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Strategies for the Solid-Phase Diversification of Poly-L-proline-Type II Peptide Mimic Scaffolds and Peptide Scaffolds Through Guanidinylation

Stevenson Flemer, Alexander Wurthmann, Ahmed Mamai, and José S. Madalengoitia*

Department of Chemistry, University of Vermont, Burlington, Vermont 05405

jose.madalengoitia@uvm.edu

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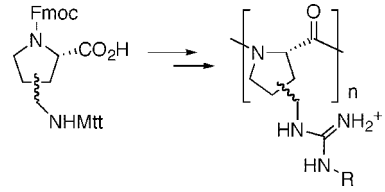

A strategy for the solid-phase diversification of PPII mimic scaffolds through guanidinylation is presented. The approach involves the synthesis *N*-Pmc-*N*′-alkyl thioureas as diversification reagents. Analogues of Fmoc-Orn(Mtt)-OH can be incorporated into a growing peptide chain on Wang resin. Side chain deprotection with 1% TFA/CH_2Cl_2 followed by EDCI-mediated reaction of *N*-Pmc-*N*′-alkyl thioureas with the side chain amine affords arginine analogues with modified guanidine head groups. The scope, limitations, and incidental chemistry are discussed.

Introduction

Within the past 15 years, poly-L-proline type II (PPII) helices have been recognized as playing an essential role in multiple cellular signaling pathways. For example, SH3 domains, some SH2 domains, WW domains, EVH1 domains, MHC class II proteins, as well as other proteins bind peptide ligands in the PPII conformation or similar extended conformations.[1] PPII helices are predominantly located on the surface of proteins and are characterized by an extended structure that makes them well suited for mediating protein−protein interactions.[2] Our interest in the development of PPII mimics stems from their potential utility in unraveling complex signaling cascades that involve PPII recognition and binding.

Interestingly, while the name poly-L-proline type II is derived from the conformation of polyproline, PPII helices in globular proteins are often composed of amino acids other than proline and often it is these nonprolyl residues that are critical for recognition of a PPII helix by a receptor.[1] Therefore, a PPII mimic must not only adopt the peptide backbone conformation of polyproline, it must also be able to incorporate the nonprolyl side chain functionality required for receptor binding. Our strategy for mimicking the PPII secondary structure involves first the synthesis of proline-templated amino acids (PTAAs) in which the proline pyrrolidine ring serves as a template for the nonprolyl amino acid side chains (Figure 1). The synthesis of PTAA oligomers from PTAAs then affords peptides that populate the PPII conformation in solution and possess the

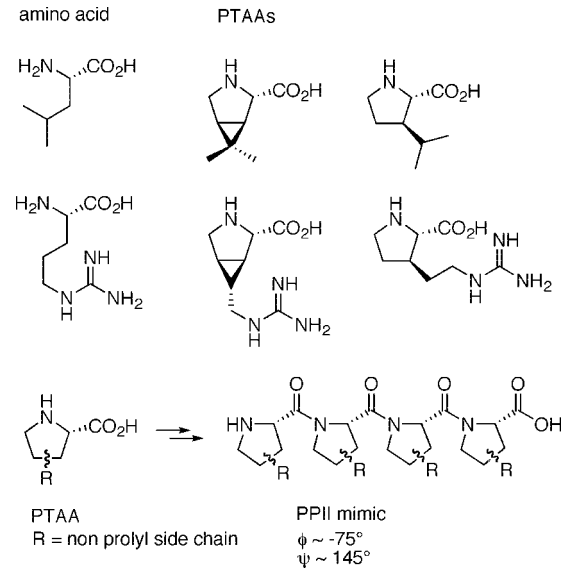

FIGURE 1. Design of PPII mimics from PTAAs.

* Author to whom correspondence should be addressed.

(1) (a) Kay, B. K.; Williamson, M. P.; Sudol, M. *FASEB* **2000**, *14*, 231. (b) Siligardi, G.; Drake, A. F. *Peptide Sci.* **1995**, *37*, 281. (c) Sudol, M.; Sliwa, K.; Russo, T. *FEBS Lett.* **2001**, *490*, 190. (d) Ball, L. J.; Jarchau, T.; Oshkinat, H.; Walter, U. *FEBS Lett.* **2002**, *513*, 45.

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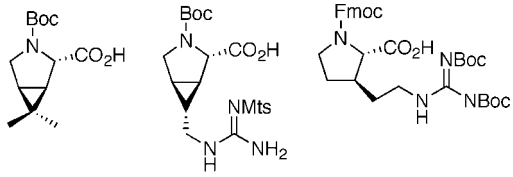

FIGURE 2. Representative PTAAs.

nonprolyl functionality necessary for recognition by the receptor. This design strategy has been validated by solution studies that confirm that oligoPTAAs do indeed populate the PPII conformation in water as well as organic solvents.[3]

Since PTAAs are essentially amino acids, it is possible that PPII mimic libraries could be constructed from a diversity of PTAAs through the strategies already developed for the synthesis of peptide libraries. One potential drawback to this goal, however, is the labor involved in the synthesis of the PTAAs. For example, the synthesis of PTAAs **1**−**3** requires 11 to 13 linear steps (Figure 2).[3a,b,4] More functionalized PTAAs by necessity require more steps to synthesize. Thus, the assembly of a large collection of PTAAs can involve a significant synthetic effort. To minimize the labor involved in the synthesis of PPII mimic libraries, we have introduced the concept of a "diversifiable" PTAA, a PTAA that can be selectively deprotected on a solid support and then diversified through an appropriate reaction. Since arginine is critical to many PPII helix−receptor interactions, our initial efforts have been directed toward developing methods for the facile synthesis of arginine analogues.

In our first communication on this approach, we reported the results of our initial studies on the diversification of proline-templated ornithines through a guanidinylation reaction that then affords arginine analogues.[5] The strategy involved the incorporation of Boc-PTAA(Fmoc)-OH (**4**) into a growing peptide, followed by deprotection of the side chain Fmoc group (Scheme 1). The side chain amine was then guanidinylated with *N*-alkyl-*N*′-ethoxycarbonyl thioureas (RNHC═SNHCO$_2$Et) in a reaction promoted by EDCI. After cleavage from the solid support, the peptides **8** were obtained in good yield and purity. These studies showed that the chemistry was amenable to solid-phase diversification, proceeded in good yield, and was open to the incorporation of a large number of guanidine substituents. However, the chemistry was not compatible with Fmoc solid-phase peptide synthesis and the guanidine groups possessed a protecting group (CO$_2$Et) that is not easily removed. In this full account of our work, we report the establishment of a general strategy for the diversification of PPII mimic scaffolds through a guanidinylation reaction that is amenable to Fmoc solid-phase peptide synthesis and affords peptides that possess fully deprotected guanidine groups. Furthermore, the chemistry is equally adaptable to proline-templated ornithines as well as ornithine itself. It is worth noting that arginine is a ubiquitous residue that is critical to the bioactivity of numerous peptides such as cell-penetrating peptides, HIV-Tar RNA binding peptides, amphiphilic antibacterial peptides, RGD peptides, etc.[6] The chemistry developed in this program allows access to the most diverse collection of arginine analogues yet assembled with which to study bioactive peptides such as the ones noted above that possess critical arginine residue(s).

Results and Discussion

The development of a method for the diversification of PPII mimic scaffolds through iterative guanidinylation reactions must take careful account of a suitable protecting group strategy. As such, we envisioned that PTAAs possessing Fmoc-N-terminal protection and side chain Mtt (methyltrityl) protection (**10**) would be compatible with Fmoc solid-phase peptide synthesis. After incorporation of the PTAA into a growing peptide chain, the Mtt group can be removed orthogonally with 1% TFA/CH_2Cl_2 without cleavage of the peptide from Wang resin.[7] Next, we envisioned that guanidinylation of the deprotected residue could then be accomplished with an *N*-alkyl-*N*′-Pmc thiourea (**10**) and EDCI similarly to the strategy described in Scheme 1. Although several guanidinylation protocols have been described in the literature,[8] we selected the development of *N*-alkyl-*N*′-sulfonyl[9] thioureas as guanyl-transfer agents as they would offer a flexible and expedient diversification method. Although our initial studies had employed a carbamoyl guanidine-protecting group, an advantage of sulfonyl-based protecting groups (such as Pmc) is that they sufficiently deactivate the nucleophilicity of the guanidine group allowing clean extension of a peptide. An additional advantage to the Pmc group is that it is stable to Mtt deprotection conditions (1% TFA/CH_2Cl_2), but can be removed with TFA-based cleavage cocktails. Thus, after guanidinylation of a resin-bound ornithine PTAA with an *N*-Pmc-*N*′-alkyl thiourea **9**, Fmoc deprotection of the N-terminus may then proceed followed by coupling of another Fmoc-PTAA(Mtt)-OH residue (Scheme 2). Mtt deprotection of this residue will then set up another round of diversification. This strategy should therefore be adaptable to iterative rounds of PTAA coupling and diversification affording large and complex libraries.

Synthesis of *N*-Alkyl-*N*′-Pmc Thioureas. The synthesis of the *N*-alkyl-*N*′-Pmc thioureas was accomplished through a modification of the classical conditions for the synthesis of *N*-sulfonyl isothiocyanates as has been previously disclosed (Scheme 3).[10,11] Commercially available PmcCl (**14**) was treated with ammonia in CH_2Cl_2 to give the corresponding sulfonamide **15** in quantitative yield. Sulfonamide **15** was then allowed to react with KOH and CS_2 in a CS_2/benzene mixture with azeotropic removal of water to afford the intermediate **16**, using a modification of the procedure described by Barton.[10] Treatment of this mixture with phosgene then afforded the critical Pmc-isothiocyanate **17** in 75% yield. Without the azeotropic removal of water, the yield for this transformation averaged

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(8) (a) Powell, D. A; Ramsden, P. D; Batey, R. A. *J. Org. Chem.* **2003**, *68*, 2300. (b) Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 8432. (c) Linton, B. R.; Carr, A. J.; Orner, B. P.; Hamilton, A. D. *J. Org. Chem.* **2000**, *65*, 1566.

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(10) Barton, D. H. R.; Fontana, G.; Yang, Y. *Tetrahedron* **1996**, *52*, 2705.

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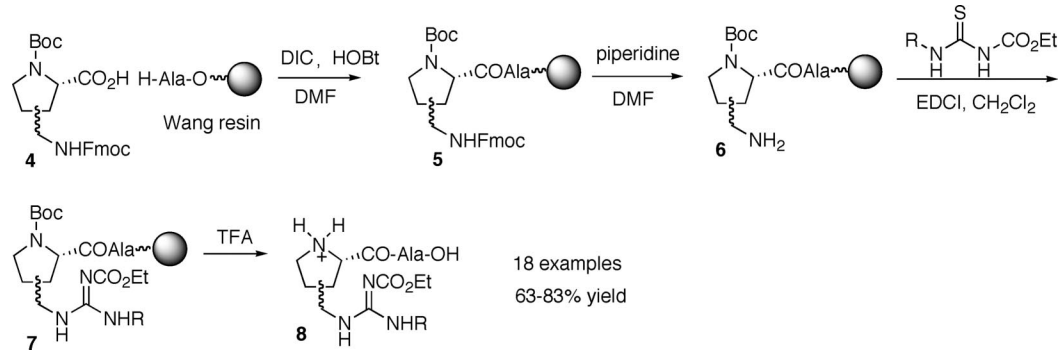
SCHEME 1



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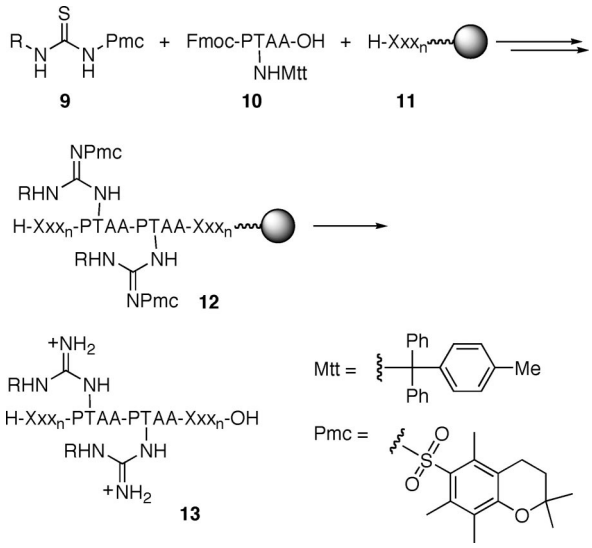
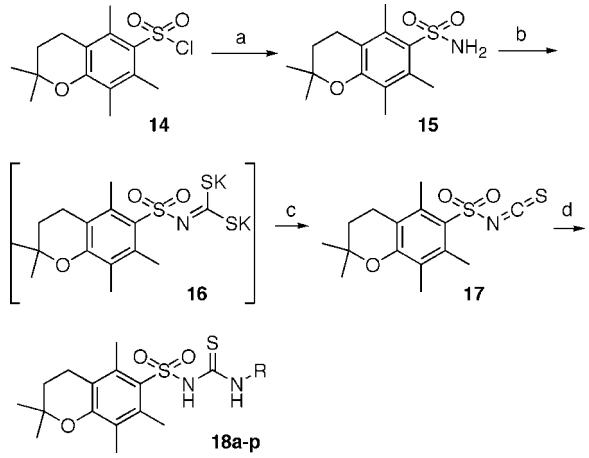


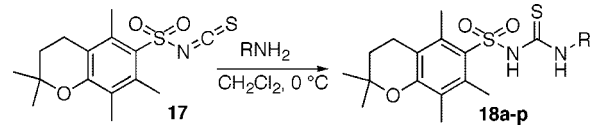
SCHEME 3[a]

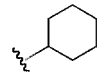
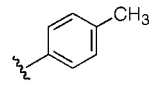
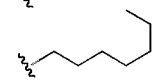
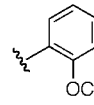
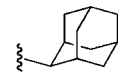
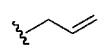
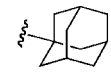
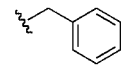
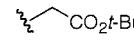
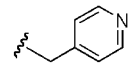
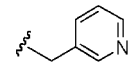
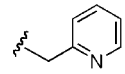
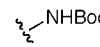


[a] Reagents and conditions: (a) NH_3, CH_2Cl_2 (100%); (b) KOH, 1:4 CS_2/benzene, D.S. trap; (c) phosgene, toluene, 0 °C (75%); (d) RNH_2, CH_2Cl_2 (58−100%).

∼54%. The TLC of the isothiocyanate revealed the presence of a small amount of an impurity, most likely a dimer since *N*-sulfonyl isothiocyanates are known to dimerize.[12] An analytical sample of Pmc-isothiocyanate could be obtained by flash chromatography on silica gel, but is significantly decreased, which is most likely due to decomposition of the highly reactive Pmc-isothiocyanate. We have found, however, that the crude *N*-Pmc-isothiocyanate is homogeneous enough to be used without further purification. The final step of the synthesis involves reaction of *N*-Pmc-isothiocyanate (**17**) with various amines to afford *N*-alkyl-*N*′-Pmc thioureas **18a**−**p** in good to excellent yields (Table 1).[11]

TABLE 1. Reaction of *N*-Pmc Isothiocyante with Amines



#	R	yield	#	R	yield
18a		94%	**18b**		99%
18c		88%	**18d**		95%
18e		81%	**18f**		96%
18g		100%	**18h**		58%
18i		88%	**18j**		86%
18k		69%	**18l**		100%
18m		58%	**18n**		88%
18o		95%	**18p**		83%

Synthesis of PTAAs. To increase the spatial diversity of PPII mimic libraries, the synthesis of PTAAs with different side chain orientations was desirable. Accordingly, the synthesis of several differential substituted, appropriately protected PTAAs was undertaken for these studies. The synthesis of PTAA **24** based on the 3-azabicyclo[3.1.0]hexane system begins with the tricycle **19**, available in multigram quantities from pyroglutamic acid

(12) Dickore, K.; Kuhle, E. *Angew. Chem., Int. Ed.* **1965**, *4*, 430.

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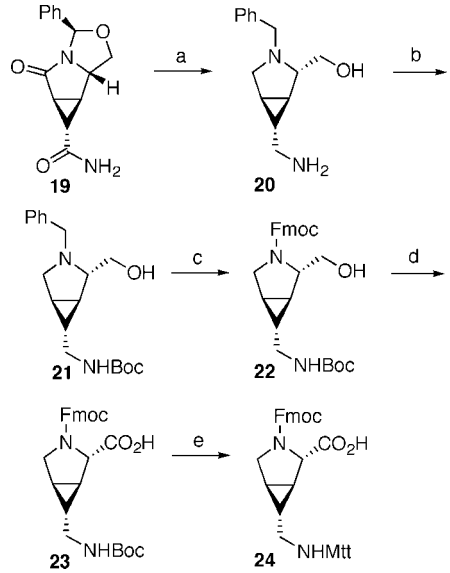
SCHEME 4[a]



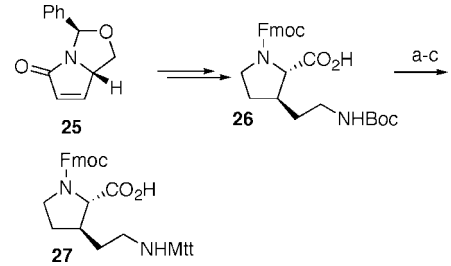
[a] Reagents and conditions: (a) LAH, THF reflux; (b) Boc_2O (68% from 19); (c) (i) H_2, Pd−C, (ii) FmocCl (44%); (d) TEMPO, $NaClO_2$, NaOCl (100%); (e) (i) TFA, (ii) MttCl, Et_3N, (iii) MeOH, 55 °C (65%).

(Scheme 4).[13] Reduction of the two amides and oxazolidine functions with LAH in THF at reflux afforded the bicycle **20**.[13] The resulting primary amine was subsequently protected with Boc_2O giving carbamate **21** in 68% yield from the primary amide **19**. *N*-Debenzylation of the tertiary amine was accomplished via hydrogenolysis in H_2 atmosphere over Pd−C. Reprotection of the resultant secondary amine with FmocCl in dioxane/saturated aqueous $NaHCO_3$ then gave the carbamate **22** in 44% yield over two steps. The primary alcohol was then smoothly oxidized to the carboxylic acid **23** with TEMPO, $NaClO_2$, and bleach in quantitative yield.[14] All that then remained to complete the synthesis of PTAA **24** was Boc-deprotection of the side chain nitrogen and reprotection of the resultant primary amine with an Mtt group. Boc deprotection of **23** was accomplished with neat TFA. The resulting TFA salt was then allowed to react with excess MttCl and Et_3N thus protecting both the carboxylate and amine groups. To obtain the side chain Mtt-protected amino acid, the Mtt ester was selectively methanolized at 50 °C affording the Mtt-protected PTAA **24** in 65% yield from the Boc-protected PTAA **23**.[7]

The synthesis of the 3-substituted ornithine **27** was accomplished from the Boc-protected PTAA **26** that is readily synthesized from the bicyclic lactam **25** (Scheme 5).[4] As above, the side chain Boc-group was deprotected with TFA. The resulting TFA salt was allowed to react with excess MttCl (2.5 equiv) and Et_3N (3 equiv) to protect both the side chain amine and the carboxylic acid group. Selective methanolysis of the Mtt ester was then accomplished at 50 °C to give the PTAA **27** in 56% yield from **26**.[7]

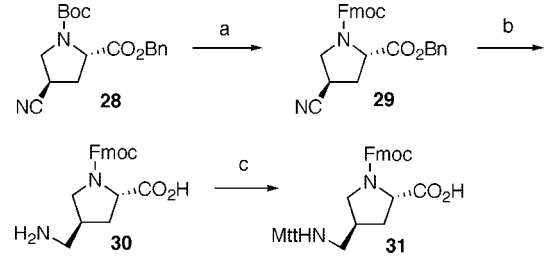
The synthesis of the 4-substituted PTAA **31** begins with the known nitrile **28**[15] that is suitably functionalized for transformation into **31** (Scheme 6). The first issue to be addressed was Fmoc-protection of the α-nitrogen. Accordingly, Boc-deprotection of the α-nitrogen was accomplished with TFA followed by Fmoc-reprotection of the resulting secondary amine with

SCHEME 5[a]



[a] Reagents and conditions: (a) TFA; (b) MttCl, Et_3N; (c) MeOH, 50 ° C.

SCHEME 6[a]



[a] Reagents and conditions: (a) (i) TFA, (ii) FmocCl (98%); (b) (i) H_2, Pd−C, (ii) H_2, PtO_2 (80%); (c) (i) MttCl, Et_3N, (ii) MeOH, 50 ° C (45%).

FmocCl in a dioxane/saturated aqueous $NaHCO_3$ solution affording the Fmoc carbamate **29** in 98% yield. Next, it was surmised that the reduction of the nitrile could be accomplished with concomitant deprotection of the benzyl ester under standard hydrogenolysis conditions. However, when this transformation was attempted (H_2, Pd−C, even at elevated pressures >250 psi), the product was obtained debenzylated, but with the nitrile intact. To reduce the nitrile, the product was resubjected to hydrogenation conditions, but with PtO_2 as the catalyst to obtain the primary amine in 80% yield for both steps. Finally, Mtt protection of the side chain nitrogen was effected through the two-step procedure involving Mtt protection of both the primary amine and carboxylate groups followed by selective solvolysis of the Mtt ester as above to give the PTAA **31** in 45% yield for both steps.

The synthesis of the other 4-substituted PTAA **37** was accomplished from the alcohol **32** derived from 4-hydroxyproline.[16] Tosylation of the 4-position with inversion of configuration was accomplished under typical Mitsunobu conditions with *p*-TsOMe, Ph_3P, and DEAD in 53% yield.[17] Displacement of the tosylate with NaN_3 in DMF at 60 °C was easily accomplished to afford the azide **34** in 87% yield. Next, the α-nitrogen was Boc deprotected with TFA and Fmoc reprotected with FmocCl in a dioxane/saturated aqueous $NaHCO_3$ solution to give the product **35** in 84% yield for both steps. Reduction of the azide to the primary amine and deprotection of the benzyl ester was accomplished with H_2, and Pd−C, furnishing the amino acid **36**, which was used without further purification. Mtt protection of the side chain nitrogen was again accomplished through the diprotection of the amine and carboxylate groups with MttCl (2.5 equiv) and DIPEA (3 equiv) in DMF followed by selective methanolysis of the Mtt ester at 50 °C to give the Fmoc-Mtt-protected PTAA **37** in 70% yield (Scheme 7).[7]

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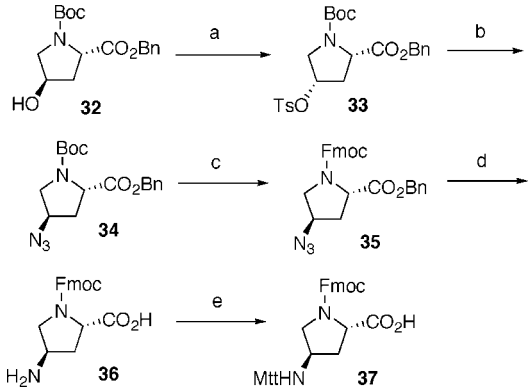
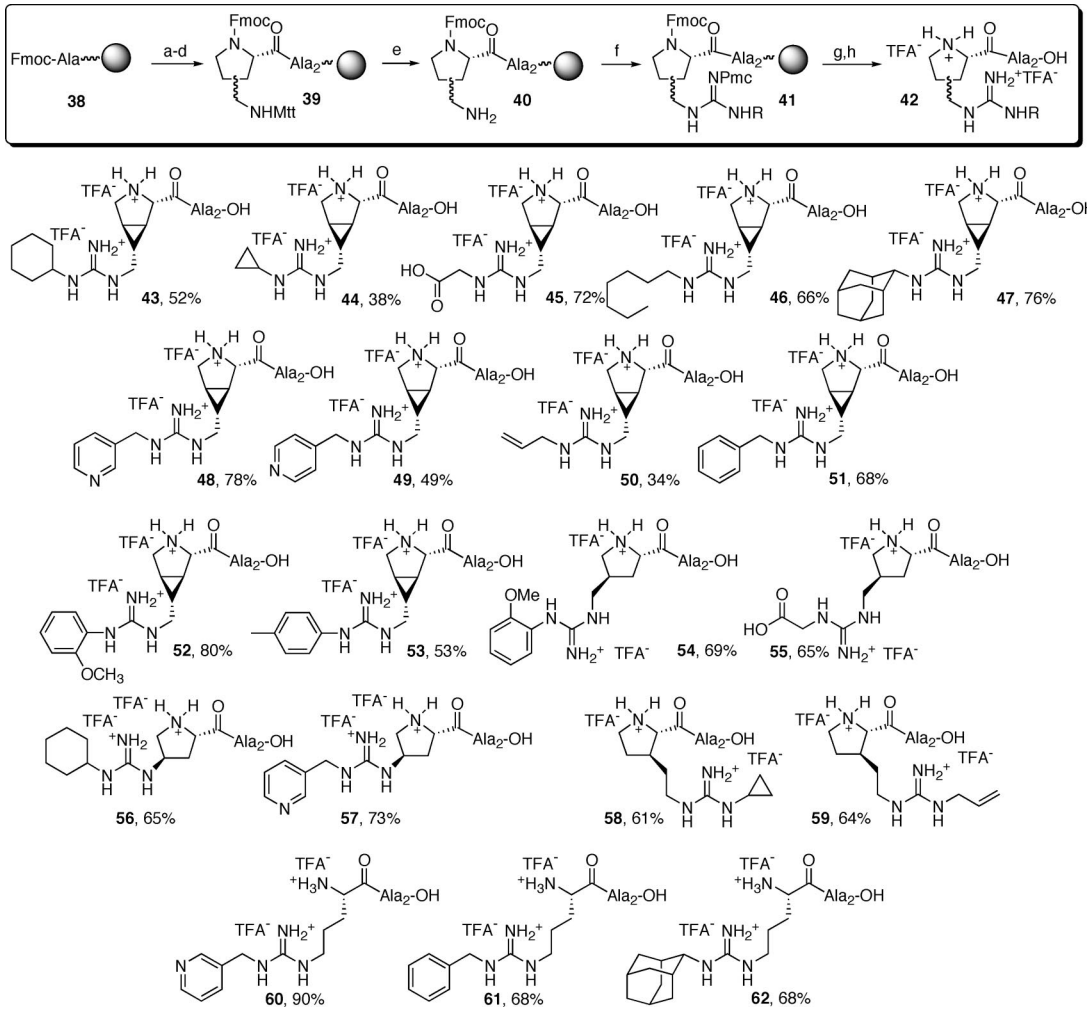
SCHEME 7[a]



[a] Reagents and conditions: (a) *p*-TsOMe, PPh_3, DEAD (53%); (b) NaN_3, DMF, 60 ° C (87%); (c) (i) TFA, (ii) FmocCl, $NaHCO_3$, dioxane/H_2O (84%); (d) H_2, Pd−C, MeOH (98%); (e) (i) MttCl, $(i\text{-Pr})_2$NEt, DMF, (ii) MeOH, 60 ° C (70%).

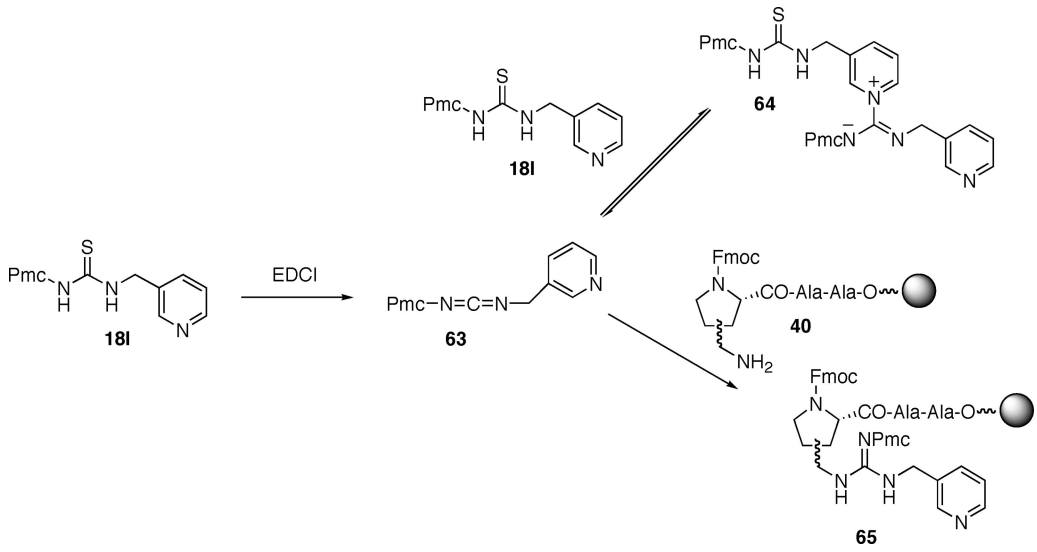
Scope of the Solid-Phase Guanidinylation. To assess the scope and limitations of the diversification strategy, we first investigated the diversification of a single PTAA (**24**) with all thioureas (**18a**−**p**) to look for trends in the reactivity of the thioureas (entries **43**−**53**, Scheme 8). We then explored the diversification of PTAAs **27**, **31**, and **37** and natural ornithine with randomly selected *N*-alkyl-*N*′-Pmc thioureas to illustrate the versatility of the method (entries **54**−**62**, Scheme 8). Beginning with Fmoc-Ala-Wang resin, the amino terminus was deprotected with 20% piperidine/DMF. Fmoc-Ala-OH was coupled with DIC/HOBt and the N-terminus was deprotected to give the resin bound Ala-Ala dipeptide. Next, one of the PTAAs **24**, **27**, **31**, **37**, or Fmoc-Orn(Mtt) was coupled to the Ala-Ala-dipeptide giving the trimer **39** suitably protected for diversification. It is worth noting that since the synthesis of the PTAAs **24**, **27**, **31**, and **37** requires 11 to 13 steps from commercially available starting materials, the couplings were performed with 1.3 equiv of PTAA instead of the typical 3 to 5 equiv of amino acid normally used in solid-phase peptide synthesis. In all instances, ninhydrin and TNBS tests showed the couplings were complete within 2 h. Next, Mtt-side chain deprotection was accomplished with 1% TFA/CH_2Cl_2 to give the side chain deprotected ornithine residue **40**. The Mtt deprotection can be visually monitored since the Mtt cation is yellow. We have found that an average of six 5-min washes was required to obtain a colorless deprotection solution (in agreement with a published study).[18] We next explored the guanidinylation reaction with the *N*-alkyl-*N*′-Pmc thioureas (1.5 equiv) and EDCI (4 equiv) in DMF. A key advantage of the

SCHEME 8[a]



[a] Reagents and conditions. (a) 20% piperidine/DMF; (b) Fmoc-Ala-OH, DIC, HOBt; (c) 20% piperidine/DMF; (d) **24**, **27**, **31**, **37**, or Fmoc-Orn(Mtt)-OH, DIC, HOBt; (e) 1% TFA/CH_2Cl_2; (f) **18a**−**p**, EDCI, DMF; (g) 20% piperidine/DMF; (h) TFA.

SCHEME 9



guanidinylation reaction is that since the guanyl group is transferred Pmc-protected, a Kaiser or TNBS resin test can be used to monitor the reaction. Accordingly, some trends in the reactivity of the thioureas became evident (discussed in more detail below). Most guanidinylations were complete within 2 h, but some required as long as 12 h. To complete the synthesis of the model peptides, the amino terminus was deprotected with 20% piperidine/DMF and the peptides were cleaved and deprotected with 95% TFA/H_2O.

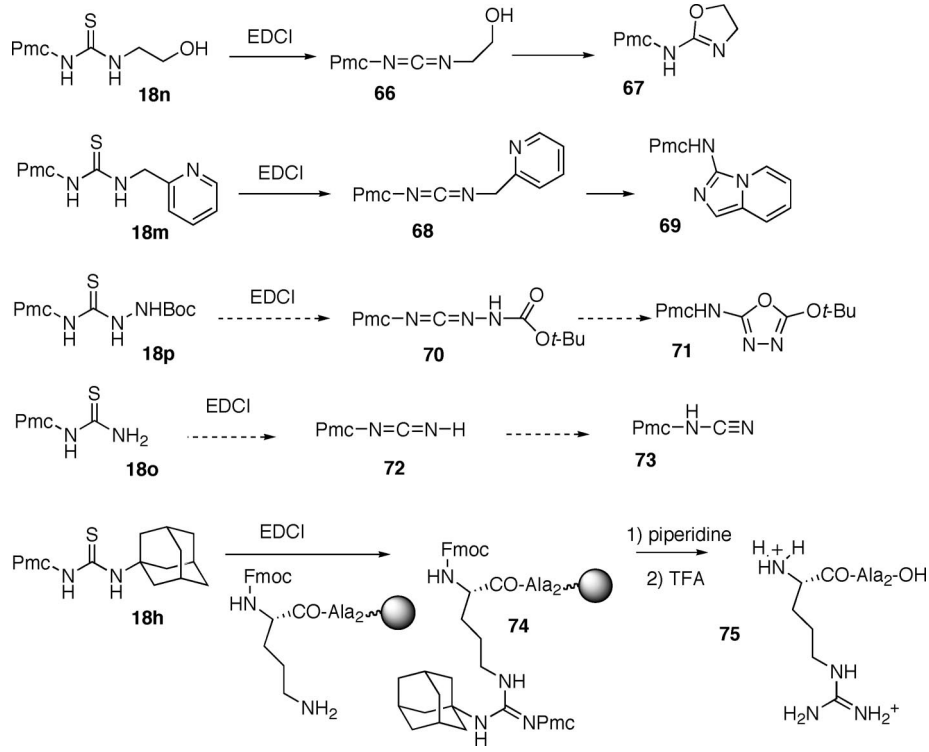
Scheme 8 demonstrates the ease and flexibility with which a wide array of arginine analogues may be incorporated into a peptide. Yields ranged from 34% to 90% for the eight-step sequence. It should first be noted that the diversification chemistry works to yield arginine PTAAs based on the 3-aza bicyclo[3.1.0]hexane system (**43**−**53**), the 4-substituted PTAAs (**54**−**57**), the 3-substituted PTAAs (**58**, **59**), as well as natural ornithine (**60**−**62**). We desired to ascertain if the diversification chemistry afforded crude compounds in enough purity for high throughput screening. Thus, after cleavage and deprotection, the tripeptides were triturated with Et_2O and purity was determined by HPLC. From this simple procedure, most compounds were obtained in >85% purity. Among the tripeptides that were not obtained in acceptable levels of purity were **45** and **55**, derived from the *t*-Bu-ester thiourea **18j** that was designed to undergo *t*-Bu deprotection under the cleavage conditions. In addition, tripeptides **48**, **49**, and **60** derived from the heterocyclic thioureas **18k** and **18*l*** were not obtained in acceptable levels of purity. That is not to say that diversification of resin-bound PTAAs with these thioureas does not work, but rather that peptides derived from these PTAAs would require purification prior to screening.

As previously noted, information about the relative rates of the guanidinylation reactions could be obtained through a standard ninhydrin test. Most guanidinylation reactions proceeded to completion in 2 h; however, more hindered thioureas such as the 2-adamantyl derived **18f** required 8 h. One surprising observation was that reaction of the *N*-benzyl-*N*′-Pmc thiourea **18j** required 2 h to proceed to completion, while the isosteric pyridyl derivatives **18k** and **18*l*** required at least 12 h to give a negative ninhydrin test. Clearly, since the thioureas **18k** and **18*l*** are isosteric with **18j** the pyridyl nitrogen is involved in retarding the rate of the guanidinylation reaction. The reaction most likely proceeds through the formation of the highly reactive *N*-sulfonyl carbodiimide **63** (Scheme 9). In a normal course, the carbodiimide **63** may then react with a resin-bound PTAA **40** giving the arginine analogue **65**. We believe that the high reactivity of carbodiimide **63** facilitates attack by the pyridyl nitrogen of another thiourea (or carbodiimide) establishing an equilibrium with the adduct **64**. The effect of the competing reaction is to lower the concentration of carbodiimide **63**, thus slowing the rate of the solid-phase guanidinylation reaction.

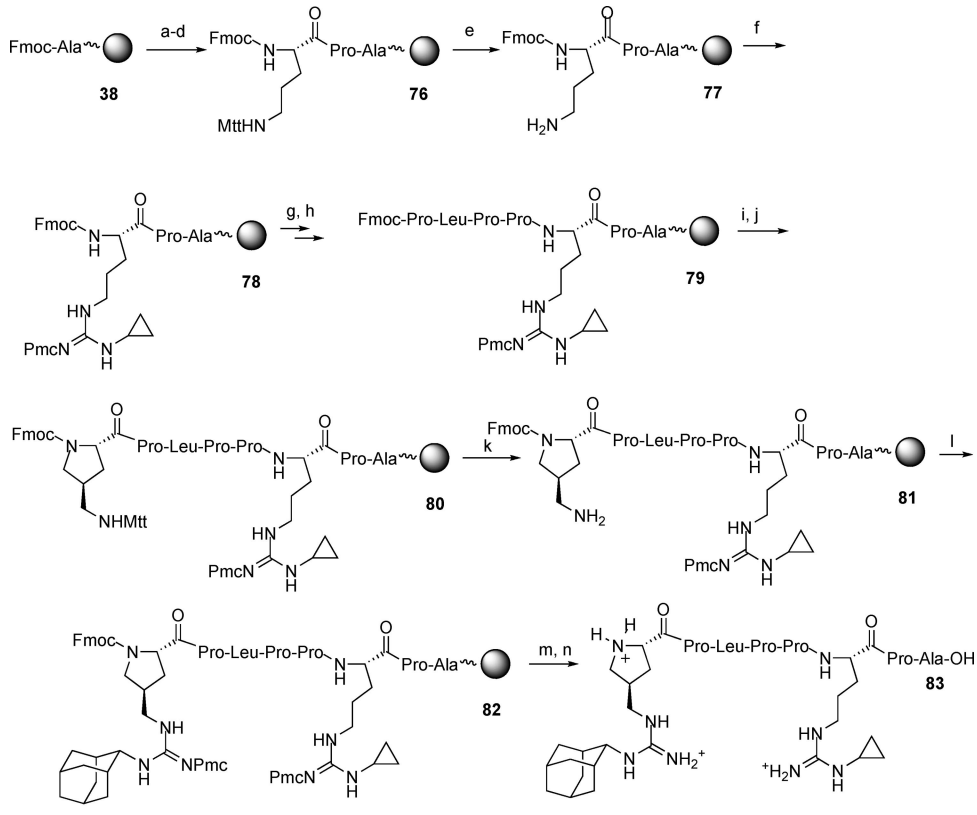
Other thioureas such as **18m**, **18n**, **18o**, and **18p** failed to give a negative ninhydrin test even after prolonged reaction times or after the use of 4 molar excess of thiourea. In the case of thioureas **18m** and **18n**, we propose that the guanidinylation reaction failed due to intramolecular cyclization pathways (Scheme 10). Indeed, from the attempted guanidinylation of a resin-bound PTAA with the 2-pyridyl-derived thiourea **18m**, we isolated the cyclic guanidine **69**,[11] which is consistent with attack of the pyridyl nitrogen on the carbodiimide carbon. Similarly, from the attempted guanidinylation of a resin-bound PTAA with the ethanolamine-derived thiourea **18n**, we isolated the cyclic isourea **67**,[11] which is consistent with attack of the hydroxyl-group on the carbodiimide carbon. The reaction of hydrazine-derived thiourea **18p** with a resin-bound PTAA also failed to give a negative ninhydrin test. In this case, we hypothesize that intramolecular attack of the carbonyl oxygen on the carbodiimide carbon resulted in the heterocycle **71**, although we did not isolate this product. We also investigated solid-phase guanidinylation of PTAAs with the thiourea **18o** since this would afford after cleavage and deprotection the arginine analogues without a guanidine substituent. However, this reaction also failed to give a negative ninhydrin test most likely because the in situ generated carbodiimide **72** quickly tautomerizes to the less reactive cyanamide **73**. We were, however, unable to isolate the cyanamide **73**. Interestingly, the guanidinylation of resin-bound ornithine with the highly hindered 1-adamantyl thiourea **18h** did proceed to completion in 8 h. However, after cleavage and deprotection, the product **75** was obtained without the adamantyl group. Apparently, the acidic deprotection conditions promote solvolysis of the adamantyl group through the intermediacy of the tertiary carboca-

(18) Li, D.; Elbert, D. L. *J. Pept. Res.* **2002**, *60*, 300.

SCHEME 10



SCHEME 11[a]



[a] Reagents and conditions: (a) piperidine/DMF; (b) Fmoc-Pro-OH, DIC, HOBt; (c) piperidine/DMF; (d) Fmoc-Orn(Mtt)-OH, DIC, HOBt; (e) 1% TFA/CH_2Cl_2; (f) **18b**, EDCI, Et_3N; (g) piperidine/DMF; (h) Fmoc-Xxx-OH, DIC, HOBt; repeat three more times; (i) piperidine/DMF; (j) **31**, DIC, HOBt; (k) 1% TFA/CH_2Cl_2; (l) **18f**, EDCI, Et_3N; (m) piperidine/DMF; (n) TFA.

tion. Thus, although not originally intended to, the thiourea **18h** serves as a surrogate for thiourea **18o** in affording arginine analogues without a guanidine substituent.

To highlight the feasibility of our approach in the synthesis of PPII combinatorial libraries through iterative rounds of PTAA coupling and diversification, we synthesized a peptide in which

to positions have undergone rounds of PTAA coupling and diversification. We selected a peptide Arg-Pro-Leu-Pro-Pro-Arg-Pro-Ala as a model based on the peptide Arg-Lys-Leu-Pro-Pro-Arg-Pro that has been shown by the Schreiber group to bind to the PI3K SH3 domain in the PPII conformation.[19] The synthesis begins with Fmoc-Ala-Wang resin using a primer Ala to avoid diketopiperazine formation (Scheme 11). Fmoc deprotection and coupling with Fmoc-Pro-OH followed by Fmoc-deprotection and coupling with Fmoc-Orn(Mtt)-OH affords the resin-bound tripeptide **76**. Mtt deprotection is accomplished by washing with 1% TFA (6 × 5 min) to give the tripeptide **77** with the deprotected side chain. The EDCI-mediated reaction of the tripeptide with *N*-Pmc-*N*′-cyclopropyl thiourea **18b** afforded the guanidine **78**. Extension of the peptide through the subsequent coupling of Pro, Pro, Leu, Pro, and PTAA **31** gave the peptide **80**. Mtt deprotection of the side chain amine was accomplished with 1% TFA (6 5 5 min) to give the deprotected amine **81**. Reaction of the amine **81** with *N*-Pmc-*N*′-(2-adamantyl) thiourea **18f** gave the diguanidine **82**. Finally, Fmoc-deprotection of the amino-terminus and cleavage and deprotection with TFA gave the fully deprotected peptide **83**. HPLC analysis of the peptide **83** revealed that **83** was obtained in 96% purity.

In summary, we have devised an approach for the facile synthesis of arginine-rich PPII mimic libraries. The strategy involves incorporation of ornithine analogues possessing Fmoc-N^{α} protection and Mtt-side chain protection into a resin-bound peptide. After incorporation of the ornithine analogues into a growing peptide chain, the side chain can be deprotected with 1% TFA/CH_2Cl_2 and diversified with *N*-alkyl-*N*′-Pmc thioureas in a reaction promoted by EDCI. After TFA-cleavage from the solid support, the peptides are obtained in good yields and purity. Scheme 8 highlights the vast array of arginine analogues that can be generated through this approach. Furthermore, our approach is suitable for the synthesis of complex libraries through iterative rounds of coupling and diversification as demonstrated by the synthesis of a model peptide.

Experimental Section

(1*S*,2*S*,5*S*,6*R*)-3-Aza-3-benzyl-6-[((*tert*-butyl)oxycarbonyl)aminomethyl]bicyclo[3.1.0]hexyl-2-methanol (21). To a 14/20 100 mL round-bottomed flask was added powdered $LiAlH_4$ (0.31 g, 8.0 mmol) along with anhydrous THF (25 mL) and a magnetic stirring bar. The flask was fitted with a condenser and purged with nitrogen. The LAH slurry was stirred vigorously while 1.38 g (5.36 mmol) of **19** (dissolved in 10 mL of anhydrous THF) was added dropwise through a septum at the top of the condenser. During the addition process, the slurry began to boil. At the end of the addition process, heat was applied to the reaction mixture, and it was allowed to stir at reflux for 1 h. At the end of this time, the heat source was removed, and sat. aq. Na_2SO_4 was added dropwise until ∼10 mL had been added. The reaction mixture was then filtered through celite, and the solid filtered material was washed with 4 × 20 mL of EtOAc. The organic washes were then combined with the filtered supernatant, and the solvent was removed in vacuo. The residue was taken up in 100 mL of EtOAc and partitioned against 100 mL of water. The organic portion was dried over $MgSO_4$ and the solvent was removed in vacuo. The resulting primary amino alcohol **20** was carried through the Boc-protection sequence without purification. A solution of di-*tert*-butyl dicarbonate (1.20 g, 5.51 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a 0 °C solution of primary amine LAH reduction product (1.25 g, 5.40 mmol) in DCM (30 mL). After 30 min, the reaction was allowed to rise to room temperature. The solvent was removed in vacuo, and the crude product was purified on silica gel with 5:45:50 MeOH/EtOAc/Hex affording 1.21 g of carbamate **21** (68% for two steps) as a colorless solid: mp 83−86 °C; $[\alpha]^{25}_D$ −2.34 (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.19−7.33 (m, 5H), 4.72 (br s, 1H), 3.78 (d, J = 13.6 Hz, 1H), 3.68 (d, J = 13.6 Hz, 1H), 3.50−3.61 (m, 2H), 3.15 (dd, J = 4.6 Hz, J = 10.0 Hz, 1H), 2.96−3.08 (m, 3H), 2.61 (d, J = 10.0 Hz, 1H), 1.45 (s, 9H), 1.36 (m, 1H), 1.33 (dd, J = 3.1 Hz, J = 7.4 Hz, 1H), 1.00 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8, 139.8, 128.3, 128.1, 126.9, 79.2, 67.0, 62.9, 58.0, 55.3, 42.8, 28.4, 26.6, 25.7, 23.1 ppm; IR (film) 3348, 1693 cm^{-1}; MS (CI) *m*/*z* 333 (MH). HRMS calcd for $C_{19}H_{29}N_2O_3$ $[M + H]^+$ 333.2178, found 333.2171.

(1*S*,2*S*,5*S*,6*R*)-3-Aza-3-[(fluoren-9-ylmethyl)oxycarbonyl]-6-[((*tert*-butyl)oxycarbonyl)aminomethyl]bicyclo[3.1.0]hexyl-2-methanol (22). Compound **21** (0.80 g, 3.25 mmol) was added to a 100 mL round-bottomed flask along with 0.17 g (5 mol %) of 10% Pd/C, a magnetic stirring bar, and 30 mL of 2:1 AcOH/EtOAc. The reaction flask was purged with H_2 and the mixture was stirred overnight with double balloon pressure additional H_2. At the end of this time, the reaction mixture was filtered through celite, concentrated, and taken to pH 13 with 30% aq. KOH. This aqueous solution was then extracted with CH_2Cl_2 (3 × 30 mL), dried over $MgSO_4$, and concentrated to afford the debenzylated product as a light yellow oil that was carried through the Fmoc-protection step without further purification. The debenzylated amino alcohol and 0.54 g (6.50 mmol) of $NaHCO_3$ in 1:1 dioxane/H_2O (30 mL) was cooled to 0 °C and FmocCl (0.92 g, 3.57 mmol, 1.1 equiv) was added in small portions over 1 h. The mixture was then allowed to come to room temperature and was stirred for an additional hour. At the end of this time, the reaction mixture was partitioned between EtOAc (100 mL) and water (100 mL), and the organic phase was separated. The aqueous layer was re-extracted with EtOAc (2 × 50 mL), and the organic portions were combined, dried over $MgSO_4$, and concentrated. The crude **22** was purified on silica gel with 1:4:5 MeOH/EtOAc/Hex affording 0.67 g of product **22** (44% for two steps) as a colorless foam: mp 58−62 °C; $[\alpha]^{25}_D$ −2.8 (*c* 1.05, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 7.79 (d, J = 7.5 Hz, 2H), 7.58 (t, J = 7.6 Hz, 2H), 7.86−7.42 (m, 2H), 7.25−7.32 (m, 2H), 4.54 (m, 1H), 4.40 (dd, J = 5.7 Hz, J = 10.7 Hz, 0.5H), 4.35 (dd, J = 6.2 Hz, J = 10.7 Hz, 0.5H), 4.20 (q, J = 5.6 Hz, 1H), 3.80 (m, 0.5H), 3.45−3.63 (m, 2H), 3.27−3.44 (m, 2H), 3.25 (m, 0.5H), 2.90−3.02 (m, 2H), 1.27−1.60 (m, 11H), 0.53 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.0, 155.8, 154.8, 143.9, 143.8, 141.3, 128.3, 127.7, 127.0, 126.8, 124.9, 124.7, 119.9, 80.7, 79.4, 67.1, 66.5, 65.1, 64.0, 61.8, 60.8, 48.3, 47.9, 47.3, 42.0, 28.4, 28.2, 24.2, 23.5, 22.3, 21.8, 21.0, 20.3 ppm; IR (film) 3355, 1689 cm^{-1}; MS (CI) *m*/*z* 487 (M + Na). HRMS calcd for $C_{27}H_{32}N_2O_5Na$ $[M + Na]^+$ 487.2209, found 487.2192.

(1*S*,2*S*,5*S*,6*R*)-3-Aza-3-[(fluoren-9-ylmethyl)oxycarbonyl]-6-[((*tert*-butyl)oxycarbonyl)aminomethyl]bicyclo[3.1.0]hexane-2-carboxylic Acid (23). Starting alcohol **22** (0.63 g, 1.36 mmol) was dissolved in acetonitrile (10 mL) and added to a 50 mL round-bottomed flask containing 0.67 M aq. NaH_2PO_4 buffer (10 mL) and TEMPO (15 mg, 0.08 mmol). The reaction mixture was brought to 35 °C and 5% aq. NaOCl (40 μL diluted with an additional 2 mL of H_2O) and $NaClO_2$ (0.31 g, 2.72 mmol, dissolved in 4 mL of H_2O) were added dropwise over 30 min. The reaction mixture was then allowed to stir overnight at 35 °C. At the end of this time, the reaction mixture was allowed to come to room temperature and was poured into 20 mL of ice-cold aq. sat. Na_2SO_3, followed by extraction with EtOAc (4 × 20 mL). The organic portions were combined and extracted with 3 × 15 mL sat. aq. $NaHCO_3$. The aqueous layers were combined and acidified at 0 °C to pH 3 with concd HCl. The aqueous portion was extracted with 4 × 20 mL of EtOAc, and the organic fractions were dried over $MgSO_4$ and concentrated in vacuo to afford the carboxylic acid **23** (100%) as a colorless foam in 0.65 g yield: mp 95−100 °C dec; $[\alpha]^{25}_D$ −3.1 (*c* 1.0, CH_3OH); ^1H NMR

(19) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933.

(500 MHz, $CDCl_3$) δ 7.72 (dd, J = 7.5 Hz, J = 19.1 Hz, 2H), 7.50−7.58 (m, 2H), 7.32−7.40 (m, 2H), 7.25−7.31 (m, 2H), 4.72 (br s, 1H), 4.38−4.50 (m, 1.5H), 4.30−4.38 (m, 1.5H), 4.22 (t, J = 6.9 Hz, 0.5H), 4.14 (t, J = 6.5 Hz, 0.5H), 3.70 (d, J = 10.6 Hz, 0.5H), 3.56−3.65 (m, 1.5H), 2.96−3.20 (m, 2H), 1.69 (m, 0.5H), 1.64 (m, 0.5H), 1.38−1.55 (m, 10H), 0.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 175.0, 156.0, 155.5, 154.8, 143.9, 143.7, 141.2, 127.64, 127.59, 127.0, 125.0, 119.9, 81.2, 79.6, 67.5, 61.3, 60.8, 48.6, 48.2, 47.2, 42.6, 41.7, 29.6, 28.3, 25.3, 24.3, 22.4, 21.0, 20.2 ppm; IR (film) 3356, 1689 cm^{-1}; MS (CI) m/z 501 (M + Na). HRMS calcd for $C_{27}H_{30}N_2O_6Na$ [M + Na]$^+$ 501.2002, found 501.1861.

(1*S*,2*S*,5*S*,6*R*)-3-Aza-3-[(9-fluorenylmethyl)oxycarbonyl]-6-[((4-methylphenyl)diphenylmethyl)aminomethyl]bicyclo[3.1.0]hexane-2-carboxylic Acid (24). *N*(α)-Fmoc-*N*(δ)-Boc-protected carboxylic acid **23** (0.62 g, 1.30 mmol) was dissolved in CH_2Cl_2 (4 mL) in a 50 mL round-bottomed flask, and trifluoroacetic acid (8 mL) was added dropwise to the resulting solution. The reaction was allowed to stir for 30 min, at which time the solvent was removed in vacuo to give the *N*(ϵ)-Boc-deprotected intermediate that was converted without further purification to the corresponding Mtt-protected product. The *N*(δ)-Boc-deprotected residue was dissolved in 2:1 $CHCl_3$/DMF (15 mL) in a 50 mL round-bottomed flask and brought to 0 °C. DIPEA (0.90 mL, 5.2 mmol) was added dropwise over 15 min followed by dropwise addition of Mtt-chloride (0.84 g, 2.86 mmol) dissolved in 5 mL of CH_2Cl_2 over 10 min. The reaction mixture was allowed to slowly reach room temperature overnight. Methanol (2.5 mL) was then added, and the temperature was raised to 50 °C for 2 additional hours. At the end of this time, the reaction mixture was allowed to cool to room temperature, and was poured into a 250 mL separatory funnel containing EtOAc (50 mL) and 0.5 M aq. citric acid (80 mL). Following separation of the organic layer, the aqueous phase was extracted with additional EtOAc (2 × 50 mL). The organic fractions were combined, dried over $MgSO_4$, and concentrated to yield the crude product. Purification was carried out by silica gel chromatography with 1:4:5 MeOH/EtOAc/Hex as eluent to give 0.53 g of PTAA **24** (65% for two steps) as a colorless foam: mp 150−160 °C dec; $[\alpha]^{25}_D$ −2.5 (*c* 0.84, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (t, J = 8.1 Hz, 2H), 7.54−7.65 (m, 2H), 7.22−7.40 (m, 16H), 7.15 (dd, J = 6.0 Hz, J = 7.6 Hz, 2H), 4.26−4.38 (m, 2.5H), 4.22 (m, 1H), 4.12 (m, 0.5H), 3.58 (m, 1H), 3.50 (s, 1H), 2.56 (m, 1H), 2.44 (m, 0.5H), 2.37 (m, 0.5H), 2.30 (d, J = 3.2 Hz, 3H), 1.56 (m, 0.5H), 1.50 (m, 0.5H), 1.37 (m, 0.5H), 1.33 (m, 0.5H), 0.77 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 175.1, 155.6, 154.9, 144.7, 144.1, 144.0, 143.8, 141.2, 136.5, 128.7, 128.6, 128.0, 127.6, 127.1, 126.7, 125.2, 119.9, 110.5, 79.8, 71.8, 67.5, 67.4, 62.0, 61.5, 48.7, 48.4, 47.2, 45.8, 25.9, 24.8, 22.2, 21.5, 20.8, 20.6 ppm; IR (film) 3062, 1705 cm^{-1}; MS (CI) m/z 635 (MH). HRMS calcd for $C_{42}H_{39}N_2O_4$ [M + H]$^+$ 635.2910, found 635.2898.

***trans*-3-[2-((4-Methylphenyl)diphenylmethyl)aminoethyl]-*N*-(9-fluorenylmethyl)-L-proline (27).** The procedure is identical with that previously described for the protecting group conversion of **23** to **24**. Starting from PTAA **26** (1.00 g, 2.08 mmol) afforded 0.75 g of PTAA **27** (56% for two steps) as a colorless foam: mp 120−128 °C dec; $[\alpha]^{25}_D$ −1.2 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.59−7.65 (m, 2H), 7.50 (dd, J = 7.5 Hz, J = 16.8 Hz, 1H), 7.45 (dd, J = 2.7 Hz, J = 7.4 Hz, 1H), 7.09−7.33 (m, 16H), 6.99 (dd, J = 3.2 Hz, J = 8.2 Hz, 2H), 4.15−4.23 (m, 1.5H), 4.07 (t, J = 2.7 Hz, 0.5H), 4.00 (m, 1H), 3.89 (d, J = 4.9 Hz, 0.5H), 3.75 (d, J = 4.9 Hz, 0.5H), 3.40 (m, 0.5H), 3.23−3.37 (m, 1.5H), 2.51 (m, 1H), 2.43 (m, 1H), 2.24 (m, 0.5H), 2.09−2.22 (m, 3.5H), 1.82 (m, 1H), 1.72 (m, 1H), 1.61 (m, 0.5H), 1.52 (m, 0.5H), 1.31 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 175.9, 155.2, 154.7, 144.8, 144.2, 144.0, 143.8, 141.2, 141.1, 136.2, 128.6, 127.9, 127.6, 127.4, 127.0, 126.6, 119.8, 119.6, 71.3, 67.5, 65.3, 64.7, 47.1, 45.9, 45.6, 42.3, 41.1, 33.6, 30.1, 29.0, 20.8 ppm; IR (film) 3063 cm^{-1}, 1702 cm^{-1}; MS (CI) m/z 637 (MH). HRMS calcd for $C_{42}H_{42}N_2O_4$ [M + H]$^+$ 637.3066, found 637.3035.

***trans*-4-Cyano-*N*-[(fluoren-9-ylmethyl)oxycarbonyl]-L-proline Benzyl Ester (29).** The nitrile **28**[15] (2.22 g, 6.72 mmol) was dissolved in trifluoroacetic acid (40 mL) and maintained at rt for 30 min. The TFA was removed under reduced pressure. To this residue was added a 1:1 solution of saturated aqueous $NaHCO_3$ (5.00 g, 59.5 mmol) and FmocCl (2.08 g, 8.04 mmol). After 12 h, the reaction mixture was extracted with EtOAc (3 × 250 mL). The combined fractions were dried ($MgSO_4$) and concentrated. The crude oil was purified by flash column chromatography eluted with hexanes and ethyl acetate (2:1) affording the product **29** (2.98 g, 98%) as a colorless solid. Mp 52−55 °C; $[\alpha]^{25}_D$ −2.76 (*c* 0.86, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77−7.72 (m, 2H), 7.55−7.47 (m, 2H), 7.41−7.23 (m, 9H), 5.26−4.94 (m, 2H), 4.58 (d, J = 2.5 Hz, 0.5H), 4.46−4.40 (m, 1.5H), 4.34(d, J = 6.4 Hz, 0.5H), 4.23 (t, J = 6.7 Hz, 0.5H), 4.00 (t, J = 6.3 Hz, 0.5H), 3.95−3.88 (m, 1H), 3.73 (t, J = 9.1 Hz, 0.5H), 3.64 (t, J = 9.4 Hz, 0.5H), 3.25−3.15 (m, 1H), 2.55−2.44 (m, 1H), 2.41−2.33 (m, 1H); ^{13}C NMR 125 MHz, $CDCl_3$) δ 170.9, 154.0, 153.6, 143.8, 143.7, 143.5, 143.3, 141.2, 135.0, 134.9, 129.6, 128.3, 127.8, 127.1, 124.9, 120.0, 119.9, 118.6, 67.9, 67.8, 67.4, 58.2, 57.8, 49.5, 48.9, 47.0, 34.7, 33.5, 27.0, 26.2 ppm; IR (film) 3065, 2957, 2248, 1746, 1709 cm^{-1}; HRMS calcd for $C_{28}H_{24}N_2O_4Li$ [M + Li]$^+$ 459.1896, found 459.1893.

***trans*-4-[((4-Methylphenyl)diphenylmethyl)aminomethyl]-*N*-[(fluoren-9-ylmethyl)oxycarbonyl]-L-proline Benzyl Ester (31).** The nitrile **29** (9.00 g, 20.0 mmol) was dissolved in EtOH (100 mL, dried over molecular sieves) and Pd/C (10% w/w, 2.11 g, 10 mol %) and PtO_2 (0.408 g, 10 mol%) were added. H_2 (250 psi) was applied. After 12 h the catalyst was removed by filtration through celite. The solvent was removed under reduced pressure to afford the amino acid **30** (5.5 g) as a colorless foam that was used without further purification. The amino acid **30** (1.00 g, 2.73 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C and DIPEA (1.52 mL, 8.74 mmol) was added dropwise over 10 min. Mtt-Cl (2.00 g, 6.83 mmol) was then added as a solution in DMF (10 mL) and the mixture was allowed to warm to room temperature. After 12 h, MeOH (50 mL) was added and the reaction mixture was warmed to 50 °C for 2 h. The product was then partitioned between water and EtOAc (2 × 100 mL). The organic fractions were combined and dried ($MgSO_4$) before the solvent was removed under reduced pressure. Purification of the residue by flash chromatography eluting with CH_2Cl_2:MeOH (97:3) afforded the product as a colorless solid (0.760 g, 45%). Mp 162−165 °C; $[\alpha]^{25}_D$ −4.61 (*c* 1.20, CH_3OH); ^{1}H NMR (500 MHz, 9:1 CD_3OD:$CDCl_3$) δ 8.71−7.66 (m, 22H), 4.51−4.00 (m, 4H), 3.71−3.53 (t, J = 8.7 Hz, 0.5H), 3.53−3.42 (t, J = 8.7 Hz, 0.5H), 3.02 (s, 1H), 2.97−2.85 (t, J = 8.9 Hz, 0.5H), 2.85−2.66 (t, J = 8.9 Hz, 0.5H), 2.07−1.82 (m, 1H), 1.71 (d, J = 15.7 Hz, 3H), 1.54 (m, 3H), 1.43−1.26 (q, J = 10.0 Hz, 0.5H), 1.26−1.07 (q, J = 10.0 Hz, 0.5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.9, 175.7, 155.8, 154.5, 145.7, 145.0, 144.2, 144.0, 143.9, 142.5, 141.8, 141.4, 136.3, 136.1, 128.7, 128.6, 128.0, 127.8, 127.6, 127.1, 126.6, 126.5, 125.1, 120.0, 119.9, 79.1, 71.4, 70.8, 67.9, 67.5, 59.7, 59.2, 51.0, 50.8, 50.7, 47.3, 46.5, 46.3, 38.6, 37.0, 35.3, 33.7, 30.4, 29.7, 21.0, 16.3 ppm. IR (film), 3061, 3021, 2949, 2246, 1684, 1598 cm^{-1}. HRMS calcd for $C_{41}H_{37}N_2O_4Li_2$ [M + 2Li − H]$^+$ 635.3073, found 635.3073.

***trans*-4-Azido-*N*-[(fluoren-9-ylmethyl)oxycarbonyl]-L-proline Benzyl Ester (35).** The Boc-protected azide **34**[17] (6.5 g, 18.8 mmol) was dissolved in TFA:CH_2Cl_2 (1:1, 100 mL) and maintained at rt for 30 min. The solvent was then removed by rotary evaporation and a mixture of saturated $NaHCO_3$ (1.5 g, 17.9 mmol) and FmocCl (5.32 g, 20.6 mmol) was added. After being stirred for 12 h, the reaction mixture was extracted into EtOAc (2 × 250 mL). The organic fractions were combined, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography eluting with EtOAc/hexanes (1:4) and afforded the product as a colorless oil (7.4 g, 84%). $[\alpha]^{25}_D$ −4.59 (*c* 10.55, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 7.7 Hz, 2H), 7.60−7.52 (m, 2H), 7.42−7.27 (m, 9H), 5.26−5.04 (m, 2H), 4.56 (t, J = 7.1 Hz, 0.5H), 4.48−4.09

(m, 4H), 4.02 (t, J = 6.1 Hz, 0.5H), 3.79−3.74 (m, 1H), 3.70 (d, J = 10.9 Hz, 0.5H), 3.59 (d, J = 10.8 Hz, 0.5H), 2.37 (m, 1H), 2.20 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 154.4, 143.9, 143.8, 143.4, 141.2, 141.1, 135.3, 135.1, 128.5, 128.4, 128.0, 127.6, 127.0, 124.9, 124.8, 119.9, 119.8, 77.3, 77.0, 76.8, 67.6, 67.0, 66.9, 59.2, 58.4, 57.8, 57.5, 51.7, 51.2, 47.0, 36.3, 35.1 ppm; IR (film) 3065, 2951, 2104, 1748, 1709 cm^{-1}. MS (CI) m/z 469.5 (MH). Anal. Calcd for $C_{27}H_{24}N_4O_4$: C, 69.22; H, 5.16; N, 11.96. Found: C, 69.52; H, 5.12; N, 11.68.

***trans*-4-[((4-Methylphenyl)diphenylmethyl)amino]-*N*-[(fluoren-9-ylmethyl)oxycarbonyl]-L-proline (37).** *trans*-4-azido-*N*-[(fluoren-9-ylmethyl)oxycarbonyl]-L-proline benzyl ester (**35**) (17.0 g, 36.3 mmol) was dissolved in EtOH (150 mL). Pd/C (10% w/w, 4.62 g, 12 mol %) was added and H_2 (350 psi) was applied. The reaction mixture was stirred for 12 h after which time the catalyst was filtered through celite and rinsed with MeOH (250 mL). The solvent was removed by rotary evaporation to yield the reduced product **36** as a colorless solid (crude yield: 12.51 g; 98%) that was used without further purification. The amino alcohol **36** (1.00 g, 2.84 mmol) was dissolved in DMF (15 mL) and DIPEA (1.59 mL, 9.09 mmol) was added dropwise at 0 °C. Mtt-Cl (2.07 g, 7.10 mmol) was dissolved in DMF (15 mL) and added dropwise to the reaction mixture. The reaction was allowed to reach room temperature and stirred overnight. After 12 h MeOH (50 mL) was added and the reaction mixture was warmed to 50 °C for 2 h. The product mixture was then partitioned between water (500 mL) and EtOAc (500 mL). The layers were separated and the aqueous layer was washed with EtOAc (500 mL). The combined organic fractions were washed with water (4 × 500 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by flash chromatography eluting with CH_2Cl_2:MeOH (97:3) afforded the product **37** as a colorless solid (1.20 g, 69.9%). Mp 145−149 °C; $[\alpha]^{25}_D$ 2.05 (c 0.72, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99−6.94 (m, 22H), 4.36−4.02 (m, 4.5H), 3.41−3.23 (m, 1.5H), 3.22−3.01 (m, 1H), 2.90−2.78 (m, 0.5 Hz), 2.76−2.67 (t, J = 9.4 Hz, 0.5H), 2.31 (s, 0.5H), 2.29−2.23 (m, 1.5H), 2.20 (s, 1H), 1.88−1.74 (q, J = 10.1 Hz, 0.5H), 1.73−1.65 (q, J = 10.1 Hz, 0.5H), 1.65−1.53 (m, 0.5H), 1.43−1.32 (m, 0.5H), 1.32−1.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 174.7, 155.9, 154.2, 146.5, 144.3, 144.1, 143.8, 143.3, 141.3, 137.3, 136.2, 128.8, 128.5, 128.4, 128.1, 127.9, 127.8, 127.1, 127.0, 126.6, 125.3, 120.0, 78.6, 77.3, 77.1, 76.8, 70.9, 68.1, 67.3, 62.6, 62.1, 58.2, 57.6, 52.8, 51.9, 47.3, 47.1, 38.2, 36.9, 31.6, 29.7, 20.9, 14.6, 14.4 ppm; IR (film) 3062, 2951, 2248, 1700 cm^{-1}. HRMS calcd for $C_{40}H_{35}N_2O_4Li_2$ $[M + 2Li - H]^+$ 621.2917, found 621.2924.

General Procedure for the Preparation of Trimer Peptides (43−62). Wang resin-Ala(Fmoc) (0.8 mmol/g, 50 mg, 0.04 mmol) was swelled in DMF (3 mL) for 10 min. After removal of solvent, the resin was treated with 20% piperidine in DMF (3 mL, 3 × 10 min), followed by multiple washing with DMF (3 × 3 mL). The deprotected beads were then incubated with Fmoc-Ala-OH (3 equiv), HOBt (3 equiv), and DIC (3 equiv) for 1 h in DMF (3 mL). Following this coupling procedure, the beads were washed with DMF (3 × 3 mL), and then Fmoc-deprotected with 20% piperidine in DMF (3 mL, 3 × 10 min). After multiple washings with DMF (3 × 3 mL), the resin was treated with Fmoc-PTAA(Mtt)-OH (1.5 equiv), HOBt (1.5 equiv), and DIC (1.5 equiv) for 4 h in DMF (3 mL). After PTAA coupling, the side chain amine was then Mtt-deprotected with 1% TFA in CH_2Cl_2 (2 mL, 7 × 10 min). The resin was washed (2 × 3 mL of CH_2Cl_2, 1 × 3 mL of 1% piperidine/DMF, 2 × 3 mL of DMF), then was incubated with representative *N*-Pmc-*N*′-substituted thiourea **18** (1.5 equiv) and EDCI (4.5 equiv) for 2−3 h in DMF (3 mL) (note: thioureas **18e** and **18h** required greater concentrations and longer reaction times: typically 4 equiv of thioureas and 24 h of reaction time for completion). The completed peptide sequence was then Fmoc-deprotected by using 20% piperidine/DMF (3 mL, 3 × 10 min) followed by repeated washing with CH_2Cl_2 (5 × 3 mL). Cleavage of the peptide from the resin and Pmc deprotection was carried out by stirring the resin in 100% TFA (3 mL) for 4 h. The mixture was filtered through glass wool, and the supernatant was concentrated in vacuo to yield the crude peptide residue, which was triturated with diethyl ether and carefully decanted to yield the representative peptide as a colorless powder. **43** (13.7 mg, 52%): ^{1}H NMR (500 MHz, CD_3OD) δ 4.55 (br s, 1H), 4.33−4.40 (m, 2H), 3.49−3.60 (m, 2H), 3.27−3.39 (m, 2H), 2.95 (m, 1H), 2.13 (m, 1H), 1.86−1.95 (m, 2H), 1.84 (m, 1H), 1.72−1.80 (m, 2H), 1.62 (m, 1H), 1.24−1.46 (m, 11H), 1.20 (m, 1H); MS (ESI) m/z 423 (MH); HRMS calcd for $C_{20}H_{35}N_6O_4$ 423.2720, found 423.2714 $[M + H]^+$.

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Supporting Information Available: Detailed experimental procedures, characterization data for compounds **15**, **17**, **18a**−**p**, **44**−**62**, **68**, and **69**, copies of ^{1}H spectra for all compounds, ^{13}C spectra all compounds except **43**−**62**, and mass spectra for compounds **43**−**62** and **83**. This material is available free of charge via the Internet at http://pubs.acs.org.

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