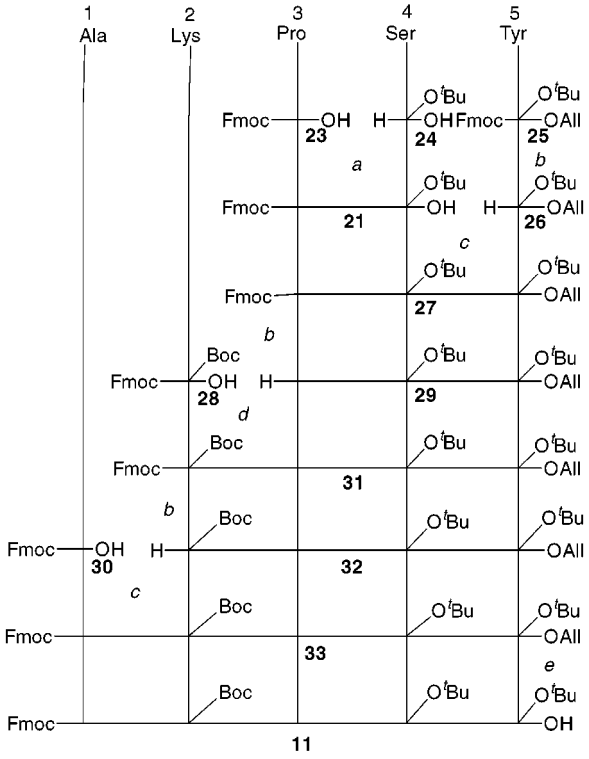
Scheme 2



Scheme 3



prepared according to Scheme 3. Formation of the activated, *N*-hydroxysuccinimide esters is readily achieved using NHS and DCC.[33] Reaction of the resulting NHS esters with a free amino acid, in the presence of a hindered nonnucleophilic base, gives good yields of the dipeptide acids, which do not require purification. Such dipeptide acids are useful carboxyl components in the formation of tripeptides via a [2 + 1] coupling. Care must be taken to monitor and minimize racemization in these reactions. However, in our hands, this approach is preferable to the alternative [1 + 2] strategy described above, wherein diketopiperazine formation is a troublesome side reaction.



a (i) acid, NHS, DCC, CH_2Cl_2; (ii) amino acid, DMF, $^iPr_2NEt$; *b* Et_2NH, CH_2Cl_2; *c* BOP, $^iPr_2NEt$, CH_2Cl_2; *d* BroP, $^iPr_2NEt$, CH_2Cl_2; *e* $Pd(PPh_3)_4$, dimedone, THF.

Figure 3.

The ultimately successful strategy for our synthesis of pentapeptide **11** is summarized in Figure 3. Treatment of Fmoc-Tyr(O^{t}Bu)-OAll (**25**) with diethylamine effected removal of the Fmoc group. Amine **26**, generated in situ[34] was coupled with dipeptide acid **21** using Castro's BOP reagent.[35] While tripeptide **27** appeared as a single species by ^{13}C NMR, we were vigilant toward the possibility of epimerization of the Ser residue during this coupling. The potential problem was highlighted in a recent paper by Di Fenza et al., which described racemization studies of Fmoc-Ser(O^{t}Bu)-OH during stepwise continuous-flow solid-phase peptide synthesis.[36] We synthesized Fmoc-Pro-D-Ser(O^{t}Bu)-Tyr(O^{t}Bu)-OAll, to determine whether it was present in product **27**. The two diastereoisomeric tripeptides had essentially identical ^{1}H NMR spectra and could not be separated by HPLC under a variety of conditions. Fortunately, the ^{13}C NMR spectra demonstrated that **27** and its diastereomer were two different compounds, which were not contaminated by each other.

Removal of the Fmoc group from tripeptide **27** was performed in situ. Coupling of the Lys residue to give **31**

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(34) Colucci, W. J.; Tung, R. D.; Petri, J. A.; Rich, D. H. *J. Org. Chem.* **1990**, *55*, 2895−2903.

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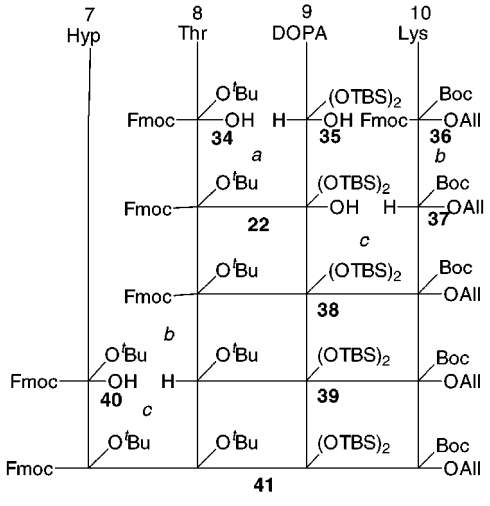
(36) Di Fenza, A.; Tancredi, M.; Galoppini, C.; Rovero, P. *Tetrahedron Lett.* **1998**, *39*, 8529−8532.



a (i) acid, NHS, DCC, CH_2Cl_2; (ii) amino acid, DMF, $^i Pr_2NEt$; *b* Et_2NH, CH_2Cl_2; *c* BOP, $^i Pr_2NEt$, CH_2Cl_2.

Figure 4.

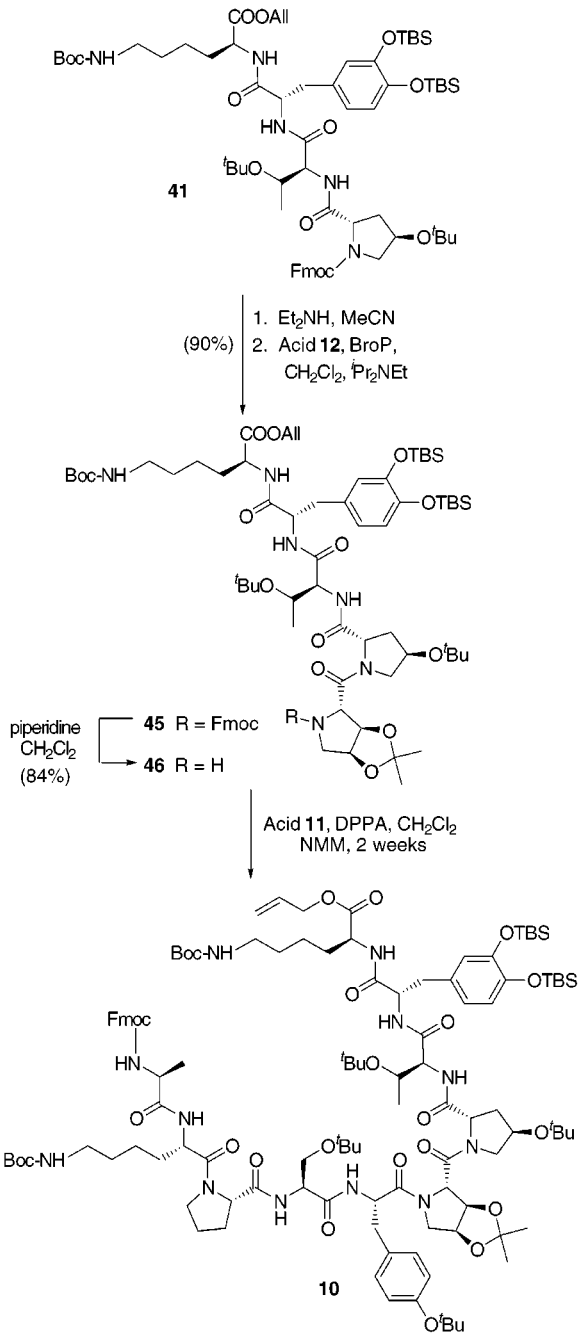
was slow and gave only a 48% yield using the BOP reagent. This is presumably due to the secondary nature of the amine in the Pro residue,[37] which serves as the nucleophile in this reaction. A much-improved yield was obtained using the BroP[38] reagent. The *N*-terminal Ala residue was appended, in an uneventful manner, to give fully protected pentapeptide **33** in 85% overall yield. Treatment of **33** with Pd(0), with added dimedone,[28] gave the free acid **11**, which was obtained in good yield after HPLC purification.

Synthesis of the 7−10 Tetrapeptide. The synthesis of tetrapeptide **41** is outlined in Figure 4. Dipeptide acid **22** was coupled with amine **37** (generated in situ from **36**), in an analogous fashion to that described above. The *trans*-4-hydroxyproline (Hyp) residue was appended using the BOP reagent to give tetrapeptide **41**.

Assembly of the Decapeptide. The final steps of the synthesis are summarized in Scheme 4. We previously reported on the behavior of DHP building block **12** as the carboxyl component in peptide bond formation.[32] On the basis of these model studies, the *N*-terminus of tetrapeptide **41** was deprotected using diethylamine. The resulting amine was coupled with DHP acid **12**, using the BroP reagent to give pentapeptide **45** in excellent yield.

Attempts to deprotect the *N*-terminus of **45 i**n situ and proceed directly with the [5 + 5] coupling were unsuccessful, largely because of solubility problems. The Fmoc group was therefore removed using piperidine, and amine **46** was purified by flash chromatography prior to further reaction. Our previous work investigating the behavior of DHP as an amino component in coupling reactions was rather discouraging.[32] The sterically hindered nature of the amine, the rigidity of the bicyclic system, and inductive electron withdrawl by the oxygen substituents on the pyrrolidine ring conspire to make the dihydroxy-

Scheme 4



proline a poor nucleophile.[39] The lethargic nature of amide bond formation reactions involving DHP-based amino components means that side reactions can be competitive. Fortunately, minimal racemization was observed when DPPA[40] was employed as coupling reagent.

Reaction of equimolar quantities of the 1−5 pentapeptide acid **11** and 6−10 pentapeptide amine **46**, in the presence of diphenylphosphoryl azide (DPPA) and 1 equiv of *N*-methylmorpholine, gave rise to two products over the course of 2 weeks. The less polar compound was

(37) Wijkmans, J. C. H. M.; Kruijtzer, J. A. W.; van der Marel, G. A.; van Boom, J. H.; Bloemhoff, W. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 394−397.

(38) (a) Castro, B.; Dormoy, J. R. *Bull. Soc. Chim. Fr.* **1973**, 3359−3361. (b) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669−672. (c) Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437−2446.

(39) For a comprehensive review of difficult peptide coupling reactions see: Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243−2266.

(40) (a) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205. (b) Benoiton, N. L.; Kuroda, K.; Chen, F. M. F. *Int. J. Pept. Protein Res.* **1982**, *20*, 81−86.

derived from carboxyl component **11**, and its identity is yet to be confirmed. The more polar compound was the desired decapeptide **10**. Conventional RP-HPLC, using MeCN−H_2O−TFA solvent systems, was not effective for purification. The crude product mixture could be dissolved in MeCN−H_2O mixtures and injected onto the column, but the decapeptide was never eluted. The choice of protecting groups and the fact that one-third of the peptide bonds are tertiary amides[41] leads to an extremely hydrophobic molecule, which appears to be retained within the C-18 column packing. Lloyd-Williams, Alberico, and Giralt have described strategies for the purification of very hydrophobic peptides.[42] While they did not rate normal phase (SiO_2) HPLC highly for their purposes, this approach turned out to be satisfactory for the isolation and purification of decapeptide **10**. The identity of the decapeptide was confirmed by HRMS and ^{1}H NMR spectroscopy.

Summary

We have successfully completed a fragment condensation synthesis of decapeptide **10**, in a convergent and efficient manner. This represents an important first step toward the controlled synthesis of oligopeptides related to Mefp1. Future plans involve the selective deprotection of the two terminii and the condensation of two decapeptides, in solution, to give a 20-mer. Another approach that may prove useful is the convergent solid-phase method, wherein one fragment is attached to a solid support.[43] It will be interesting to see how the size of the peptides affects their conformation and adhesive properties.

Experimental Section

General. Fmoc-Ala-OH, Fmoc-Hyp(O^{t}Bu)-OH, and Fmoc-Thr(O^{t}Bu)-OH were obtained from Bachem. Fmoc-Pro-OH, L-DOPA, and *tert*-butyldimethylsilyl chloride were obtained from Lancaster. H-Ser(O^{t}Bu)-OH, Fmoc-Tyr(O^{t}Bu)-OH, and Fmoc-Lys(ϵ-Boc)-OH were obtained from Sigma. *N*-Hydroxysuccinimide, $Pd(PPh_3)_4$, BOP reagent, and cesium carbonate were obtained from Acros. DPPA was obtained from Fluka, and dimedone was obtained from Aldrich. Dicyclohexylcarbodiimide and allyl bromide were obtained from Riedel de Haën. BroP reagent was prepared according to ref 38a. DOPA-$(OTBS)_2$ was prepared according to ref 30b. Dichloromethane and acetonitrile were freshly distilled from CaH_2. DMF was dried and distilled from BaO and stored over 4 Å molecular sieves. Methanol was dried and distilled from magnesium turnings and stored over 4 Å molecular sieves. Piperidine, diisopropylethylamine, triethylamine, *N*-methylmorpholine, and diethylamine were each dried and distilled from CaH_2 and stored over KOH pellets.

General Procedure for the Synthesis of Dipeptide Acids. *N*-Hydroxysuccinimide (1.0 equiv) was added to a suspension of the Fmoc-protected amino acid (1.0 equiv) in dry CH_2Cl_2 (∼8 mL per mmol) at 0 °C under N_2. DCC (1.0 equiv) was added, and the mixture was stirred at 0 °C for 20 min, warmed to room temperature, and stirred for 4.5 h. The suspension was filtered through a plug of cotton in a Pasteur pipet, washing well with CH_2Cl_2. The filtrate was concentrated to about one-fifth of its original volume, stoppered, and left to stand in the refrigerator for 2 h. The mixture was filtered again and concentrated to give a colorless foam. This was dissolved in dry DMF (∼2.5 mL per mmol), and the solution was cooled to 0 °C under N_2. The free amino acid, with appropriate side chain protection (1.0 equiv), was added, followed by the dropwise addition of diisopropylethylamine (1.0 equiv). The mixture was gradually allowed to warm to room temperature and stirred for 17 h. The mixture was filtered, if necessary, through a plug of cotton in a Pasteur pipet, rinsing with EtOAc. The mixture was diluted with EtOAc (∼40 mL per mmol) and washed with 5% aqueous HCl (equal volume). The aqueous layer was extracted with EtOAc (∼40 mL per mmol). The organic layers were combined, washed with water (∼80 mL per mmol), filtered through $MgSO_4$, and concentrated.

Fmoc-Pro-Ser(O^{t}Bu)-OH (21). According to the general procedure above on a scale of 1.19 mmol to give **21** as a colorless foam (570 mg, 95%): TLC R_f 0.25 (9:1 CH_2Cl_2−MeOH); $[\alpha]^{20}_D = -27.1°$ (*c* 1.06, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 200 MHz, 350K) δ 1.06 (s, 9H), 1.70−2.25 (m, 4H), 3.38−3.50 (m, 2H), 3.53 (dd, J = 9.2, 4.6 Hz, 1H), 3.66 (dd, J = 9.2, 4.6 Hz, 1H), 4.20−4.48 (m, 4H), 7.28−7.44 (m, 4H), 7.62−7.67 (m, 2H), 7.84 (d, J = 7.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz, 350K) δ 22.9, 26.6, 29.9, 46.4, 46.5, 52.5, 59.3, 61.2, 66.5, 72.2, 119.4, 119.5, 124.6, 124.7, 126.6, 126.7, 127.1, 128.4, 140.3, 143.4, 143.6, 153.8, 170.9, 171.3; HRMS (DCI) calcd for $(M + H)^+$ $C_{27}H_{33}N_2O_6$ 481.233860, obsd 481.23305.

Fmoc-Thr(O^{t}Bu)-DOPA(OTBS)$_2$-OH (22) According to the general procedure above on a scale of 0.94 mmol to give **22** as a colorless foam (704 mg, 94%): TLC R_f 0.47 (2:1 EtOAc−hexane); $[\alpha]^{20}_D = +33.0°$ (*c* 1.27, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.07 (s, 12H), 0.87 (s, 18H), 0.96 (d, J = 6 Hz, 3H), 1.09 (s, 9H), 2.79−3.23 (m, 2H), 4.04−4.16 (m, 3H), 4.28 (d, J = 6.6 Hz, 2H), 4.70 (m, 1H), 5.95 (br s, 1H), 6.59−6.67 (m, 3H), 7.15−7.34 (m, 4H), 7.50 (m, 2H), 7.66 (d, J = 7.3 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 1.5, 16.3, 18.3, 18.4, 25.9, 28.0, 36.7, 47.1, 53.4, 58.3, 66.7, 67.0, 75.5, 119.9, 121.0, 121.9, 122.1, 125.1, 127.0, 127.7, 128.7, 141.3, 143.7, 143.9, 146.1, 146.8, 155.9, 169.4, 175.7; HRMS (FAB) calcd for M^+ $C_{44}H_{65}N_2O_8Si_2$ 805.42798, obsd 805.4328.

General Procedure for the Synthesis of Allyl Esters. Cesium carbonate (0.5 equiv) was added to a suspension of the Fmoc-protected amino acid (1.0 equiv) in dry methanol (∼5 mL per mmol). The suspension soon gave way to a homogeneous solution, which was stirred at room temperature under N_2 for 2 h. The mixture was concentrated to give a colorless foam, which was dissolved in DMF (∼5 mL per mmol). Allyl bromide (1.2 equiv) was added,and the mixture was stirred at room temperature under N_2 for 18 h. The mixture was partitionned between EtOAc (50 mL per mmol) and water (50 mL per mmol). The organic layer was washed with brine (50 mL per mmol), dried over $MgSO_4$, filtered, and concentrated.

Fmoc-Tyr(O^{t}Bu)-OAll (25). According to the general procedure above on a scale of 0.54 mmol. The residue was purified by flash chromatography, eluting with 5:1 hexanes−EtOAc to give Fmoc-Tyr(O^{t}Bu)-OAll (**25**) (540 mg, 98%) as an oil: TLC R_f 0.29 (5:1 hexanes−EtOAc); $[\alpha]^{20}_D = -10.1°$ (*c* 1.25, EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.32 (s, 9H), 3.08 (d, J = 5.8 Hz, 2H), 4.21 (t, J = 6.8 Hz, 1H), 4.30−4.48 (m, 2H), 4.59−4.71 (m, 3H), 5.21−5.34 (m, 2H), 5.76−5.93 (m, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 7.27−7.44 (m, 4H), 7.57 (d, J = 7.2 Hz, 2H), 7.77 (dd, J = 6.8, 0.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.8, 37.6, 47.1, 54.8, 66.0, 66.9, 78.3, 119.0, 119.9, 124.1, 125.0, 126.9, 127.6, 129.7, 130.4, 131.3, 141.2, 143.7, 143.8, 154.4, 155.4, 171.2; HRMS (FAB) calcd for $(M + H)^+$ $C_{31}H_{34}NO_5$ 500.24370, obsd 500.24495.

Fmoc-Lys(ϵ-Boc)-OAll (36). According to the general procedure above on a scale of 1.07 mmol. The residue was purified by flash chromatography, eluting with 3:1 hexanes−ethyl acetate, increasing the polarity to 1:1 hexanes−ethyl acetate, to give Fmoc-Lys(ϵ-Boc)-OAll (**36**) (508 mg, 94%) as a colorless foam: R_f 0.23 (3:1 hexanes−ethyl acetate); $[\alpha]^{20}_D$ =

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−10.3° (*c* 1.12, EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 (s, 9H), 1.17−1.78 (m, 6H), 3.02 (q, J = 5.8 Hz, 2H), 4.13 (t, J = 7.0 Hz, 1H), 4.29−4.34 (m, 2H), 4.56 (d, J = 5.6 Hz, 2H), 5.17 (dd, J = 10.4, 1.5 Hz, 1H), 5.24 (dd, J = 17.2, 1.5 Hz, 1H), 5.40 (br d, J = 7.6 Hz, 1H), 5.79 (ddt, J = 17.2, 10.4, 5.6 Hz, 1H), 7.17−7.35 (m, 4H), 7.52 (d, J = 7.0 Hz, 2H), 7.67 (d, J = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.3, 28.4, 29.5, 32.1, 40.0, 47.1, 53.7, 65.9, 66.9, 79.1, 118.9, 119.9, 125.0, 127.0, 127.6, 131.4, 141.2, 143.7, 143.8, 156.0 (2C), 172.1; HRMS (FAB) calcd for M^+ $C_{29}H_{36}N_2O_6$ 508.25733, obsd 508.25893.

Fmoc-Pro-Ser(O^{t}Bu)-Tyr(O^{t}Bu)-OAll (27). Diethylamine (2 mL) was added to a suspension of Fmoc-Tyr(O^{t}Bu)-OAll (**25**) (125 mg, 0.270 mmol, 1.00 equiv) in dry acetonitrile (2 mL). The solution was stirred at room temperature under N_2 for 30 min and then concentrated. The residue was taken up in acetonitrile (3 mL) and concentrated again. The residue was dissolved in dry CH_2Cl_2 (3 mL) and cooled to 0 °C. Diisopropylethylamine (113 μL, 84 mg, 0.648 mmol, 2.40 equiv) was added, followed by Fmoc-Pro-Ser(O^{t}Bu)-OH (**21**) (136 mg, 0.283 mmol, 1.0 equiv), and finally BOP reagent (125 mg, 0.283 mmol, 1.05 equiv). The flask was flushed with N_2, stoppered, and left to stir for 3 days. The orange solution was concentrated, and the product was isolated by flash column chromatography, eluting with 1:1 EtOAc−hexanes to give tripeptide **27** as a colorless oil (194 mg; 97%): TLC R_f 0.30 (1:1 EtOAc−hexanes); $[\alpha]^{20}_D$ = −5.0° (*c* 0.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.12 (s, 9H), 1.30 (s, 9H), 1.8−2.3 (m, 2H), 3.05 (m, 2H), 3.39−3.77 (m, 6H), 4.24−4.50 (m, 6H), 4.54 (d, J = 5.8 Hz, 2H), 4.80 (m, 1H), 5.20 (dd, J = 10.3, 1.3 Hz, 1H), 5.25 (d, J = 17.1, 1.3 Hz, 1H), 5.72−5.89 (m, 1H), 6.86 (d, J = 8.3 Hz, 2H), 6.98−7.00 (m, 2H), 7.30−7.48 (m, 4H), 7.55−7.58 (m, 2H), 7.76 (d, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.6, 27.3, 28.8, 29.1, 37.4, 47.0, 47.2, 53.0, 53.5, 60.8, 61.0, 65.8, 67.7, 74.0, 78.3, 118.7, 119.9, 124.0, 125.0, 127.0, 127.7, 129.7, 130.5, 131.5, 141.2, 143.9, 154.4, 155.8, 170.7, 171.7, 172.4; HRMS (FAB) calcd for $(M + H)^+$ $C_{43}H_{54}N_3O_8$ 740.39111, obsd 740.39289.

Fmoc-Lys(ϵ-Boc)-Pro-Ser(O^{t}Bu)-Tyr(O^{t}Bu)-OAll (31). Diethylamine (2 mL) was added to a suspension of tripeptide **27** (88 mg, 0.119 mmol, 1.00 equiv) in acetonitrile (2 mL). The solution was stirred at room temperature under N_2 for 30 min and then concentrated. The residue was taken up in acetonitrile (5 mL) and concentrated again. The residue was dissolved in CH_2Cl_2 (3 mL). Fmoc-Lys(ϵ-Boc)-OH (**28**) (59 mg, 0.125 mmol, 1.05 equiv) was added, followed by diisopropylethylamine (31 μL, 23 mg, 0.178 mmol, 1.5 equiv) and BroP reagent (48 mg, 0.125 mmol, 1.05 equiv). The mixture was stirred at room temperature under N_2 for 3 h and then concentrated. The residue was applied to a flash column and eluted with 3:1 EtOAc−hexanes to give tetrapeptide **31** as an oil (114 mg; 99%): TLC R_f 0.27 (2:1 EtOAc−hexanes); t_R 13.6 min (85% MeCN; 15% H_2O with 0.1% TFA) at 0.6 mL min^{-1} on a 4.6 mm C18 column); $[\alpha]^{20}_D$ = −7.4° (*c* 0.92, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.17 (s, 9H), 1.32 (s, 9H), 1.43 (s, 9H), 1.8−2.3 (m, 8H), 3.08 (m, 4H), 3.30−3.43 (m, 1H), 3.55−3.84 (m, 5H), 4.21 (t, J = 7.0 Hz, 1H), 4.37 (d, J = 7.0 Hz, 2H), 4.30−4.62 (m, 5H), 4.57 (d, J = 7.0 Hz, 2H), 4.85 (m, 1H), 5.21−5.33 (m, 2H), 5.67 (d, J = 8.5 Hz, 1H), 5.68−5.99 (m, 1H), 6.88 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 7.26−7.44 (m, 4H), 7.60 (d, J = 7.6 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.0, 24.9, 27.2, 28.5, 28.7, 29.3, 29.5, 32.1, 37.1, 40.0, 47.0, 47.3, 52.1, 52.9, 53.3, 60.2, 61.0, 65.7, 66.8, 74.3, 78.4, 78.9, 118.7, 119.8, 124.0, 125.0, 126.9, 127.6, 129.6, 130.6, 131.4, 141.1, 143.6, 143.8, 154.4, 156.1, 169.9, 170.8, 171.1, 171.8; HRMS (FAB) calcd for $(M + H)^+$ $C_{54}H_{74}N_5O_{11}$ 968.53848, obsd 968.53776.

Fmoc-Ala-Lys(ϵ-Boc)-Pro-Ser(O^{t}Bu)-Tyr(O^{t}Bu)-OAll (33). Diethylamine (2 mL) was added to a solution of tetrapeptide **31** (114 mg, 0.118 mmol, 1.00 equiv) in acetonitrile (2 mL). The solution was stirred for 30 min at room temperature under N_2 and then concentrated. The residue was taken up in acetonitrile (5 mL) and concentrated again. The residue was dissolved in CH_2Cl_2 (3 mL), and Fmoc-Ala-OH (**30**) (38 mg, 0.124 mmol, 1.05 equiv) was added, followed by triethylamine (25 μL, 18 mg, 0.177 mmol, 1.50 equiv) and then BOP reagent (55 mg, 0.124 mmol, 1.05 equiv). The mixture was stirred at room temperature for 3 h and then concentrated. The residue was applied to a flash column and eluted with CH_2Cl_2 and then 2% MeOH in CH_2Cl_2 to give pentapeptide **33** as an oil (115 mg; 94%): TLC R_f 0.40 (2:1 EtOAc−hexanes); $[\alpha]^{20}_D$ = −13.1° (*c* 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (s, 9H), 1.28 (d, J = 8.6 Hz, 4H), 1.31 (s, 9H), 1.41 (s, 9H), 1.60−2.54 (m, 6H), 3.06 (d, J = 5.4 Hz, 2H), 3.34 (t, J = 7.8 Hz, 1H), 3.63−3.94 (m, 2H), 4.23 (q, J = 6.8 Hz, 1H), 4.27−4.50 (m, 4H), 4.55 (d, J = 5.7 Hz, 2H), 4.70−4.89 (m, 2H), 5.01 (br s, 1H), 5.20 (dd, J = 6.4 Hz, 1.2 Hz, 1H), 5.27 (dd, J = 13.0, 1.1 Hz, 1H), 5.58 (br d, 1H), 5.75−5.92 (m, 1H), 6.88 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 7.30−7.43 (m, 4H), 7.59 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 19.1, 21.8, 24.9, 27.2, 28.3, 28.7, 29.1, 31.7, 36.6, 37.3, 39.9, 47.0, 47.4, 50.3, 52.8, 53.4, 60.1, 61.0, 65.7, 67.0, 74.1, 78.2, 78.7, 118.7, 119.8, 123.9, 125.0, 126.9, 127.5, 129.6, 130.5, 131.3, 141.1, 143.6, 143.8, 154.2, 155.8, 156.0, 169.5, 169.7, 170.6, 171.2, 172.1; HRMS (FAB) calcd for $(M + H)^+$ $C_{57}H_{79}N_6O_{12}$ 1039.57556, obsd 1039.57726.

Fmoc-Ala-Lys(ϵ-Boc)-Pro-Ser(O^{t}Bu)-Tyr(O^{t}Bu)-OH (11). $Pd(PPh_3)_4$ (13 mg, 0.011 mmol, 0.1 equiv) and dimedone (46 mg, 0.329 mmol, 3.0 equiv) were added to a solution of ester **33** (114 mg, 0.110 mmol, 1.0 equiv) in THF (4 mL), and the solution was stirred at room temperature, under N_2, in a flask wrapped in aluminum foil, for 2 h. The mixture was concentrated, and the orange residue was purified by RP-HPLC to give pentapeptide acid **2** (83 mg, 75%) as a colorless solid. HPLC conditions: 10 μm C-18 column, 21 mm diameter, flow rate 12 mL min^{-1}, using 75% MeCN in H_2O (with 0.1% added TFA); t_R 10.5 min. TLC R_f 0.37 (9:1 CH_2Cl_2−MeOH); $[\alpha]^{20}_D$ = −16.9° (*c* 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.13 (s, 9H), 1.30 (s, 9H), 1.42 (s, 9H), 1.80−2.05 (m, 6H), 3.05−3.68 (m, 8H), 4.20−4.72 (m, 12 H), 6.87 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 7.26−7.42 (m, 4H), 7.57 (br m, 2H), 7.75 (d, J = 7.0 Hz, 2H); HRMS (FAB) calcd for M^+ $C_{54}H_{75}N_6O_{12}$ 999.54430, obsd 999.545187.

Fmoc-Thr(O^{t}Bu)-DOPA(OTBS)$_2$-Lys(ϵ-Boc)-OAll (38). A solution of **36** (421 mg, 0.828 mmol, 1.00 equiv) in acetonitrile (3 mL) and diethylamine (3 mL) was stirred at room temperature under N_2 for 30 min and then concentrated. The residue was slurried with acetonitrile and concentrated a second time. A solution of dipeptide acid **22** (700 mg, 0.869 mmol, 1.05 equiv) in CH_2Cl_2 (3 mL; 1 mL rinse) was added to the residue under N_2. *N*-Methylmorpholine (137 μL, 126 mg, 1.24 mmol, 1.5 equiv) was added, followed by BOP reagent (384 mg, 0.869 mmol, 1.05 equiv). The mixture was stirred at room temperature for 3 days and then concentrated. The product was isolated from the residue by flash chromatography, eluting with 3:1 hexanes−EtOAc, increasing the polarity to 2:1 hexanes−EtOAc to give **38** (800 mg, 90%). TLC R_f 0.33 (2:1 hexanes−EtOAc); $[\alpha]^{20}_D$ = +2.6° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.17 (s, 12H), 0.9−1.9 (m, 6H), 0.96 (s, 18H), 1.05 (d, J = 6.2 Hz, 3H), 1.19 (s, 9H), 1.43 (2, 9H), 1.43 (s, 9H), 2.98−3.00 (m, 2H), 3.07 (q, J = 6.1 Hz, 2H), 4.09−4.15 (m, 2H), 4.22 (t, J = 7.0 Hz, 1H), 4.38 (d, J = 7.0 Hz, 2H), 4.55 (q, J = 6.8 Hz, 1H), 4.61 (dd, J = 5.8 Hz, 2H), 5.26 (dd, J = 10.4, 1.2 Hz, 1H), 5.33 (dd, J = 17.2, 1.2 Hz, 1H), 5.89 (ddt, J = 17.2, 10.4, 5.6 Hz, 1H), 6.63 (dd, J = 8.1, 2.0 Hz, 1H), 6.68 (d, J = 6.0 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 7.23 (br d, J = 8.0 Hz, 1H), 7.31 (t, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.60 (d, J = 7.3 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 1.3, 16.8, 18.3, 22.3, 25.8, 28.0, 28.3, 29.6, 31.9, 36.8, 40.1, 47.1, 52.2, 54.1, 58.5, 65.8, 66.5, 66.9, 75.6, 79.0, 118.9, 119.9, 120.8, 122.0, 125.0, 127.0, 127.6, 129.1, 131.5, 141.2, 143.6, 143.8, 145.9, 146.8, 155.9, 169.1, 170.4, 171.3; HRMS (FAB) calcd for M^+ $C_{58}H_{88}N_4O_{11}Si_2$ 1073.6066, obsd 1073.6127.

Fmoc-Hyp(O^{t}Bu)-Thr(O^{t}Bu)-DOPA(OTBS)$_2$-Lys(ϵ-Boc)-OAll (41). Diethylamine (2 mL) was added to a solution of tripeptide **38** (136 mg, 0.127 mmol, 1.00 equiv) in acetonitrile (2 mL). The solution was stirred for 30 min at room temperature under N_2 and then concentrated. The residue was taken up in acetonitrile (4 mL) and concentrated again. The residue was dissolved in CH_2Cl_2 (4 mL), and Fmoc-Hyp(O^{t}Bu)-OH (**40**)

(55 mg, 0.133 mmol, 1.05 equiv) added, followed by triethylamine (27 μL, 19 mg, 0.190 mmol, 1.50 equiv) and then BOP reagent (59 mg, 0.133 mmol, 1.05 equiv). The mixture was stirred at room temperature for 3.5 h and then concentrated. The product was isolated from the residue by flash chromatography, eluting with 1:1 EtOAc−hexanes to give tetrapeptide **41** (116 mg, 74%) as a foam: TLC R_f 0.62 (1:1 hexanes−EtOAc); $[\alpha]^{20}_D = -9.1°$ (*c* 1.34, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 12H), 0.96 (s, 18H), 1.04 (d, J = 6.3 Hz, 3H), 1.10 (s, 9H), 1.10−1.80 (m, 6H), 1.21 (s, 9H), 1.41 (s, 9H), 2.12−2.22 (m, 2H), 2.93−3.05 (m, 3H), 3.41 (dd, J = 10.6, 4.7 Hz, 1H), 3.75 (br s, 1H), 4.20−4.64 (m, 8H), 4.59 (d, J = 5.5 Hz, 2H), 4.83 (br s, 1H), 5.23 (dd, J = 10.4, 1.0 Hz, 1H), 5.30 (d, J = 17.2 Hz. 1H), 5.82−5.89 (m, 1H), 6.62−6.68 (m, 3H), 6.82 (br d, J = 7.8 Hz, 1H), 7.12 (br s, 1H), 7.30 (t, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.76 (d, J = 7.4 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.1, 14.2, 18.4, 29.9, 22.3, 22.6, 25.8, 27.0, 28.0, 28.2, 28.4, 29.4, 29.6, 29.9, 31.7, 31.8, 36.6, 37.6, 39.3, 40.1, 47.0, 52.1, 53.9, 54.2, 57.9, 59.7, 60.3, 65.7, 67.8, 69.4, 74.0, 75.1, 78.8, 118.7, 119.9, 120.6, 122.0, 121.9, 125.0, 127.0, 127.6, 129.6, 131.6, 141.2, 143.7, 145.7, 146.8, 155.5, 155.9, 169.4, 170.5, 171.3, 171.8; HRMS (FAB) calcd for M^+ $C_{67}H_{104}N_5O_{13}Si_2$ 1242.71692, obsd 1242.71669.

Fmoc-DHP[$O_2CH(CH_3)_2$]-Hyp(O^{t}Bu)-Thr(O^{t}Bu)-DOPA-(OTBS)$_2$-Lys(ϵ-Boc)-OAll (45). Diethylamine (1.0 mL) was added to a suspension of tetrapeptide **41** (53 mg, 0.043 mmol, 1.0 equiv) in acetonitrile (1.0 mL). The mixture was stirred at room temperature under N_2 for 30 min and then concentrated. The residue was taken up in acetonitrile (2 mL) and concentrated again. A solution of compound **12** (18 mg, 0.045 mmol, 1.05 equiv) in CH_2Cl_2 (2 mL; 1 mL rinse) was added to the residue under N_2. Diisopropylethylamine (11 μL, 8.3 mg, 0.06 mmol, 1.5 equiv) was added to the solution immediately, and then BroP reagent (9 mg, 0.022 mmol, 1.10 equiv) was added. The flask was flushed with N_2, stoppered, and stirred at room temperature for 48 h. The solution was concentrated, and the residue was applied to a flash column and eluted with 2:1 EtOAc−hexanes to give pentapeptide **45** (55 mg, 90%): TLC R_f 0.30 (1:1 hexanes−EtOAc); $[\alpha]^{20}_D = -12.9°$ (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.18 (s, 12H), 0.97 (s, 18H), 1.04 (d, J = 6.7 Hz, 3H), 1.11 (s, 3H), 1.18 (s, 15H), 1.30−1.40 (m, 3H), 1.42 (s, 9H), 1.48 (d, J = 5.5 Hz, 3H), 1.60−1.80 (m, 4H), 1.95−2.30 (m, 2H), 3.01 (dd, J = 14.4, 6.0 Hz, 4H), 3.30 (dd, J = 10.0, 5.8 Hz, 1H), 3.63−4.02 (m, 4H), 4.15−4.87 (m, 15H), 5.22−5.36 (m, 2H), 5.79−5.98 (m, 1H), 6.60−6.75 (m, 3H), 7.03 (br, 1H), 7.30−7.43 (m, 4H), 7.53 (d, J = 7.2 Hz, 1H), 7.61 (dd, J = 7.2, 3.5 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H); HRMS (FAB) calcd for M^+ $C_{75}H_{114}N_6O_{16}Si_2$ 1411.79081, obsd 1411.79473.

Pentapeptide Amine 46. Piperidine (0.5 mL) was added to a solution of pentapeptide **45** (45 mg, 0.032 mmol) in CH_2Cl_2 (2 mL). The solution was stirred at room temperature under N_2 for 15 min and then concentrated. The residue was dissolved in a minimum quantity of CH_2Cl_2 and loaded onto a short flash column. The column was eluted with 2:1 EtOAc−hexanes to elute the Fmoc−piperidine adduct, then with 9:1 CH_2Cl_2−MeOH to elute the secondary amine **46** as an oil (32 mg, 84%): TLC R_f 0.59 (9:1 CH_2Cl_2−MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.17 (s, 6H), 0.97 (s, 18H), 1.03 (d, J = 6.4 Hz, 3H), 1.17 (s, 9H), 1.18 (s, 9H), 1.30−1.50 (m, 9H), 1.43 (s, 9H), 1.60−1.80 (m, 4H), 1.95−2.30 (m, 2H), 2.85−3.10 (m, 4H), 3.63−4.02 (m, 4H), 4.15−4.87 (m, 15H), 5.22−5.36 (m, 2H), 5.79−5.98 (m, 1H), 6.60−6.75 (m, 3H), 7.03 (br, 1H); HRMS (FAB) calcd for $(M + H)^+$ $C_{60}H_{105}N_6O_{14}Si_2$ 1189.72273, obsd 1189.72396.

Decapeptide 10. A solution of acid **11** (26.9 mg, 0.027 mmol, 1.00 equiv) in CH_2Cl_2 (1 mL; 0.5 mL rinse) was added to amine **46** (32 mg, 0.027 mmol, 1.00 equiv). *N*-Methylmorpholine (3 μL, 0.028 mmol, 1.05 equiv) was added, followed by DPPA (5.8 μL, 0.027 mmol, 1.00 equiv). The flask was flushed with N_2, stopped, and left to stir at room temperature. After 4 days, another portion of DPPA (2.0 μL) was added, and stirring was continued for a further 10 days. The residue was concentrated, and the product was isolated from the reaction mixture by flash chromatography, eluting with 2−3% MeOH in CH_2Cl_2. A mixture of the decapeptide and a slightly less polar compound were separated by HPLC, eluting with 2−10% MeOH in CH_2Cl_2 over 20 min at 4.7 mL min^{-1} on a 10 mm silica column. The decapeptide eluted at 10.4 min: TLC R_f 0.34 (9:1 CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07−0.18 (m, 12 H, 4 × $SiCH_3$), 0.80−1.52 (m, 14 H, 2 × [Hβ, Hγ of Lys] and 2 × CH_3 of DHP), 0.90−0.97 (m, 18H, 2 × $SiC(CH_3)_3$), 1.03 (d, J = 6.1 Hz, 0.7H, Thr CH_3), 1.06 (d, J = 6.1 Hz, 0.3H, Thr CH_3), 1.13−1.20 (m, 18H, $OC(CH_3)_3$ of Thr and Ser), 1.25−1.32 (m, 21H, CH_3 of Ala and $OC(CH_3)_3$ of Tyr and Hyp), 1.41−1.44 (m, 18H, 2 × Boc of Lys), 1.49−2.65 (m, 8 H, Hβ and Hγ of Pro, Hβ of Hyp and Hϵ of Lys), 2.78 (dd, J = 13.6, 5.6 Hz, 1H), 2.97−3.07 (m, 4H), 3.25 (dd, J = 13.0 Hz, 1H), 3.43 (dd, J = 13.1, 5.0 Hz, 1H), 3.49−3.54 (m, 1H), 3.59−3.70 (m, 2H), 3.73 (dd, J = 10.0, 4.2 Hz, 1H), 3.72−3.79 (m, 3H), 4.04−4.07 (m, 1H), 4.22−4.76 (m, 16H), 4.88 (td, J = 15.0, 9.0 Hz, 1H), 5.23−5.33 (m, 2H), 5.74−5.93 (m, 1H), 6.62−6.73 (m, 3H), 6.85 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.30 (app. t, J = 7.2 Hz, 2H), 7.39 (app. t, J = 7.2 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.75 (d, J = 7.2 Hz, 2H), 8.04 (d, J = 6.6 Hz, 1H), 8.09 (d, J = 10.2 Hz, 1H), 8.85 (d, J = 9.0 Hz, 1H); HRMS (FAB) calcd for $(M + H)^+$ $C_{113}{}^{13}C$ $H_{177}N_{12}O_{25}Si_2$ 2171.25200, obsd 2171.25151.

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Supporting Information Available: Tables of ^{13}C NMR data, with proposed assignments, for compounds **21**, **22**, **25**, **27**, **31**, **36**, **38** and **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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